

**THE ROLE OF SOIL MICROBES IN ALLEVIATING
ABIOTIC STRESSES IN ECONOMICALLY
IMPORTANT CROP PLANTS**

**THESIS SUBMITTED TO
DELHI TECHNOLOGICAL UNIVERSITY
FOR THE AWARD OF THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN
BIOTECHNOLOGY**

**SUBMITTED BY
MADHULIKA SINGH
2K18/PHD/BT/511**

**UNDER THE GUIDANCE OF
PROF. JAI GOPAL SHARMA
&
PROF. BHOOPANDER GIRI**



**DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
DELHI-110042
INDIA**

2024

Copyright © Delhi Technological University-2024

ALL RIGHTS RESERVED

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Department of Biotechnology
Shahbad Daulatpur, Bawana Road, Delhi-110042, India



DECLARATION

This is to declare that the research work embodied in this thesis entitled **“The Role of Soil Microbes in Alleviating Abiotic Stresses in Economically Important Crop Plants”** submitted to Delhi Technological University, Delhi-110042, in fulfillment of the requirement for the award of the degree of Doctor of Philosophy is based on the original research work carried out by me under the supervision of Prof. Jai Gopal Sharma, Department of Biotechnology, Delhi Technological University, Delhi, India, and Co-supervision of Prof. Bhoopander Giri, Department of Botany, Swami Shraddhanand College, University of Delhi, Delhi, India. The extent of information derived from the existing literature has been indicated in the body of the thesis at appropriate places giving the source of information. I declare that I have devotedly acknowledged, given credit, and refereed to the research workers wherever their work has been cited in the text and the body of the thesis. Every effort has been made to make sure that the scientific contributions of others are appropriately cited. To the best of my knowledge, this research work has not been submitted in part or full for the award of any degree or diploma in Delhi Technological University or any other University/Institution.

Date: 29 April 2024

Place: Delhi

mamylh
29/4/24
MADHULIKA SINGH
Research Scholar
Reg. No. 2K18/PHD/BT/511

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Department of Biotechnology
Shahbad Daulatpur, Bawana Road, Delhi-110042, India



CERTIFICATE

This is to certify that the Ph.D. thesis entitled "**The Role of Soil Microbes in Alleviating Abiotic Stresses in Economically Important Crop Plants**" submitted to Delhi Technological University, Delhi-110042, in fulfillment of the requirement for the award of the degree of **Doctor of Philosophy** is based on the original research work carried out by the candidate carried under the supervision of Prof. Jai Gopal Sharma, Department of Biotechnology, Delhi Technological University, Delhi, India, and Co-supervision of Prof. Bhoopander Giri, Department of Botany, Swami Shraddhanand College, University of Delhi, Delhi, India. It is further certified that the work embodied in this thesis has neither partially nor fully been submitted to any other University or Institution for the award of any degree or diploma.


Prof. Jai Gopal Sharma 29/04/2024
(Supervisor)

Department of Biotechnology
Delhi Technological University
Delhi-110042


29/4/2024

Prof. Bhoopander Giri
(Co-supervisor)
Department of Botany
Swami Shraddhanand College
University of Delhi
Delhi-110036


29.04.24
Prof. Yasha Hasija
Head and DRC Chairman
Department of Biotechnology
Delhi Technological University
Delhi-110042

Dedicated to My Father
Dr. Mahendra Nath Singh

ACKNOWLEDGEMENTS

The satisfaction and euphoria that accompany the successful completion of any task would be incomplete without the mention of people who made it possible because "*Success is the abstract of hard work and perseverance, but steadfast of all is encouraging guidance.*" The success of our efforts could not have been achieved without the guidance and encouragement of all those who served as beacons of light.

I would like to express my heartfelt gratitude to Prof. Jai Gopal Sharma, my supervisor at the Department of Biotechnology, Delhi Technological University. Throughout my Ph.D. journey, he has been the most influential figure. I am truly grateful for his unwavering commitment to science, outstanding guidance, and discipline that has significantly contributed to my growth as a scientist and an individual over the past five years.

I sincerely appreciate Prof. Bhoopander Giri, my co-supervisor at the Department of Botany, Swami Shraddhanand College, University of Delhi. His meticulous review of every aspect of my work has played a pivotal role in ensuring its excellence. I am grateful for critical appraisal, perceptive comments, and insightful discussions, which were instrumental in guiding me through the process of writing and revising my thesis. I value his patience, understanding, and constructive criticism immensely. I am grateful to Prof. Pravir Kumar, former Head of the Department of Biotechnology, for providing me with the necessary facilities to complete my research work.

I want to express my special and heartfelt thanks to Prof. Ashok K. Prasad, Former Head, Department of Chemistry, University of Delhi, Delhi for his support and positive enthusiasm throughout my research. My sincere thanks to Prof. K.S. Rao for providing the photosynthesis analyzer instrument facility in the Department of Botany, University of Delhi. I also thank Prof. Ratul Baishya for providing the leaf area meter facility in the Department of Botany, University of Delhi. My sincere gratitude to Prof. P.L. Uniyal for always being an inspiration and support.

It is my sincere pleasure to thank Prof. Ajit Verma Department of Microbiology, Amity University, Noida, India for his training in culture techniques for *Piriformospora indica*. My sincere appreciation goes out to Prof. Parveen Garg, Principal, Swami Shraddhanand College, University of Delhi for his constant support during my research work. My research work would not have been possible without the support provided by

Dr. D.V. Bhardwaj, Administrative Officer, Swami Shraddhanand College, University of Delhi. A special thanks to Dr. Anjana Kapoor, Department of Microbiology, Swami Shraddhanand College, University of Delhi for her kind help during my research career.

My sincere thanks to all the faculty members of the Department of Biotechnology, Delhi Technological University, Delhi, and lab colleagues especially Ms. Neha Tiwari for their positive cooperation and support during my research work. My sincere thanks to Aavnish Kumar, Neelesh Kumar, and Guddu Kumar for their help during my statistical problems and research work.

I am grateful to the All India Institute of Medical Sciences, New Delhi for extending their microscopy facilities. I am thankful to the Department of Soil Sciences and Agricultural Chemistry at the Indian Agricultural Research Institute in Delhi for the analysis of the physio-chemical parameters of soil samples. I extend my sincere thanks to National Seeds Corporation Limited, Beej Bhawan, Pusa Complex, New Delhi, India for providing seeds for my research work. I would like to acknowledge the Department of Microbiology, IARI, New Delhi for providing the consortium of plant-growth-promoting bacteria and Arbuscular Mycorrhizal Fungi.

I especially thank Mr. Jaipal, Mr. Pramod, and Mr. Devender ji, for their kind help in growing plants for my research work. I am thankful to Mr. Ganesh Giri, Mr. Rajkumar, and Mr. Sandeep Kumar for their laboratory assistance. They have been a huge support throughout my research period.

This thesis is simply impossible without the support of my husband Dr. Sunil Kumar Singh who has always been the guiding force in my life. His unending support kept me moving easily with all hazards disappearing miraculously. He taught me the importance of learning, believing in myself, and instilling in me the importance of learning. My deepest gratitude to my mother Mrs. Prabha Singh, for her blessings and love. I am proud of my son Rohan Singh for his unwavering love and support throughout my research work.

Date: 29 April 2024

Place: Delhi

monish
29/4/24
Madhulika Singh

ABSTRACT

The present study aimed to investigate the role of *Piriformospora indica*, arbuscular mycorrhizal fungi (AMF), and plant growth-promoting bacteria (PGPB) in alleviating drought and salinity stress in HD-2967 wheat and Maharaja BIO-9637 maize variety. In a completely randomized design experiment, wheat and maize plants were subjected to different water regimes viz. 75, 55, 45, and 35% field capacity (FC) and different levels of salinity viz. 0, 50, 100, and 200 mM NaCl under greenhouse conditions. Under different water regimes and salinity levels, microbial inoculation significantly enhanced the morphological, physiological, biochemical, and ultrastructural characteristics of the wheat and maize plants. Plants inoculated with PGPB, *P. indica*, and AMF showed increased shoot and root length, shoot and root biomass, leaf area, photosynthetic rate, transpiration rate, stomatal conductance, and internal CO₂ as compared to uninoculated plants under all water regimes and salinity levels. The PGPB, *P. indica*, and AMF-inoculated wheat and maize plants accumulated higher content of glycine betaine, total sugars, trehalose, proline, putrescine, spermidine, carotenoids, proteins, α -tocopherol, and showed a decrease in lipid peroxidation, relative membrane permeability, and lipoxygenase enzyme (LOX) activity as compared to uninoculated plants. Besides, PGPB and *P. indica* wheat and maize plants showed a higher level of antioxidant enzymes viz., superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX) than uninoculated plants under drought and salinity stress. However, AMF inoculation did not affect CAT activity in maize plants under drought and salinity stress, and wheat plants under salinity stress and its activity decreased with increasing levels of stress. Microbial inoculation helped wheat and maize plants to overcome water stress and salinity-induced deficiency of macronutrients (Ca²⁺, Mg²⁺, and K⁺) and micronutrients (Cu, Mn²⁺, Fe, and Zn²⁺), and reduced damage to the cell ultrastructure (plasma membrane and chloroplasts). Comparing the potential of microbial inoculants to increase growth, nutritional, biochemical, physiological, and ultrastructural changes, the PGPB-inoculated wheat and maize plants showed greater drought and salinity resilience followed by AMF and *P. indica* inoculated plants. These microbial inoculants offer a significant potential to meet the challenges of sustainable agriculture under drought and salinity conditions.

CONTENTS

	<i>Page No.</i>
<i>List of Tables</i>	<i>i</i>
<i>List of Figures</i>	<i>iii</i>
<i>List of Abbreviations</i>	<i>vii</i>
1. Introduction	1
2. Review of Literature	7
2.1 Effect of drought stress	7
2.2 Effect of salinity stress	7
2.3 Plant adaption to resist abiotic stress	8
2.4 Strategies to minimize crop loss due to abiotic stress	9
2.4.1 <i>Piriformospora indica</i>	10
2.4.2 Arbuscular mycorrhizal fungi	13
2.4.3 Plant growth-promoting bacteria	13
2.5 Role of microbes in alleviating abiotic stress in plants	16
2.5.1 Plant growth and biomass	16
2.5.2 Nutrient uptake	18
2.5.2.1 Macronutrients	18
2.5.2.2 Micronutrients	23
2.5.3 Physiological changes	25
2.5.4 Biochemical changes	28
2.5.4.1 Photosynthetic pigments	28
2.5.4.2 Osmolytes	29
2.5.4.3 Protein	31
2.5.4.4 α -tocopherol	33
2.5.4.5 Membrane damage	34
2.5.5 Antioxidant response	34
2.5.6 Ultrastructural changes	35
2.5.6.1 Chloroplast	35
2.5.6.2 Plasma membrane	37

3.	Materials and Methods	40
3.1	Plant materials	40
3.1.1	Habit	40
3.1.2	Economic importance	41
3.2	Microbial inoculum	42
3.3	Experimental design, growth condition, and soil	44
3.3.1	Wheat plants subjected to drought stress treatment	44
3.3.2	Wheat plants subjected to salinity stress treatment	46
3.3.3	Maize plants subjected to drought stress treatment	48
3.3.4	Maize plants subjected to salinity stress treatment	50
3.4	Measurements	52
3.4.1	Objective I	
3.4.1.1	Estimation of AMF and <i>P. indica</i> colonization	52
3.4.1.2	Estimation of plant growth parameters	52
3.4.1.3	Elemental analysis of tissue	52
3.4.1.4	Estimation of physiological changes	53
3.4.1.5	Estimation of biochemical changes	53
3.4.1.5.1	Photosynthetic pigments	53
3.4.1.5.2	Osmolytes	53
3.4.1.5.3	Protein	55
3.4.1.5.4	α -tocopherol	56
3.4.1.5.5	Membrane damage	56
3.4.2	Objective II	57
3.4.2.1	Determination of ultrastructural changes	57
3.4.3	Objective III	58
3.4.3.1	Enzymatic antioxidants	58
4.	Results and Discussion	
4.1	Part I: The role of soil microbes in alleviating drought stress in wheat	59
4.1.1	Results	59
4.1.1.1	Microbial colonization	59
4.1.1.2	Growth parameters	61
4.1.1.3	Mineral nutrients content	61

4.1.1.4	Physiological parameters	65
4.1.1.5	Biochemical parameters	66
4.1.1.5.1	Photosynthetic pigment content	66
4.1.1.5.2	Osmolyte content	67
4.1.1.5.3	Protein	70
4.1.1.5.4	α -tocopherol content	70
4.1.1.5.5	Membrane damage	71
4.1.1.6	Antioxidant enzyme activity	72
4.1.1.7	Cell ultrastructural studies	74
4.1.1.7.1	Chloroplast	74
4.1.1.7.2	Plasma membrane	77
4.1.2	Discussion	80
4.2	Part II: The role of soil microbes in alleviating salinity stress in wheat	90
4.2.1	Results	90
4.2.1.1	Microbial colonization	90
4.2.1.2	Growth parameters	90
4.2.1.3	Mineral nutrients content	92
4.2.1.4	Physiological parameters	96
4.2.1.5	Biochemical parameters	97
4.2.1.5.1	Photosynthetic pigment content	97
4.2.1.5.2	Osmolyte content	98
4.2.1.5.3	Protein	101
4.2.1.5.4	α -tocopherol content	101
4.2.1.5.5	Membrane damage	102
4.2.1.6	Antioxidant enzyme activity	103
4.2.1.7	Cell ultrastructural studies	105
4.2.1.7.1	Chloroplast	105
4.2.1.7.2	Plasma membrane	108
4.2.2	Discussion	111
4.3	Part III: The role of soil microbes in alleviating drought stress in maize	120
4.3.1	Results	120
4.3.1.1	Microbial colonization	120
4.3.1.2	Growth parameters	122
4.3.1.3	Mineral nutrients content	122

4.3.1.4	Physiological parameters	124
4.3.1.5	Biochemical parameters	125
4.3.1.5.1	Photosynthetic pigment content	125
4.3.1.5.2	Osmolyte content	126
4.3.1.5.3	Protein	129
4.3.1.5.4	α -tocopherol content	130
4.3.1.5.5	Membrane damage	130
4.3.1.6	Antioxidant enzyme activity	132
4.3.1.7	Cell ultrastructural studies	133
4.3.1.7.1	Chloroplast	134
4.3.1.7.2	Plasma membrane	137
4.3.2	Discussion	140
4.4	Part IV: The role of soil microbes in alleviating salinity stress in maize	149
4.4.1	Results	149
4.4.1.1	Microbial colonization	149
4.4.1.2	Growth parameters	151
4.4.1.3	Mineral nutrients content	151
4.4.1.4	Physiological parameters	155
4.4.1.5	Biochemical parameters	156
4.4.1.5.1	Photosynthetic pigment content	156
4.4.1.5.2	Osmolyte content	157
4.4.1.5.3	Protein	159
4.4.1.5.4	α -tocopherol content	160
4.4.1.5.5	Membrane damage	161
4.4.1.6	Antioxidant enzyme activity	162
4.4.1.7	Cell ultrastructural studies	164
4.4.1.7.1	Chloroplast	164
4.4.1.7.2	Plasma membrane	167
4.4.2	Discussion	170
5.	Summary and Conclusion	179
	References	187
	List of Publication	223
	Conferences	226

LIST OF FIGURES

<i>Figure No.</i>	<i>Title</i>	<i>Page No.</i>
2.1	Causal factors and impact of drought and salinity on plant growth.	8
2.2	Protective mechanisms used by microbes in mitigation of drought and salinity stress	10
3.1	Microscopic view of (A) <i>Azotobacter chroococcum</i> (B) <i>Lactococcus lactis</i> (C) <i>Enterobacter asburiae</i> bacteria after gram staining	42
3.2	Arbuscular mycorrhizal fungi (A-E) and <i>P. indica</i> (F) spores as seen under a microscope	43
3.3	Experimental set-up showing the influence of drought stress on (A) un-inoculated wheat plants (control); (B) <i>P. indica</i> -inoculated wheat plants; and (C) PGPB-inoculated wheat plants (D) AMF-inoculated wheat plants after 80 days of sowing	45
3.4	Experimental set-up showing the influence of salinity stress on (A) un-inoculated wheat plants (control); (B) <i>P. indica</i> -inoculated wheat plants; and (C) PGPB-inoculated wheat plants (D) AMF-inoculated wheat plants after 80 days of sowing	47
3.5	Experimental set-up showing the influence of drought stress on (A) un-inoculated maize plants (control); (B) <i>P. indica</i> -inoculated maize plants; and (C) PGPB-inoculated maize plants (D) AMF-inoculated maize plants after 80 days of sowing	49
3.6	Experimental set-up showing the influence of salinity stress on (A) un-inoculated maize plants (control); (B) <i>P. indica</i> -inoculated maize plants; and (C) PGPB-inoculated maize plants (D) AMF-inoculated maize plants after 80 days of sowing	51
4.1	Microscopic observation of root colonization of (A) AMF-inoculated wheat plants and (B) <i>P. indica</i> -inoculated wheat plants under drought stress	59
4.2	Effects of drought and microbial inoculation in wheat on (A) photosynthetic rate; (B) stomatal conductance; (C) transpiration rate; and (D) internal CO ₂	65

Figure No.	Title	Page No.
4.3	Effects of drought and microbial inoculation in wheat on the content of (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; and (D) carotenoid.	66
4.4	Effects of drought and microbial inoculation in wheat on the content of (A) glycine betaine; (B) total sugar; (C) trehalose; (D) proline; (E) putrescine; and (F) spermidine	68
4.5	Effects of drought and microbial inoculation in wheat on the content of (A) protein; and (B) α -tocopherol	70
4.6	Effects of drought and microbial inoculation in wheat on (A) MDA content; and (B) relative permeability (C) LOX activity	72
4.7	Effects of drought and microbial inoculation in wheat on the activity of (A) SOD; (B) CAT; and (C) APX	73
4.8	Transmission electron micrographs of chloroplast in mesophyll cells of (A) Control wheat plants not exposed to drought stress (75% FC) (B) Control wheat plants subjected to 55% FC (C) Control wheat plants exposed to 45% FC (D) Control wheat plants subjected to 35% FC (E) <i>P. indica</i> inoculated wheat plants not exposed to drought stress (75% FC) (F) <i>P. indica</i> inoculated wheat plants subjected to 55% FC (G) <i>P. indica</i> inoculated wheat plants subjected to 45% FC (H) <i>P. indica</i> inoculated wheat plants subjected to 35% FC	75
4.9	Transmission electron micrographs of chloroplast in mesophyll cells of (A) PGPB-inoculated wheat plants not exposed to drought stress (75% FC) (B) PGPB-inoculated wheat plants subjected to 55% FC (C) PGPB-inoculated wheat plants exposed to 45% FC (E) AMF-inoculated wheat plants not exposed to drought stress (75% FC) (F) AMF-inoculated wheat plants subjected to 55% FC (G) AMF-inoculated wheat plants subjected to 45% FC (H) AMF-inoculated wheat plants subjected to 35% FC	76
4.10	Transmission electron micrographs of the cell wall and plasma membrane in mesophyll cells of (A) Control wheat plants not exposed to drought stress (75% FC) (B) Control wheat plants subjected to 55% FC stress (C) Control wheat plants exposed to 45% FC stress (D) Control wheat plants subjected to 35% FC stress (E) <i>P. indica</i> inoculated wheat plants not exposed to drought stress (75% FC) (F) <i>P. indica</i> inoculated wheat plants subjected to 55% FC stress (G) <i>P. indica</i> inoculated wheat plants subjected to 45% FC stress (H) <i>P. indica</i> inoculated wheat plants subjected to 35% FC stress	78

Figure No.	Title	Page No.
4.11	Transmission electron micrographs of the cell wall and plasma membrane in mesophyll cells of (A) PGPB-inoculated wheat plants not exposed to drought stress (75% FC) (B) PGPB-inoculated wheat plants subjected to 55% FC stress (C) PGPB-inoculated wheat plants exposed to 45% FC (D) PGPB-inoculated wheat plants subjected to 35% FC stress (E) AMF-inoculated wheat plants not exposed to drought stress (75% FC) (F) AMF-inoculated wheat plants subjected to 55% FC (G) AMF-inoculated wheat plants subjected to 45% FC stress (H) AMF-inoculated wheat plants subjected to 35% FC stress	79
4.12	Microscopic observation of root colonization of (A) AMF-inoculated wheat plants and (B) <i>P. indica</i> -inoculated wheat plants under salinity stress	90
4.13	Effects of salinity and microbial inoculation in wheat on (A) photosynthetic rate; (B) stomatal conductance; (C) transpiration rate; and (D) internal CO ₂	96
4.14	Effects of salinity and microbial inoculation in wheat on the content of (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; and (D) carotenoid	97
4.15	Effects of salinity and microbial inoculation in wheat on the content of (A) glycine betaine; (B) total sugar; (C) trehalose; (D) proline; (E) putrescine; and (F) spermidine	100
4.16	Effects of salinity and microbial inoculation in wheat on the content of (A) protein; and (B) α -tocopherol	101
4.17	Effects of salinity and microbial inoculation in wheat on (A) MDA content; and (B) relative permeability (C) LOX activity	103
4.18	Effects of salinity and microbial inoculation in wheat on the activity of (A) SOD; (B) CAT; and (C) APX	104
4.19	Transmission electron micrographs of chloroplast in mesophyll cells of (A) Control wheat plant not exposed to NaCl (B) Control wheat plants subjected to 50 mM NaCl (C) Control wheat plants not exposed to 100 mM NaCl (D) Control wheat plants subjected to 200 mM NaCl (E) <i>P. indica</i> inoculated wheat plants not exposed to NaCl (F) <i>P. indica</i> inoculated wheat plants subjected to 50 mM NaCl (G) <i>P. indica</i> inoculated wheat plants subjected to 100 mM NaCl (H) <i>P. indica</i> inoculated wheat plants subjected to 200 mM NaCl	106

<i>Figure No.</i>	<i>Title</i>	<i>Page No.</i>
4.20	Transmission electron micrographs of chloroplast of mesophyll cells of (A) PGPB-inoculated wheat plants not exposed to NaCl (B) PGPB-inoculated wheat plants subjected to 50 mM NaCl (C) PGPB-inoculated wheat plants exposed to 100 mM NaCl (D) PGPB-inoculated wheat plants subjected to 200 mM NaCl (E) AMF-inoculated wheat plants not exposed to NaCl (F) AMF-inoculated wheat plants subjected to 50 mM NaCl (G) AMF-inoculated wheat plants subjected to 100 mM NaCl (H) AMF-inoculated wheat plants subjected to 200 mM NaCl	107
4.21	Transmission electron micrographs of the mesophyll cell wall and plasma membrane (A) Control wheat plants not exposed to NaCl (B) Control wheat plants subjected to 50 mM NaCl (C) Control maize plants exposed to 100 mM NaCl (D) Control wheat plants subjected to 200 mM NaCl (E) <i>P. indica</i> wheat inoculated plants not exposed to NaCl (F) <i>P. indica</i> inoculated wheat plants subjected to 50 mM NaCl (G) <i>P. indica</i> inoculated wheat plants subjected to 100 mM NaCl (H) <i>P. indica</i> inoculated wheat plants subjected to 200 mM NaCl	109
4.22	Transmission electron micrographs of the mesophyll cell wall and plasma membrane: (A) PGPB-inoculated wheat plants without NaCl treatment (B) PGPB-inoculated wheat plants subjected to 50 mM NaCl (C) PGPB-inoculated wheat plants subjected to 100 mM NaCl (F) AMF-inoculated wheat plant subjected to 50 mM NaCl (G) AMF-inoculated wheat plants subjected to 100 mM NaCl (H) AMF-inoculated wheat plants subjected to 200 mM NaCl	110
4.23	Microscopic observation of root colonization of (A) AMF-inoculated maize plants and (B) <i>P. indica</i> -inoculated maize plants under drought stress	120
4.24	Effects of drought and microbial inoculation in maize on (A) photosynthetic rate; (B) stomatal conductance; (C) transpiration rate; and (D) internal CO ₂	125
4.25	Effects of drought and microbial inoculation in maize on the content of (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; and (D) carotenoid	126
4.26	Effects of drought and microbial inoculation in maize on the content of (A) glycine betaine; (B) total sugar; (C) trehalose; (D) proline; (E) putrescine; and (F) spermidine	129

Figure No.	Title	Page No.
4.27	Effects of drought and microbial inoculation in maize on the content of (A) protein; and (B) α -tocopherol	130
4.28	Effects of drought and microbial inoculation in maize on (A) MDA content; and (B) relative permeability (C) LOX activity	131
4.29	Effects of drought and microbial inoculation in maize on the activity of (A) SOD; (B) CAT; and (C) APX	133
4.30	Transmission electron micrographs of chloroplast in mesophyll cells of (A) Control maize plants not exposed to drought stress (75% FC) (B) Control maize plants subjected to 55% FC (C) Control maize plant exposed to 45% FC (D) Control maize plants subjected to 35% FC (E) <i>P. indica</i> inoculated maize plants not exposed to drought stress (75% FC) (F) <i>P. indica</i> maize inoculated plants subjected to 55% FC (G) <i>P. indica</i> inoculated maize plants subjected to 45% FC (H) <i>P. indica</i> inoculated maize plants subjected to 35% FC	135
4.31	Transmission electron micrographs of chloroplast in mesophyll cells of (A) PGPB-inoculated maize plants not exposed to drought stress (75% FC) (B) PGPB-inoculated maize plants subjected to 55% FC (C) PGPB-inoculated maize plants exposed to 45% FC (D) PGPB-inoculated maize plants subjected to 35% FC (E) AMF-inoculated maize plants not exposed to drought stress (75% FC) (F) AMF-inoculated maize plants subjected to 55% FC (G) AMF-inoculated maize plants subjected to 45% FC (H) AMF-inoculated maize plants subjected to 35% FC	136
4.32	Transmission electron micrographs of the cell wall and plasma membrane in mesophyll cells of (A) Control maize plants not exposed to drought stress (75% FC) (B) Control plants subjected to 55% FC stress (C) Control maize plants exposed to 45% FC stress (D) Control maize plants subjected to 35% FC stress (E) <i>P. indica</i> inoculated maize plants not exposed to drought stress (75% FC) (F) <i>P. indica</i> inoculated maize plants subjected to 55% FC stress (G) <i>P. indica</i> inoculated maize plants subjected to 45% FC stress (H) <i>P. indica</i> inoculated maize plants subjected to 35% FC stress	138

Figure No.	Title	Page No.
4.33	Transmission electron micrographs of the cell wall and plasma membrane in mesophyll cells of (A) PGPB-inoculated maize plants not exposed to drought stress (75% FC) (B) PGPB-inoculated maize plants subjected to 55% FC stress (C) PGPB-inoculated maize plants exposed to 45% FC (D) PGPB-inoculated maize plants subjected to 35% FC stress (E) AMF-inoculated maize plants not exposed to drought stress (75% FC) (F) AMF-inoculated maize plants subjected to 55% FC (G) AMF-inoculated maize plants subjected to 45% FC stress (H) AMF-inoculated maize plants subjected to 35% FC stress	139
4.34	Microscopic observation of root colonization of (A) AMF-inoculated maize plants and (B) <i>P. indica</i> -inoculated maize plants under salinity stress	149
4.35	Effects of salinity and microbial inoculation in maize on (A) photosynthetic rate; (B) stomatal conductance; (C) transpiration rate; and (D) internal CO ₂	155
4.36	Effects of salinity and microbial inoculation in maize on the content of (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; and (D) carotenoid	156
4.37	Effects of salinity and microbial inoculation in maize on the content of (A) glycine betaine; (B) total sugar; (C) trehalose; (D) proline; (E) putrescine; and (F) spermidine	159
4.38	Effects of salinity and microbial inoculation in maize on the content of (A) protein; and (B) α -tocopherol	160
4.39	Effects of salinity and microbial inoculation in maize on (A) MDA content; and (B) relative permeability (C) LOX activity	161
4.40	Effects of salinity and microbial inoculation in maize on the activity of (A) SOD; (B) CAT; and (C) APX	163
4.41	Transmission electron micrographs of chloroplast in mesophyll cells of (A) Control maize plants not exposed to NaCl (B) Control maize plants subjected to 50 mM NaCl (C) Control maize plants not exposed to 100 mM NaCl (D) Control maize plant subjected to 200 mM NaCl (E) <i>P. indica</i> inoculated maize plant not exposed to NaCl (F) <i>P. indica</i> inoculated maize plant subjected to 50 mM NaCl (G) <i>P. indica</i> inoculated maize plant subjected to 100 mM NaCl (H) <i>P. indica</i> inoculated maize plant subjected to 200 mM NaCl	165

<i>Figure No.</i>	<i>Title</i>	<i>Page No.</i>
4.42	Transmission electron micrographs of the chloroplast of mesophyll cells of (A) PGPB-inoculated maize plants not exposed to NaCl (B) PGPB-inoculated maize plants subjected to 50 mM NaCl (C) PGPB-inoculated maize plants exposed to 100 mM NaCl (D) PGPB-inoculated maize plants subjected to 200 mM NaCl (E) AMF-inoculated maize plants not exposed to NaCl (F) AMF-inoculated maize plants subjected to 50 mM NaCl (G) AMF-inoculated maize plants subjected to 100 mM NaCl (H) AMF-inoculated maize plants subjected to 200 mM NaCl	166
4.43	Transmission electron micrographs of the mesophyll cell wall and plasma membrane (A) Control maize plants not exposed to NaCl (B) Control maize plants subjected to 50 mM NaCl (C) Control maize plants exposed to 100 mM NaCl (D) Control maize plants subjected to 200 mM NaCl (E) <i>P. indica</i> inoculated maize plants not exposed to NaCl (F) <i>P. indica</i> inoculated maize plants subjected to 50 mM NaCl (G) <i>P. indica</i> inoculated maize plants subjected to 100 mM NaCl (H) <i>P. indica</i> inoculated maize plants subjected to 200 mM NaCl	168
4.44	Transmission electron micrographs of the mesophyll cell wall and plasma membrane: (A) PGPB-inoculated maize plants without NaCl treatment (B) PGPB-inoculated maize plants subjected to 50 mM NaCl (C) PGPB-inoculated maize plants subjected to 100 mM NaCl (D) PGPB-inoculated maize plants subjected to 200 mM NaCl (E) AMF-inoculated maize plants not exposed to NaCl (F) AMF-inoculated maize plants subjected to 50 mM NaCl (G) AMF-inoculated maize plants subjected to 100 mM NaCl (H) AMF-inoculated maize plants subjected to 200 mM NaCl	169

LIST OF TABLES

<i>Table No.</i>	<i>Titles</i>	<i>Page No.</i>
2.1	Growth, nutrient, physiological, and biochemical status of microbial inoculated plants compared to uninoculated plants under drought stress	11
2.2	Growth, nutrient, physiological, and biochemical status of microbial inoculated plants compared to uninoculated plants under salinity stress	14
3.1	Classification of AMF and <i>P. indica</i>	44
3.2	Physio-chemical properties of soil used for growing wheat plants under drought stress	45
3.3	Physio-chemical properties of soil used for growing wheat plants under salinity stress	47
3.4	Physio-chemical property of soil used for growing maize plants under drought stress	49
3.5	Physio-chemical property of soil used for growing maize plants under salinity stress	51
4.1	Influence of drought stress and microbe inoculations on percentage microbe colonization, length, and biomass of shoot and root in wheat plants	60
4.2	Influence of water stress and microbial inoculation on the content of macro-nutrients and micro-nutrients in leaves of the wheat plants	64
4.3	Effect of drought, <i>P. indica</i> , drought $\times$ <i>P. indica</i> , PGPB, drought $\times$ PGPB, AMF, drought $\times$ AMF on physiological processes, photosynthetic pigments, protein, α -tocopherol, membrane damage, osmolytes contents, and antioxidant enzyme activity in wheat plants	80
4.4	Influence of salinity stress and microbe inoculations on percentage microbe colonization, length, and biomass of shoot and root in wheat plants	91
4.5	Influence of salinity stress and microbial inoculation on the content of macro-nutrients and micronutrients in leaves of the wheat plants	95

<i>Table No.</i>	<i>Titles</i>	<i>Page No.</i>
4.6	Effect of NaCl, <i>P. indica</i> , NaCl × <i>P. indica</i> , PGPB, NaCl × PGPB, AMF, NaCl × AMF on physiological processes, photosynthetic pigments, protein, α-tocopherol, membrane damage, osmolytes contents, and antioxidant enzyme activity in wheat plants	111
4.7	Influence of drought stress and microbe inoculations on percentage microbe colonization, length, and biomass of shoot and root in maize plants	121
4.8	Influence of water stress and microbial inoculation on the content of macro-nutrients and micronutrients in leaves of the maize plants	123
4.9	Effect of drought, <i>P. indica</i> , drought × <i>P. indica</i> , PGPB, drought × PGPB, AMF, drought × AMF on physiological processes, photosynthetic pigments, protein, α-tocopherol, membrane damage, osmolytes contents, and antioxidant enzyme activity in maize plants	140
4.10	Influence of salinity stress and microbe inoculations on percentage microbe colonization, length, and biomass of shoot and root in maize plants	150
4.11	Influence of salinity stress and microbial inoculation on the content of macro-nutrients and micro-nutrients in leaves of the maize plants	154
4.12	Effect of NaCl, <i>P. indica</i> , NaCl × <i>P. indica</i> , PGPB, NaCl × PGPB, AMF, NaCl × AMF on physiological processes, photosynthetic pigments, protein, α-tocopherol, membrane damage, osmolytes contents, and antioxidant enzyme activity in maize plants	170

LIST OF ABBREVIATIONS

AMF	:	Arbuscular mycorrhizal fungi
APX	:	Ascorbate peroxidase
Ca ²⁺	:	Calcium
CO ₂	:	Carbon dioxide
CAT	:	Catalase
cw	:	Cell wall
cm	:	Centimeter
Cu	:	Copper
CuSO ₄ x 5H ₂ O	:	<i>Copper sulfate pentahydrate</i>
°C	:	Degree Celsius
DMSO	:	Dimethyl sulfoxide
EDTA	:	Ethylene diamine tetra-acetic acid
FC	:	Field capacity
g	:	Gram
HPLC	:	High-performance liquid chromatography
h	:	Hour
HCl	:	Hydrochloric acid
H ₂ O ₂	:	Hydrogen peroxide
IARI	:	Indian Agricultural Research Institute
ICP-MS	:	Inductively coupled plasma mass spectrometry
Fe	:	Iron
kg	:	Kilogram
kg ha ⁻¹	:	Kilogram per hectare
LOX	:	Lipoxygenase
Mg ²⁺	:	Magnesium
MDA	:	Malondialdehyde content
Mn ²⁺	:	Manganese
µg g ⁻¹	:	Microgram per gram
µl	:	Microlitre

μM	:	Micromolar
$\mu\text{mol m}^{-2}\text{s}^{-1}$	:	Micromolar per meter square per second
$\mu\text{mol min}^{-1} \text{g}^{-1} \text{fw}$	:	Micromole per minute per gram fresh weight
mg	:	Milligram
mg g^{-1}	:	Milligram per gram
$\text{mg g}^{-1}\text{fw}$	:	Milligram per gram fresh weight
mg l^{-1}	:	Milligram per litre
ml/min	:	Milliliter per minute
ml	:	Millilitre
mm	:	Millimeter
mM	:	Millimolar
min	:	Minute(s)
nm	:	Nanometre
$\text{nmol g}^{-1} \text{fw}$	:	Nanomole per gram fresh weight
HNO_3	:	Nitric acid
N	:	Nitrogen
ppm	:	Parts per million
%	:	Percent
P	:	Phosphate
PGPB	:	Plant growth promoting bacteria
pm	:	Plasma membrane
pg	:	Plastoglobule
K^+	:	Potassium
KI-I ₂	:	Potassium iodide iodine
K_2SO_4	:	Potassium sulphate
psi	:	Pound-force per square inch
ROS	:	Reactive oxygen species
rpm	:	Rotation per minute
Na^+	:	Sodium
Na_2CO_3	:	Sodium bicarbonate
NaCl	:	Sodium chloride

NaOH	:	Sodium hydroxide
SD	:	Standard deviation
H ₂ SO ₄	:	Sulphuric acid
SOD	:	Superoxide dismutase
TBA	:	Thiobarbituric acid
TCA	:	Trichloroacetic acid
V	:	Vesicle
v/v	:	Volume per volume
H ₂ O	:	Water
W	:	Watt
w/v	:	Weight per volume
Zn ²⁺	:	Zinc

1. INTRODUCTION

Abiotic stress such as drought, salinity, heat, cold, and heavy metals are serious threats to plant production and yield. Drought is a major abiotic stress that adversely affects agricultural production and threatens food security worldwide (Guo et al. 2020, Mirzabaev et al. 2023). A decline in rainfall and an increasing frequency of dry spells are contributing to drought stress (Jarrett et al. 2023). As a consequence, about 45% of agricultural fields are experiencing continuous drought conditions (Yadav et al. 2020) that eventually negatively affect plant growth by decreased transpiration, photosynthesis rates (Naservafaei et al. 2023), osmolytes (Haghighi et al. 2023) and nutrient uptake (Shah et al. 2023). Under drought stress, plants face the problem of increased electrolyte leakage, lipid peroxidation, and reactive oxygen species (ROS) production (Yu et al. 2023). Nevertheless, prolonged drought may result in irreparable damage to plant growth, resulting in reduced crop yields.

The growth, productivity, and yield of plants are also adversely affected by salinity stress (Soni and Savalia 2023). Approximately 50% of global agricultural land will be affected by salinity by 2050 (Gopalakrishnan and Kumar 2021). Several factors contribute to salinity problems, such as low rainfall, high rates of evaporation, weathering, excessive fertilizer use, and improper irrigation practices (Gopalakrishnan et al. 2020, Zhao et al. 2020). In plants exposed to salinity, changes occur in morphology, physiology, biochemistry (Silva et al. 2023), and ultrastructure that inhibit growth and development (El-Dakak et al. 2023). Additionally, salinity affects enzyme activity, stomatal conductance, photosynthesis, and reactive oxygen species (ROS) production. ROS produced by cells damages cell membranes, lipids, proteins, DNA, and RNA, as well as induces programmed cell death (Zhao et al. 2020, Hussain et al. 2021). Salinity stress adversely affects nutrient uptake, osmotic balance, membrane integrity, and the overall growth of plants (Evelin et al. 2009, Srivastava et al. 2019, Zhang et al. 2022a). Moreover, salinity stress severely affects the photosynthetic apparatus in plants through the inhibition of chlorophyll biosynthesis, leading to reduced CO₂ availability due to stomatal closure, and inflicting damage on the

photosynthetic apparatus, ultimately causing a significant decline in photosynthetic efficiency (Zahra et al. 2022).

However, to overcome the problem, the plant needs to maintain better osmotic adjustment, antioxidant defense mechanisms, stomatal closure, and increased root-shoot ratios (López-Galiano et al. 2019). Plants acquire many physiological and metabolic adaptations to resist abiotic stress, including ion toxicity (Bibi et al. 2022), osmotic shock (Umar et al. 2022), and oxidative damage (Afridi et al. 2022). Studies have shown that plants have evolved mechanisms to tolerate abiotic stress, such as the synthesis of antioxidant enzymes, polyamines, compatible solutes, osmoprotectants, and hormones (Numan et al. 2018). Plants are equipped with an efficient antioxidant system to counter salt-induced oxidative stress (Azeem et al. 2023). It includes antioxidant enzymes like SOD, CAT, and APX (Azeem et al. 2023) which can scavenge ROS and prevent membrane damage from oxidative stress. SOD is considered a first line of defense that converts superoxide into H_2O_2 . Further, CAT and APX convert H_2O_2 into H_2O . Additionally, non-enzymatic antioxidants, like polyamines, tocopherols, carotenoids, prolines (Hao et al. 2021), glutathione, ascorbic acid, polyphenols, and flavonoids (Azeem et al. 2023) play a crucial role in ROS detoxification. Carotenoids protect chloroplast ultrastructure, inhibit peroxidation of lipids, stabilize membrane integrity, and play a central role in the assembly of light-harvesting complex (Gómez-Sagasti et al. 2022). Polyamines interact with negatively charged membrane sites to inhibit membrane degradation in both grana and stroma thylakoids (Ali et al. 2020, Wang et al. 2022). Proline is an effective ROS scavenger in stressful environments, preventing cells from being damaged by oxidative stress. In addition, tocopherol prevents lipid peroxidation by scavenging free radicals in the host tissue (Evelin et al. 2013, Niki 2021). In response to abiotic stress, osmolytes accumulate, including trehalose, proline, glycine betaine, and polyamines, all of which play a key role in maintaining cell turgidity, and membrane integrity (Patel et al. 2020). Additionally, osmolytes regulate osmotic pressure and quench free radicals (Bashir et al. 2021). As a non-reducing sugar, trehalose stabilizes membranes and macromolecules under salinity stress by maintaining osmotic balance and preventing oxidative damage. Proline is a secondary amino acid that is crucial for osmotic adjustment (Noreen et al.

2019), as well as membrane stabilization in salinity stress conditions. Despite this, prolonged abiotic stress may impede the growth of plants, resulting in a reduction in yield.

Wheat (*Triticum aestivum* L.) is an important cereal crop that belongs to the family Poaceae and is considered to be the first-ever domesticated plant (Raza et al. 2019). The plant is used to make a wide range of products like bread, macaroni cake, cookies, and flour, and is also used in the production of starch, malt, dextrose, gluten, and alcohol. In 2021, global wheat production was reported to be 770 million thousand tonnes; nevertheless, wheat productivity is vulnerable to abiotic stress, and climate change is projected to cause a 1.9% reduction in world wheat production by the mid-century (Pequeno et al. 2021).

Maize (*Zea mays* L.), a key cereal crop in the Poaceae family, is grown primarily during the summer monsoon season (July to October). The hybrid maize variety exhibits a growth cycle of 110-115 days. This variety displays resistance and tolerance to prevalent diseases and pests. With a moderate plant height, it offers a high-yielding capacity. Notably, its kernels boast a productive profile characterized by an orange-yellow coloration and a semi-flint grain texture. It has potential tolerance to drought stress (Srinivasa et al. 2016). It is cultivated during the Kharif season, providing a staple food for humans and serving as livestock feed. Maize is also a key raw material for starch, glucose, and cornflake production (Sinha et al. 2019). Nevertheless, abiotic stress exerts a pronounced impact on maize productivity (Ali et al. 2022).

Various mitigation strategies have been developed to alleviate drought stress, including film farming (Mori 2015), drought-tolerant crops (Nuccio et al. 2018, Diatta et al. 2021), nanoparticles (Silva et al. 2023), super-absorbent hydrogels and biochar (Waqar et al. 2022). In recent years, the development of salt-tolerant crops has involved traditional breeding. Besides being cost-intensive, time-consuming, and labor-intensive, most of these practices require the implementation of high technicalities. There is a need for sustainable, cost-effective solutions.

Plant growth-promoting microorganisms have proven to be a preferred natural source to boost drought (Ramakrishna et al. 2020) and salinity tolerance (Desai et al. 2023) in

plants. Several endophytic fungi viz. AMF (Wang et al. 2023, Liu et al. 2023a) and *Piriformospora indica* (Azizi et al. 2021, Tsai et al. 2020) and PGPB viz. *Azotobacter*, *Bacillus*, *Enterobacter*, and *Rhizobium* (Hashemi et al. 2022, Mahreen et al. 2023, Jofre et al. 2023, Komatsu et al. 2023) can promote plant growth under drought and salt stress conditions (Karima et al. 2023, Masrahi et al. 2023, Iqbal et al. 2023, Fraj and Werbrouck 2023, Mousavi et al. 2022, Mousavi et al. 2022, Dixit et al. 2023, Desai et al. 2023, Oviya et al. 2023).

These microbes can produce many bioactive compounds and can survive under abiotic stress (Tyagi et al. 2023). *P. indica* is a plant endophyte that enhances antioxidant capacity, activates induced systemic resistance, mobilizes nutrients, and regulates plant hormone levels during stress conditions (Rajput et al. 2022, Aslani et al. 2023). In an array of host plants, *P. indica* symbiosis alleviates drought stress in plants such as *Salvia officinalis* (Aslani et al. 2023), *Solanum lycopersicum* (Azizi et al. 2021, Ghabooli and Kaboosi 2022), *Poncirus trifoliata* (Cao et al. 2022). *P. indica* symbiosis mitigates salinity stress in plants such as *Brassica napus* (Iqbal et al. 2023), *Ocimum basilicum* (Fraj and Werbrouck 2023), rice (Reshna et al. 2022).

The AMF is a filamentous fungus from the phylum “Glomeromycotina” (Giovannini et al. 2020) that forms mutualistic associations with 80% of land plants (van der Heijden et al. 2015, Chourasiya et al. 2021). AMF hyphal network proliferates several meters beyond the depletion zone and acquires soil nutrients and water (Tyagi et al. 2021), thereby boosting the nutrient uptake capacity of their partners. Besides, AMF maintains ion balance, stimulates antioxidant enzyme activity, and regulates water-holding capacities to combat drought stress (Israel et al. 2022, Bhardwaj et al. 2023, Fresno et al. 2023, Liu et al. 2023a). AMF symbiosis has been shown to alleviate drought stress in *Dalbergia sissoo* (Bhardwaj et al. 2023), *Camellia sinensis* (Liu et al. 2023a), *Trifolium repens* (Fresno et al. 2023). AMF symbiosis mitigates salinity stress in legumes (Evelin et al. 2012, Karima et al. 2023), pistachio (Afshar and Abbaspour 2023), and barley (Masrahi et al. 2023).

PGPB also imparts a beneficial effect on the plant’s photosynthetic capacity, osmolyte accumulation, leaf area, and relative water content (Fadiji et al. 2022). They could

alleviate abiotic stress due to their ability to solubilize phosphate and produce phytohormone, exopolysaccharide, and siderophore (Ilyas et al. 2020, Mahreen et al. 2023, Liu et al. 2023b, Aslani et al. 2023). Inbaraj (2021) found that PGPB-inoculated wheat, maize, and rice plants improved biomass production, photosynthetic pigments, membrane integrity, and proline content, and increased CAT and SOD activities to scavenge free radicals under abiotic stress. Furthermore, PGPB improves nutrient absorption as well as the water uptake capacity of plants growing under stress conditions (Nemeskeri et al. 2022). PGPB has been shown to alleviate drought stress in an array of host plants such as *Oryza sativa* (Mahreen et al. 2023), *Juglans regia* (Liu et al. 2023b), *Salvia officinalis* (Aslani et al. 2023) and could mitigate salinity stress in plants such as *Glycyrrhiza glabra* (Mousavi et al. 2022), *Arabidopsis* (Dixit et al. 2023), and *Vigna radiata* (Desai et al. 2023).

Under stress conditions, the effectiveness and reliability of microorganisms can be improved using microbial consortium (Krestini et al. 2020). A synergistic relationship between the PGPB consortium (*Bacillus subtilis*, *Brucella haematophilum*, and *Bacillus cereus*) has shown improved growth, proline content, photosynthetic pigments, membrane integrity, and antioxidant enzyme production in rice plants (Mahreen 2023). Similarly, the AMF consortium (*Glomus*, *Sclerocystis*, and *Acaulospora*) improved salt tolerance of *Phoenix dactylifera* by increasing nutrient uptake, photosynthetic pigments, stomatal conductance, antioxidant enzyme activity, and decreasing malondialdehyde levels (Ait-El-Mokhtar et al. 2020).

So far, no studies have been conducted to compare the effectiveness of the PGPB consortium, AMF consortium, and *P. indica* in alleviating drought and salinity stress in crop plants. The influence of PGPB consortium, AMF consortium, and *P. indica* on the antioxidant enzyme activity (SOD, CAT, and APX) and accumulation of non-enzymatic antioxidants (ascorbic acid, α -tocopherol, glutathione, and carotenoids) in host plants under abiotic stress conditions has been poorly studied (Rajput et al. 2022, Mahreen et al. 2023). No attempt has been made to link levels of osmolytes, including proline, glycine betaine, total sugars, reduced lipid peroxidation, and membrane integrity to ultrastructural damage prevention. The role of macronutrients (Ca^{2+} , Mg^{2+} , K^{+} , and Na^{+}) and micronutrients (Cu , Fe , Mn^{2+} , and Zn^{2+}) in plants under stress

conditions has been studied by some researchers (Giri et al. 2003, Giri et al. 2007, Giri and Mukerji 2004, Evelin et al. 2012, Evelin et al. 2013, Rahmani et al. 2023). However, not much attention has been paid to the role of the PGPB consortium, AMF consortium, and *P. indica* colonization in increasing nutrient uptake during drought and salinity stress. Taking this into account, the present study was undertaken to investigate the role of inoculation of PGPB (*Azotobacter chroococcum*, *Enterobacter asburiae*, and *Lactococcus lactis*), AMF (*Glomus intraradices*, *Glomus mosseae*, and *Scutellospora*) and *P. indica* on the nutrient acquisition, antioxidant response, and ultrastructural changes in drought and salt-stressed HD-2967 wheat and Maharaja BIO-9637 maize variety.

Objectives

- 1. To study the role of soil microbes on growth, changes in nutritional, biochemical, and physiological parameters of wheat and maize plants under salt and drought stresses.**
- 2. To study the role of soil microbes on cell ultrastructural changes in wheat and maize plants under salt and drought stresses.**
- 3. To study the role of soil microbes during salt and drought stresses on the antioxidant accumulation in wheat and maize plants.**

2. REVIEW OF LITERATURE

By 2050, the global population is expected to reach 9 billion, which will require a 70% increase in food production to feed them (Khandelwal et al. 2023). Plants are continuously exposed to various abiotic stresses, including extreme temperatures, salinity, heavy metals, waterlogging, drought, and ultraviolet radiation. These factors adversely affect plants' growth, productivity, and yield (Soni and Savalia 2023), disturb the soil ecosystem, and result in significant crop losses (Bera et al. 2022, Moon and Ali 2022a).

2.1 Effect of drought stress

Drought is one of the major abiotic stresses that adversely affect agricultural production and threaten food security worldwide (Guo et al. 2020; Mirzabaev et al. 2023). A decline in rainfall and an increasing frequency of dry spells contribute to drought stress (Jarrett et al. 2023). As a consequence, about 45% of agricultural fields are experiencing continuous drought conditions (Yadav et al. 2020) that eventually negatively affect plant growth by decreased transpiration and photosynthesis rates (Naservafaei et al. 2023), osmolytes (Haghighi et al. 2023), and nutrient uptake (Shah et al. 2023). Under drought stress, plants face the problem of increased electrolyte leakage, lipid peroxidation, and reactive oxygen species (ROS) production (Yu et al. 2023). Nevertheless, prolonged drought may result in irreparable damage to plant growth, resulting in reduced crop yields. However, to overcome the problem, the plant needs to maintain better osmotic adjustment, antioxidant defense mechanisms, stomatal closure, and increased root-shoot ratios (López-Galiano et al. 2019).

2.2 Effect of salinity stress

The growth, productivity, and yield of plants are adversely affected by salinity stress (Srivastava et al 2019, Soni and Savalia 2023). Approximately 50% of global agricultural land will be affected due to salinity by 2050 (Gopalakrishnan and Kumar 2021). Several factors contribute to salinity problems, including low rainfall, high evaporation rates, weathering, excessive fertilizer usage, and improper irrigation practices (Gopalakrishnan et al. 2020, Zhao et al. 2020). In plants exposed to salinity,

changes occur in morphology, physiology, biochemistry (Silva et al. 2023), and ultrastructure that inhibit growth and development (El-Dakak et al. 2023). Additionally, salinity affects enzyme activity, stomatal conductance, photosynthesis, and reactive oxygen species (ROS) production. ROS produced by cells damage cell membranes, lipids, proteins, DNA, and RNA, as well as induce programmed cell death (Zhao et al. 2020, Hussain et al. 2021). Salinity stress adversely affects nutrient uptake, osmotic balance, membrane integrity, and the overall growth of plants (Zhang et al. 2022a).

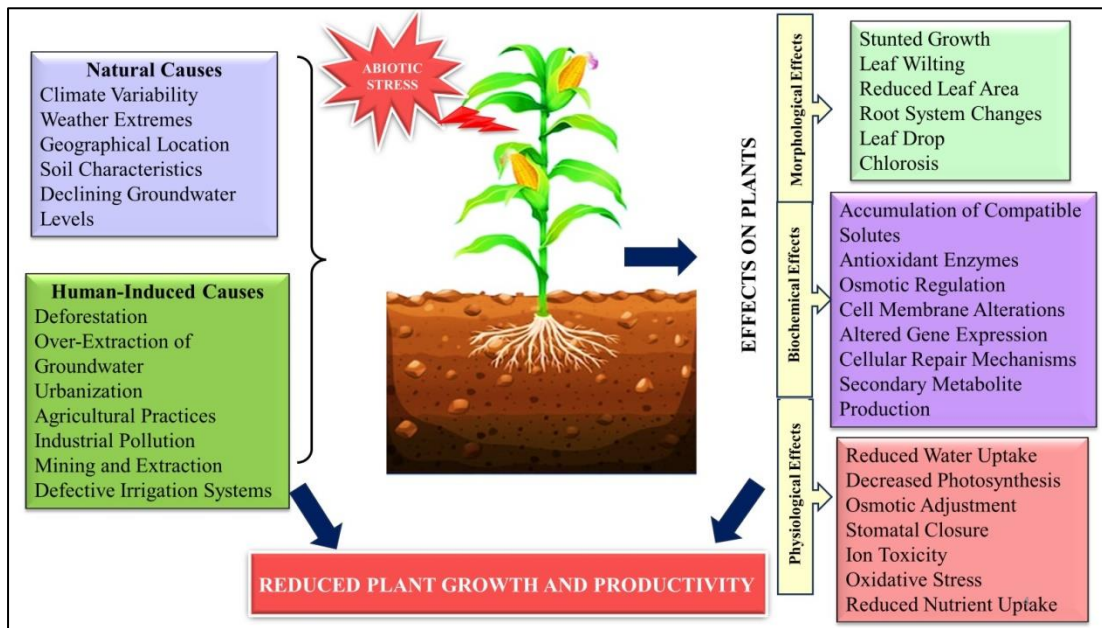


Figure 2.1: Causal factors and impact of drought and salinity on plant growth.

2.3 Plant adaption to resist abiotic stress

To cope with abiotic stresses like ion toxicity (Bibi et al. 2022), osmotic shock (Umar et al. 2022), and oxidative damage (Afridi et al. 2022), plants acquire physiological and metabolic adaptations. Studies have shown that plants have evolved mechanisms to tolerate abiotic stress, such as the synthesis of antioxidant enzymes, polyamines, compatible solutes, osmoprotectants, and hormones (Numan et al. 2018). Plants are equipped with an efficient antioxidant system to counter salt-induced oxidative stress (Azeem et al. 2023). It includes antioxidant enzymes like SOD, CAT, and APX (Azeem et al. 2023), which can scavenge ROS and prevent membrane damage from oxidative stress. SOD is considered a first line of defense that converts superoxide into H_2O_2 .

Further, CAT and APX convert H_2O_2 into H_2O . Non-enzymatic antioxidants such as polyamines, tocopherol, carotenoids, proline (Hao et al. 2021), glutathione, ascorbic acid, polyphenols, and flavonoids (Azeem et al. 2023) also perform a significant role in the detoxification of ROS. Additionally, non-enzymatic antioxidants, like polyamines, tocopherols, carotenoids, prolines (Hao et al. 2021), glutathione, ascorbic acid, polyphenols, and flavonoids (Azeem et al. 2023) play a crucial role in ROS detoxification. Carotenoids protect chloroplast ultrastructure, inhibit peroxidation of lipids, stabilize membrane integrity, and play a central role in the assembly of light-harvesting complex (Gómez-Sagasti et al. 2022). Polyamines interact with negatively charged membrane sites to inhibit membrane degradation in both grana and stroma thylakoids (Ali et al. 2020, Wang et al. 2022). Proline is an effective ROS scavenger in stressful environments, preventing cells from being damaged by oxidative stress. In addition, tocopherol prevents lipid peroxidation by scavenging free radicals in the host tissue (Niki 2021). In response to abiotic stress, osmolytes accumulate, including trehalose, proline, glycine betaine, and polyamines, all of which play a key role in maintaining cell turgidity, and membrane integrity (Patel et al. 2020). Additionally, osmolytes regulate osmotic pressure and quench free radicals (Bashir et al. 2021). As a non-reducing sugar, trehalose stabilizes membranes and macromolecules under salinity stress by maintaining osmotic balance and preventing oxidative damage. Proline is a secondary amino acid that is crucial for osmotic adjustment (Noreen et al. 2019), as well as membrane stabilization under saline conditions. Despite this, prolonged abiotic stress may impede the growth of plants, resulting in a reduction in yield.

2.4 Strategies to minimize crop loss due to abiotic stress

There are several mitigation strategies developed for reducing drought stress, including film farming (Mori 2015), drought-tolerant crops (Nuccio et al. 2018), nanoparticles (Silva et al. 2023), and superabsorbent hydrogels (Waqar et al. 2022). In recent years, several salt-tolerant crops have been developed by conventional breeding programs and recombinant DNA technologies. However, this approach is highly time-consuming and labour and cost-intensive. In the light of upcoming challenges, there is a need to develop a novel, sustainable, and cost-effective approach. In this regard, plant growth-

promoting microorganisms have proven to be a preferred natural source to boost drought (Ramakrishna et al. 2020) and salinity tolerance (Desai et al. 2023) in plants to meet the growing demand for food. Several endophytic fungi viz. AMF (Wang et al. 2023, Liu et al. 2023a) and *Piriformospora indica* (Azizi et al. 2021, Tsai et al. 2020) and PGPB viz. *Azotobacter* (Hashemi et al. 2022), *Bacillus* (Mahreen et al. 2023), *Enterobacter* (Jofre et al. 2023), and *Rhizobium* (Komatsu et al. 2023) can promote plant growth under drought conditions. Under severe saline conditions endophytic fungi viz. AMF (Evelin et al 2009, Karima et al. 2023, Afshar and Abbaspour 2023, Masrahi et al. 2023) and *P. indica* (Iqbal et al. 2023, Fraj and Werbrouck 2023), and PGPB viz. *Azotobacter* (Mousavi et al. 2022, Mousavi et al. 2022), *Bacillus* (Dixit et al. 2023), *Enterobacter* (Desai et al. 2023), and *Rhizobium* (Oviya et al. 2023) can promote plant growth. These microbes can produce many bioactive compounds and can survive under abiotic stress (Tyagi et al. 2023) (Table 2.1-2.2).

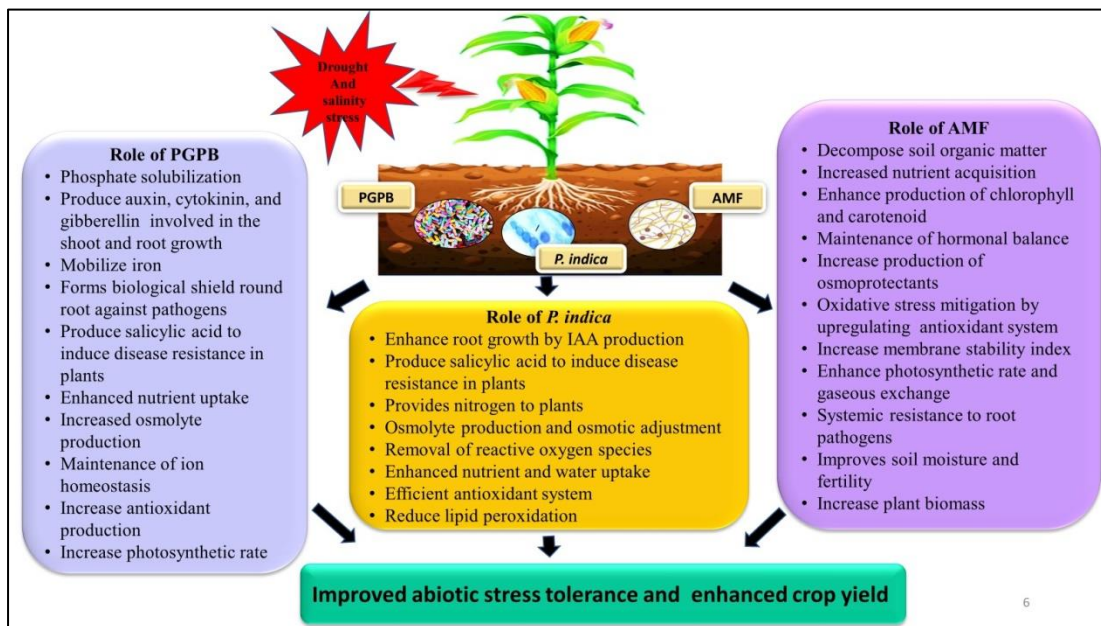


Figure 2.2: Protective mechanisms used by microbes in mitigation of drought and salinity stress

2.4.1 *Piriformospora indica*

P. indica is an endophytic root-colonizing species, discovered from orchid plants in the Thar Desert of Rajasthan, India by Prof. Ajit Verma and his co-workers, School of Life Sciences, Jawaharlal Nehru University, New Delhi. The fungus has typical pear-shaped chlamydospores. *P. indica* can be easily grown on various substrates.

Table 2.1: Growth, nutrient, physiological, and biochemical status of microbial inoculated plants compared to uninoculated plants under drought stress

Microbe	Plant species	Effects of microbe	Reference
AMF	<i>Dalbergia sissoo</i>	Increased shoot and root length and biomass, photosynthesis rate, stomatal conductance, and transpiration	Bhardwaj et al. (2023)
AMF	<i>Commiphora myrrha</i>	Increased shoot and root length and biomass	Birhane et al. (2023)
<i>Rhizophagus clarus</i>	<i>Glycine max</i>	Increased leaf area	Oliveira et al. (2022)
<i>Rhizophagus irregularis</i> , <i>Funneliformis mosseae</i>	<i>Cupressus arizonica</i>	Increased photosynthetic pigment content	Aalipour et al. (2023).
AMF	<i>Ceratonia siliqua</i>	Reduced hydrogen peroxide and malondialdehyde content, higher inorganic solutes and organic solutes	Boutasknit et al. (2020)
<i>Rhizoglyphus irregularis</i>	<i>Trifolium repens</i>	Higher α -tocopherol content	Fresno et al. (2023)
<i>Glomus versiforme</i> and <i>Bacillus methylotrophicus</i>	<i>Nicotiana tabacum</i>	Increased chlorophyll and carotenoids content, higher photosynthesis, and reduced electrolyte leakage and lipid peroxidation	Begum et al. (2022)
AMF	<i>Arachis hypogaea</i>	higher photosynthetic rate, antioxidant enzyme activity, reduced relative electrolyte conductivity malondialdehyde content, and the accumulation of reactive oxygen	Liu et al. (2023c)
<i>P. indica</i>	<i>Solanum lycopersicum</i>	Higher shoot and root length and biomass	Ghabooli and Kaboosi (2022)
<i>Serendipita indica</i> , <i>Rhizophagus intraradices</i> , and <i>Azotobacter chroococcum</i>	<i>Zea mays</i>	Higher root and shoot length and biomass, chlorophyll content, and antioxidant enzyme activities	Tyagi et al. (2023)
<i>P. indica</i>	<i>Solanum lycopersicum</i>	Enhanced dry weight, relative water content, and proline level	Azizi et al. (2021)
<i>P. indica</i>	<i>Oryza sativa</i>	Increased photosynthetic efficiency and reduced lipid peroxidation	Tsai et al. (2020)

Microbe	Plant species	Effects of microbe	Reference
<i>Providencia</i> sp. and <i>Proteus mirabilis</i>	<i>Zea mays</i>	Higher pigments, protein, phenolics, and relative water content and decreased lipid peroxidation	Vishnupradeep et al. (2022)
<i>Acinetobacter</i> , <i>calcoaceticus</i> , and <i>Penicillium</i> sp.	<i>Setaria italica</i>	Enhanced the accumulation of glycine betaine, proline, and sugars	Kour et al. (2020)
<i>Rhizobium</i>	<i>Astragalus sinicus</i>	Higher levels of polyamines	Liu et al. (2022)
PGPB	<i>Zea mays</i>	improved biomass, leaf area, proline, sugar, and protein content, decrease catalase activity	Siddique et al. 2022
<i>Bacillus endophyticus</i> , <i>Bacillus altitudinis</i> , and <i>Bacillus megaterium</i>	<i>Oryza sativa</i>	Increased sugars, proteins, proline, phenolics, potassium, calcium, abscisic acid, and indole acetic acid contents	Devarajan et al. (2021)
<i>Azotobacter chroococcum</i>	<i>Trachyspermum ammi</i>	Improved essential oil content	Hashemi et al. (2022)
<i>Bacillus subtilis</i> , <i>Brucella haematophilum</i> , and <i>Bacillus cereus</i> consortium	<i>Oryza sativa</i>	Higher proline content, chlorophyll content, relative water content, membrane stability index, and production of antioxidant enzymes	Mahreen et al. (2023)
<i>Enterobacter</i> and <i>Pseudomonas</i>	<i>Arabidopsis thaliana</i>	Increased melatonin content, reduced oxidative and membrane damage	Jofre et al. (2023)

It enhances antioxidant capacity, activates induced systemic resistance, mobilizes nutrients, and regulates plant hormone levels during stress conditions (Rajput et al. 2022, Aslani et al. 2023). In an array of host plants, *P. indica* symbiosis alleviates drought stress such as *Salvia officinalis* (Aslani et al. 2023), *Solanum lycopersicum* (Azizi et al. 2021, Ghabooli and Kaboosi 2022), *Poncirus trifoliata* (Cao et al. 2022). *P. indica* symbiosis alleviates salinity stress such as *Brassica napus* (Iqbal et al. 2023), *Ocimum basilicum* (Fraj and Werbrouck 2023), rice (Reshna et al. 2022).

2.4.2 Arbuscular mycorrhizal fungi

The AMF is a filamentous fungus from the phylum “Glomeromycotina” (Giovannini et al. 2020) that forms mutualistic associations with 80% of land plants (van der Heijden et al. 2015, Chourasiya et al. 2021). AMF symbiosis has been shown to alleviate drought stress (Zou et al 2019, Ma et al 2022, Bhardwaj et al. 2023, Liu et al. 2023a, Fresno et al. 2023) and salinity stress in various plants (Evelin et al. 2012, Giri et al. 2017, Singh et al. 2023, Karima et al. 2023, Afshar and Abbaspour 2023, Masrahi et al. 2023). AMF hyphal network proliferates several meters beyond the depletion zone and acquires soil nutrients and water (Tyagi et al, 2021), thereby boosting the nutrient uptake capacity of their partners. Besides, AMF maintains ion balance, stimulates antioxidant enzyme activity, and regulates water-holding capacities to combat drought stress (Israel et al. 2022, Bhardwaj et al. 2023, Fresno et al. 2023, Liu et al. 2023a).

2.4.3 Plant growth-promoting bacteria

Azotobacter chroococcum is a gram-negative bacterium, with spores white, cream, or yellow-brown in colour, rod-shaped, fixed nitrogen under aerobic conditions. *Lactococcus lactis* gram-positive bacteria, round in shape nonsporulating, and non-motile. *Enterobacter asburiae* is gram-negative, facultative anaerobe, rod-shaped, and nonsporulating. PGPB has been shown to alleviate drought stress (Mahreen et al. 2023, Liu et al. 2023b, Aslani et al. 2023) and salinity stress in an array of host plants (Mousavi et al. 2022, Dixit et al. 2023, Desai et al. 2023).

Table 2.2: Growth, nutrient, physiological, and biochemical status of microbial inoculated plants compared to uninoculated plants under salinity stress

Microbe	Plant species	Effects of microbe	Reference
<i>Glomus intraradices</i>	<i>Colocasia esculenta</i>	Higher chlorophyll and carotenoid content	Baltazar-Bernal et al. (2022)
<i>Rhizoglosum intraradices</i> , <i>Funneliformis mosseae</i> , <i>R. intraradices</i> , <i>R. fasciculatum</i> , <i>Gigaspora</i> sp.	<i>Pisum sativum</i>	Higher nutrient uptake, osmolyte, chlorophyll, and carotenoid content, and lower cellular leakage of electrolyte	Parihar et al. (2020)
<i>Rhizophagus irregularis</i>	<i>Pistacia vera</i>	Higher α -tocopherol and carotenoid content and antioxidant enzyme activities	Afshar and Abbaspour (2023)
<i>G. mosseae</i> and <i>Streptomyces rimosus</i>	<i>Vitis vinifera</i>	Reduced ion leakage and malondialdehyde content and increased total phenol and flavonoid content and catalase activity	Karimi and Noori (2022)
<i>Funneliformis geosporum</i>	<i>Medicago sativa</i> , <i>M. falcata</i> , <i>Trifolium repens</i> , and <i>T. alexandrinum</i>	Enhanced shoot biomass and phosphorus content	Karima et al. (2023)
AMF and phosphate solubilizing bacteria	<i>Hordeum vulgare</i>	Improved plant height, length of the spike, uptake of nitrogen, phosphorus, and potassium	Masrahi et al. (2023)
<i>Glomus</i> , <i>Sclerocystis</i> , and <i>Acaulospora</i>	<i>Phoenix dactylifera</i>	Increased nutrient uptake, photosynthetic pigments, stomatal conductance, antioxidant enzyme activity	Ait-El-Mokhtar et al. (2020)
<i>Serendipita indica</i>	<i>Ocimum basilicum</i>	Increased total phenolic, flavonoid, and total chlorophyll content	Fraj and Werbrouck (2023)
<i>Pseudomonas fluorescens</i> and <i>Azotobacter chroococcum</i>	Mustard	Increased proline and glycine-betaine content, and antioxidative enzymes activities	Khan et al. (2023)
<i>Pseudomonas plecoglossicida</i> , and <i>Bacillus subtilis</i>	<i>Persea americana</i>	Higher shoot and root length and biomass	Solórzano-Acosta et al. (2023)
<i>Bacillus safensis</i> , <i>B. subtilis</i> , and <i>B. subtilis</i>	<i>Arabidopsis</i> sp.	High total chlorophyll content, and antioxidant enzyme activities	Dixit et al. (2023)

Microbe	Plant species	Effects of microbe	Reference
<i>Cronobacter</i> and <i>Enterobacter</i>	<i>Vigna radiata</i>	Increased shoot root biomass, chlorophyll carotenoids, and proline content	Desai et al. (2023)
<i>Rhizobium phaseoli</i> , <i>R. pusense</i> , <i>Pantoea dispersa</i> , and <i>B. tequilensis</i>	<i>Arachis hypogaea</i>	Increased antioxidant enzyme activities and accumulation of osmolyte	Oviya et al. (2023)
<i>Bacillus subtilis</i> , <i>Brucella haematophilum</i> , and <i>Bacillus cereus</i>	<i>Oriyza sativa</i>	Improved growth, proline, photosynthetic pigments content, membrane integrity, and antioxidant enzyme activity	Mahreen et al. (2023)
<i>Azotobaccter sp.</i>	<i>Glycyrrhiza glabra</i>	Increased membrane stability index, polyphenol oxidase, and peroxidase activity	Mousavi et al. (2022)
<i>Enterobacter sp.</i> , <i>Bacillus sp.</i> , and <i>P. indica</i>	<i>Brassica napus</i>	Enhanced uptake of nutrients and production of growth hormone	Iqbal et al. 2023

PGPB imparts a beneficial effect on the plant's photosynthetic capacity, osmolyte accumulation, leaf area, and relative water content (Fadiji et al. 2022). They could alleviate abiotic stress due to their ability to solubilize phosphate and produce phytohormone, exopolysaccharide, and siderophore (Ilyas et al. 2020, Mahreen et al. 2023, Liu et al. 2023b, Aslani et al. 2023). Inbaraj (2021) found that PGPB-inoculated wheat, maize, and rice plants improved biomass production, photosynthetic pigments, membrane integrity, and proline content, and increased CAT and SOD activities to scavenge free radicals under abiotic stress. Furthermore, PGPB improves nutrient absorption as well as the water uptake capacity of plants growing under stress conditions (Nemeskeri et al. 2022).

2.5 Role of microbes in alleviating abiotic stress in plants

2.5.1 Plant growth and biomass

Drought and salinity stress represent substantial challenges that impose limitations on global agricultural productivity. These stressors detrimentally impact plant growth and biomass by diminishing nutrient uptake and the photosynthesis capacity of plants (Evelin et al. 2013, Ullah and Farooq 2022, Azeem et al. 2023). In such adverse conditions, soil microbes, notably mycorrhizal fungi and rhizobacteria, assume a pivotal role in shaping plant growth and development. These microbes establish symbiotic relationships with plant roots, effectively augmenting nutrient uptake and rendering essential elements more accessible to plants. This is particularly crucial during drought and salinity stress, where nutrient uptake efficiency becomes a critical determinant due to constraints posed by water scarcity and elevated salt concentrations. AMF colonization in *Dalbergia sissoo* (Bhardwaj et al. 2023), and *Commiphora myrrha* (Birhane et al. 2023) has increased plant growth and biomass under abiotic stress. Additionally, studies by Ghabooli and Kaboosi (2022) highlight that inoculating *Solanum lycopersicum* with *P. indica* under water stress conditions augments root and shoot biomass as well as root volume. The influence of AMF associations extends beyond nutrient uptake, enhancing water uptake and nutrient absorption, specifically phosphorus and nitrogen, thereby promoting comprehensive plant growth. Drought stress, with its consequential impact on water availability and growth reduction, is

mitigated by the contribution of AMF to improved water use efficiency. This is achieved by the extension of plant roots, facilitating enhanced exploration of the soil for water resources. In the case of salinity stress, which induces osmotic stress, disrupting water balance and ion homeostasis in plants, soil microbes play a crucial role in osmotic adjustment. Substances such as exopolysaccharides produced by these microbes aid in maintaining cellular turgor pressure. Khan et al. (2023) demonstrated that PGPB colonization improved shoot and root length in mustard plants under salinity stress. The facilitated ion uptake and sequestration by soil microbes prevent the toxic accumulation of ions, such as sodium, within plant tissues, contributing significantly to enhanced plant growth under salinity stress. Root architecture is pivotal for water and nutrient absorption, and soil microbes influence this by promoting lateral root growth and branching. These alterations in root morphology significantly increase the soil exploration capacity of plants, allowing for more efficient access to water and nutrients, consequently contributing to heightened shoot and root length and biomass. The modulation of plant hormones, including auxins, cytokinins, and abscisic acid (ABA), is another avenue through which soil microbes influence plant growth. ABA, particularly crucial in stress responses, plays a role in regulating stomatal closure and water use efficiency. Soil microbes can modulate ABA levels in plants, thereby influencing their ability to withstand drought and salinity stress. The resultant hormonal interactions also contribute to increased shoot growth and leaf expansion, leading to an augmentation in the leaf area. Oliveira et al. (2022) reported that AMF-inoculated soybean plants had increased leaf area as compared to the control plants during drought stress. Khan et al. (2023) reported that mustard plants inoculated with PGPB had increased leaf area during salinity stress. Both drought and salinity stress induce oxidative stress in plants, characterized by the accumulation of ROS. Soil microbes, through the production of antioxidant enzymes and compounds, play a vital role in detoxifying ROS, and preventing cellular damage. This protective mechanism against oxidative stress is essential for sustaining plant vitality under adverse environmental conditions. The improved nutrient uptake (Rajput et al. 2022), osmolyte accumulation, antioxidant activity, and decreased relative permeability led to improved environmental stress tolerance and increased plant growth in microbial-inoculated plants (Tyagi et al. 2023).

2.5.2 Nutrient uptake

Mineral nutrients play a vital role in plant growth and development. Drought and salinity stress decrease the nutrient contents of plants. This is because of decreased root development and less water availability during drought and salinity stress, therefore plants are not able to absorb essential nutrients. However, microbial-inoculated plants could enjoy increased absorption surface of extraradical hyphae for enhancing the nutrient status and thereby improving growth and productivity (Ortiz et al. 2015).

2.5.2.1 Macronutrients

Calcium is essential for membrane integrity, cell wall stability, and ion transport (de Bang et al. 2021). Drought and salinity stress induce Ca^{2+} deficiency in plants. During abiotic stress, Na^+ replaces Ca^{2+} in the membranes and cell walls, reducing cell turgidity and hydraulic conductivity. Microbial-inoculated plants are better at absorbing Ca^{2+} ions (Devarajan et al. 202, Ait-El-Mokhtar et al. 2020) during abiotic stress. Beneficial microbial partners enhance essential mineral uptake, particularly Ca^{2+} while regulating potentially harmful ions like Na^+ (Pan et al. 2020) through ion transporters and channels influenced by microbial signals. Microbial-inoculated plants employ sequestration mechanisms, potentially involving vacuolar compartmentalization, to prevent excessive Na^+ accumulation within plant cells (Wang et al. 2021). These microbes also influence root architecture, promoting lateral root growth and branching, facilitating Ca^{2+} absorption, and excluding harmful ions, such as Na^+ . The spatial arrangement of roots, influenced by microbial associations, limits Na^+ entry into the plant, favoring the selective uptake of Ca^{2+} ions. In response to salinity stress, plants activate specific genes, including those in the salt overly sensitive (SOS) pathway, which regulates ion balance. Beneficial microbes can modulate SOS gene expression, contributing to efficient Na^+ exclusion and sequestration, ultimately favoring the selective uptake of Ca^{2+} ions.

Magnesium is an essential component of chlorophyll, and it is required for photosynthesis and protein synthesis (Kleczkowski and Igamberdiev 2021). Drought and salinity stress reduced chlorophyll content and photosynthesis in plants. PGPB, AMF, and *P. indica* inoculation increased chlorophyll content and photosynthesis in plants during salinity

stress (Tisarum et al. 2020, Inbaraj 2021). However, due to increased Mg^{2+} content under drought and salinity stress, microbial-inoculated plants have higher chlorophyll content and photosynthesis. Drought stress reduced Mg^{2+} uptake in wheat, but *Azospirillum* (Parihar et al. 2020) and mycorrhizal fungi (Evelin et al 2013) could mitigate this effect by increasing the Mg^{2+} uptake under both drought and salt stress. Ca^{2+} is a strong competitor of Mg^{2+} for plasma membrane sites (Pandey and Sanyal 2021) and has more affinity for plasma membrane sites than Mg^{2+} . the strong competition between Ca^{2+} and Mg^{2+} , and the binding sites on the root plasma membrane appear to have less affinity for highly hydrated Mg^{2+} than for Ca^{2+} (Marschner, 1995). However, AMF inoculation alone or in combination with *Pseudomonas* sp. helps in mitigating NaCl-induced ionic imbalance by maintaining favorable $Ca^{2+} : Mg^{2+}$ thus maintaining a favorable nutrient profile (Hidri et al. 2019). Calcium and magnesium are divalent cations, each carrying a 2+ charge (Ca^{2+} and Mg^{2+}), and they share similar ionic radii. Their comparable charges and sizes often lead to competition for the same binding sites on membrane proteins. While Mg^{2+} is more adept at accepting charges, proteins designed to bind calcium (Ca^{2+}) can selectively attach to Ca^{2+} even in the presence of a significantly higher concentration of Mg^{2+} . Calcium-binding proteins are distinguished by charged residues and a robust structure, favoring interactions with calcium ions. Conversely, proteins binding to magnesium are characterized by an abundance of polar regions and less-than-ideal shapes, diminishing their interactions with magnesium ions (Jing et al. 2018).

Potassium maintains osmotic and membrane potential (Sardans and Peñuelas 2021) and regulates the synthesis of proteins, sugars, and enzyme activity (Hasanuzzaman et al. 2018). Abiotic stress has a negative effect on potassium uptake by plants. As Na^{+} and K^{+} have similar physiological properties such as Na^{+} and K^{+} uptake in plants primarily involves active transport through membrane-embedded transport proteins in the roots. Both ions are absorbed from the soil, and the root system regulates uptake based on soil availability. Ion channels facilitate the diffusion of Na^{+} and K^{+} in root cell membranes, contributing to plant cell osmotic potential. Despite commonalities, they are inevitable competitors. Plants exhibit selectivity in K^{+} uptake over Na^{+} . Specific transporters, like high-affinity potassium transporters (HKT) for K^{+} and sodium transporters for Na^{+} , are involved. Plants deploy mechanisms to cope with high soil salinity, including Na^{+}

exclusion from sensitive tissues, compartmentalization in vacuoles, and substituting Na^+ with K^+ in cellular processes. Consequently, cytoplasmic Na^+ competes with K^+ for binding sites, inhibiting metabolic processes that rely on K^+ . Higher osmolyte accumulation, protein synthesis, and antioxidant enzyme activity can be attributed to high K^+ accumulation (Hasanuzzaman et al. 2018) in microbial-inoculated plants under abiotic stress. AMF excludes Na^+ by discriminating its uptake from soil or plant transfer. The low Na^+ content found in AMF-treated roots can be attributed to the ability of the fungus to retain Na^+ in intraradical hyphae or compartmentalize it in root cell vacuoles. The mycorrhizal symbiosis enhances plant growth and physiological processes, thereby diluting these ions in plant tissues. PGPB enhanced K^+ uptake during abiotic stress (Aslani et al. 2021). AMF influences Na^+ translocation to the aerial parts. AMF enhances the plant's ability to absorb essential nutrients, including K^+ , which competes with sodium for uptake. By promoting K^+ uptake, AMF can help maintain a favorable ion balance in plant tissues and reduce the influx of sodium. AMF influences Na^+ translocation in plants by sequestering Na^+ into vacuoles or excluding it from the cytosol, minimizing toxic effects in the apoplast compared to the cytoplasm. AMF also assists in retracting Na^+ from the xylem, diverting it away from photosynthetic tissues to the roots (Maathuis 2013). Molecular studies on Mycorrhizal *Oryza sativa* plants exhibit enhanced Na^+ compartmentalization through the upregulation of OsNHX3 (Na^+/H^+ exchanger), and increased expression of OsSOS1 (salt overly sensitive) and OsHKT2 (high affinity K^+ transporter) for Na^+ efflux from the cytosol to apoplastic spaces (Porcel et al. 2016). The vacuolar Na^+/H^+ antiporter NHXs, present in roots and leaves, aid in Na^+ sequestration (Munns 2005). Plasma membrane Na^+/H^+ antiporter SOS1 facilitates Na^+ secretion and redistribution in roots and shoots, preventing entry into photosynthetic organs (Olías et al. 2009). Na^+/K^+ transporter HKT plays a role in diverting Na^+ from photosynthetic tissues to roots and confiscating Na^+ from the xylem (Davenport et al. 2007). Through exopolysaccharide binding to sodium ions in the soil, PGPB can confer drought tolerance to plants by decreasing sodium absorption from the soil and increasing nutrient uptake by roots (Bhagat et al. 2021).

Phosphorus (P) is an essential macroelement crucial for cellular structures, contributing significantly to DNA, RNA, ATP, and lipids, and plays a pivotal role in various plant

developmental stages, including flowering and disease resistance (Razaq et al. 2017). However, its availability in the soil solution restricts plant uptake. Salinization-induced precipitation with cations, such as Ca^{2+} , Mg^{2+} , and Zn^{2+} (Azcon-G de Aguilar et al. 1979), limits phosphorus availability, leading to salt-induced phosphorus deficiency and subsequent stunted growth in plants (Taiz and Zeiger 2006). Microbial organisms, including bacteria and fungi, are crucial for phosphorus solubilization and mineralization. Bacterial species like *Pseudomonas*, *Agrobacterium*, and *Bacillus* play significant roles in mobilizing poorly available phosphorus (Babalola and Glick 2012). Fungal strains, including *Achrothcium*, *Alternaria*, *Aspergillus*, and *Cladosporium*, also exhibit phosphorus solubilization and mineralization capabilities (Srinivasan et al. 2012, Sharma et al. 2013).

Soil fungi exhibit greater mobility within soil compared to bacteria and play a crucial role in solubilizing inorganic phosphate. They are particularly significant in this process due to their higher production and secretion of acids, including gluconic, citric, lactic, 2-keto gluconic, oxalic, tartaric, and acetic acid, compared to bacteria (Sharma et al. 2013). Enhanced P acquisition in AMF-associated plants can be attributed to several mechanisms. These include an increased availability of P in the soil facilitated by the secretion of acid and alkaline phosphatases by AMF hyphae, leading to the liberation of P from its bound form. Additionally, AMF contribute to the maintenance of intrinsic phosphate concentration (Pi) by synthesizing polyphosphates within their hyphae. The ability of AMF to uptake P at lower thresholds is facilitated by the expression of high-affinity phosphate transporter genes (GvPT, GiPT, and GmosPT). In AMF-associated plants, effective P uptake plays a crucial role in preserving cell membrane integrity, reducing ion leakage, compartmentalizing toxic ions in vacuoles, and selectively taking up ions (Evelin et al. 2012). This, in turn, mitigates the adverse effects of salinity. Inoculation with *Penicillium funiculosum*, and *Fusarium oxysporum* enhances wheat growth and phosphorus uptake in saline soil (Elgharably and Nafady 2021). Furthermore, soil amendment with AMF and biochar improves salinity tolerance, growth, and phosphorus uptake in common wheat (*Triticum aestivum* L.) (Ndiate et al. 2022).

Bacterial members deploy diverse mechanisms for P solubilization, releasing H^+ in an acid-independent mechanism (Naraian and Kumari, 2018) and excreting organic acids,

leading to soil acidification. The carboxyl and hydroxyl residues present in organic acid molecules form chelates with cations, binding to phosphate. This interaction results in a pH reduction and the release of phosphate anions. Predominantly, the organic acids secreted include gluconic, lactic, isovaleric, oxalic, malonic, succinic, citric, and propionic. Among these, several phosphate-solubilizing bacteria (PSB) such as *Pseudomonas* *Acinetobacter* and *Rhizobium* are known to produce gluconic acid and 2-ketogluconic acid (Naraian and Kumar, 2018). Microbial mineralization reactions release organic phosphates (Po), with various bacteria hydrolyzing Po to Pi using enzymes such as acid phosphatases, phytases, and phosphonatases (Sharma et al. 2013, Alori et al. 2017). Rhizosphere fluorescent *Pseudomonads* strains enhance phosphorus uptake in wheat plants under water-deficit stress (Karimzadeh et al., 2021), and the fungus *P. indica* contributes to salinity tolerance in tomatoes by ameliorating phosphorus accumulation and maintaining ion homeostasis (Ghorbani et al. 2019).

Nitrogen, a vital nutrient for plants, exists predominantly in croplands in the forms of NO_3^- and NH_4^+ (Hachiya and Sakakibara 2017). Functioning as a crucial macronutrient, soil N is indispensable for various physiological processes essential to plant growth and development. N plays a pivotal role in the composition of amino acids, proteins, and nucleic acids, serving as essential building blocks for plant cells. Its active involvement in photosynthesis, chlorophyll formation, enzyme synthesis, and coenzyme production significantly contributes to energy transfer and metabolic activities within plants (Huang et al. 2021). Moreover, N availability directly influences plant morphology, leaf expansion, and overall biomass. Maintaining adequate soil N levels is imperative for optimal plant productivity and the sustainability of agricultural systems. Adverse environmental conditions, including salinity, water deficit, low light intensity, heat, chilling, metal excess, and UV-B radiation, limit soil N availability. These stresses hinder N uptake by plants, adversely affect N assimilation in tissues, and drastically impact crop yields. Drought reduces water availability, impeding N uptake, while high soil salinity disrupts water absorption and interferes with N uptake, as Na^+ ions in saline soils compete with N for root absorption, resulting in reduced N absorption. (Dubey et al. 2021).

The mutualistic relationship between legume roots and PGPB leads to the formation of root nodules, facilitating the fixation of atmospheric N into ammonium. This symbiotic

association serves as an illustrative instance of PGPB, wherein the bacteria metabolize root exudates to supply N for amino acid synthesis. Moreover, N-fixing capabilities are evident in free-living bacteria such as *Azospirillum* and *Stenotrophomonas* (Dobbelaere and Vanderleyden 2003). Under salt stress, plants colonized by AMF exhibit increased N concentration compared to non-mycorrhizal plants (Giri and Mukerji 2004). AMF inoculation enhanced the growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress (Wang et al. 2018). In mycorrhizal plants, N is acquired by extraradical mycelium as inorganic N from the soil in the form of nitrate and is assimilated via nitrate reductase in arbuscule-containing cells (Chen et al. 2018). The resulting arginine undergoes breakdown to urea in the hyphae, ultimately being transferred to the plant as NH_4^+ with the carbon skeletons from arginine breakdown re-incorporated into the fungal carbon pools (Govindarajulu et al. 2005).

Increased N uptake in mycorrhizal plants is attributed to changes in N metabolism brought about by alterations in enzymes of N metabolism (Khan et al. 2021). Improved N nutrition has been reported to mitigate the toxic effects of Na^+ ions by reducing their uptake, and indirectly maintaining chlorophyll content (Founoune et al. 2002). In leguminous mycorrhizal plants, higher N concentration is associated with AMF-mediated promotion of nodulation, preventing premature nodule senescence during salt stress (Garg and Manchanda 2009). Mycorrhizal plants exhibit higher leghemoglobin concentration, delaying the color change in nodules under salt stress, and possess elevated Nase activity, contributing to their increased N-fixing ability. The synergistic action of mycorrhizal and nodule symbioses on infection rate, mineral nutrition, and plant growth supports the necessity of N in enhancing plant tolerance to salinity stress (Rabie and Almadini 2005).

2.5.2.2 Micronutrients

Zn is an essential micronutrient crucial for crop growth, serving as a stimulator of aldolase and a key component of carbonic anhydrase, both integral in carbon metabolism (Tsonev and Cebola Lidon 2012). Additionally, Zn plays a vital role in various biomolecules like proteins and lipids, serving as a cofactor for auxins, and thereby influencing plant nucleic acid metabolism. Application of Zn has demonstrated

positive effects on crop yield and quality, while its deficiency adversely affects yield and crop quality (Hassan et al. 2019). Fe, another micronutrient, is indispensable for all living organisms. It functions as a component in numerous enzymes involved in biochemical processes such as photosynthesis, respiration, and nitrogen fixation (Kobayashi and Nishizawa 2012). Fe deficiency impacts leghemoglobin content, nodule mass, nitrogenase activity, and bacteroid number (Garcia et al. 2015). In nature, Fe predominantly exists as ferric (Fe^{3+}), while plants absorb it as ferrous (Fe^{2+}). The availability of Fe is generally low in saline-sodic and calcareous soils worldwide (Rabhi et al. 2007). Mn is a vital micronutrient supporting plant growth and development. It plays essential roles in various cellular compartments and serves as a cofactor for the oxygen-evolving complex in photosystem II, facilitating the water-splitting reaction in photosynthesis (Alejandro et al. 2020). Cu serves as a crucial electron carrier in the electron transport system. Cu, present in organic complexes in soils, undergoes reversible changes in oxidation states. Its divalent form (Cu^{2+}) is adsorbed to soil components and forms complexes with oxygen, sulfur, and nitrogen atoms. In alkaline pH, Cu availability decreases, leading to deficiency. Cu, as a constituent of enzymes, functions as an electron carrier, catalyzing oxidation-reduction processes in cellular metabolism. It also aids in the detoxification of superoxide radicals and contributes to the lignification of plant cell walls (Pandey 2018).

Abiotic stress diminishes micronutrient solubility and mobility, establishing a depletion zone around plant roots, and leading to reduced nutrient uptake (Hussain et al. 2018). AMF enhances mineral elements (Zn, Fe, Mn, and Cu), mitigating harmful ion uptake and oxidative stress in *Cucumis sativus* L. (Hashem et al. 2018). AMF's extensive root-hyphal system shortens nutrient pathways, serving as a substrate for nutrient binding. AMF-induced pH changes in the rhizosphere modulate nutrient solubility and availability (Etesami et al. 2021). Increased sink size of Cu and Zn, driven by higher shoot P, promotes nutrient uptake and translocation to shoots in AMF-inoculated plants. AMF application alleviated salinity stress in wheat by enhancing micronutrient uptake (Zn, Fe, Cu, Mn) and regulating Na (decrease) and K (increase) uptake (Huang et al. 2023). The fungi's mycelial system absorbs nutrients like N, P, K, Zn, Cu, and Fe, some of which are transmitted to the plant. AMF-induced root colonization boosts Zn

absorption in root length units by expanding the root absorption surface through fungal hyphae growth (Gao et al. 2007).

PGPB enhances micronutrient availability for plants through mechanisms such as by production of chelating agents and soil pH reduction (Miransari 2013). In iron-deficient conditions, PGPB produces siderophores, high-affinity Fe(III)-chelating compounds that scavenge iron, making it easily accessible to plants (Kloepper et al. 1980). Siderophore-producing PGPB meets the iron needs for normal growth of iron-starved tomato plants (Grobela and Hiller 2017). Certain PGPBs, like *Bacillus*, *Pseudomonas*, and *Geobacter*, increase plant-available Mn by reducing Mn^{4+} to metabolically useful Mn^{2+} (Osorio Vega 2007). This impact on Mn availability is attributed to bacterial effects on plant growth, root secretion, and production of chelating agents like phenolic compounds and organic acids that form soluble complexes with Mn, Fe, and other micronutrients, preventing their precipitation (Etesami and Maheshwari 2018). The effects of soil microorganisms on Cu and Zn availability are influenced by compound production and plant growth, including root exudates (Iqbal et al. 2010). In general, soil microorganisms enhance micronutrient availability to salinity-stressed plants through the production of chelating agents and organic acids, promoting plant rooting systems via Indole-3-Acetic Acid production and increasing root exudates (Etesami and Maheshwari 2018). According to earlier studies, Zn^{2+} , Fe, and Mn^{2+} content in *Vitis vinifera* was increased after AMF and PGPB (Karimi and Noori 2022) during abiotic stress. Bacteria chelate and acidify soil to make Cu, Fe, Mn^{2+} , and Zn^{2+} more accessible to plants (Etesami et al. 2014).

2.5.3 Physiological changes

Photosynthesis is integral to plant growth and is therefore an important indicator of a plant's potential for growth and abiotic stress tolerance (Liang et al. 2019). In response to abiotic stress, stomatal closure decreases gas exchange and internal CO_2 levels, resulting in a decrease in photosynthetic pigment production, thereby reducing the photosynthetic rate (Liang et al. 2019, Meng et al. 2023). Moreover, the transpiration rate decreases during drought and salinity leading to reduced nutrient and water uptake. AMF colonization in *Dalbergia sissoo* seedlings under drought stress enhanced stomatal

conductance, transpiration, internal CO₂, and photosynthesis (Bhardwaj et al. 2023). *Rhizophagus irregularis* improved the salt tolerance of *Elaeagnus angustifolia* plants by improving leaf photosynthetic performance, PSII function, antioxidant capacity, and leaf chloroplast ultrastructure (Liang et al. 2021). PGPB inoculation improved plant photosynthetic efficiency by increasing the CO₂ assimilation rate, stomatal conductance, and transpiration rate (Vishnupradeep et al. 2022), and reducing structural damage to the photosynthetic apparatus by enhancing osmolyte accumulation in plants. AMF consortium (*Funneliformis mosseae* 1, *Funneliformis mosseae* 2, and *Diversispora tortuosa*) inoculation improves plant photosynthetic efficiency by improving CO₂ assimilation rate, stomatal conductance, and transpiration rate in *Zelkova serrata* plants during salinity stress (Wang et al. 2019). Co-inoculation with AMF and phosphate solubilizing fungi enhanced CO₂ assimilation rate, stomatal conductance, and transpiration rate during salinity stress (Zai et al. 2021). The establishment of an extensive root system is advantageous in supporting plant growth during early stages and extracting water from shallow soil layers, minimizing evaporation losses. Drought stress may variably impact root growth across plant species, influencing the root-to-shoot ratio. This ratio tends to increase under limited water availability, with roots displaying resilience to growth inhibition. Roots respond to drought by initiating a signal cascade through the xylem, inducing physiological changes crucial for plant adaptation. Absciscic acid (ABA), cytokinins, and ethylene participate in root-to-shoot signaling during drought stress (Batool et al. 2019) with ABA playing a central role in stomatal closure promoting the efflux of K⁺ ions from guard cells, resulting in stomatal closure, a critical adaptation to limited water supply. *Azotobacter chroococcum* and *Azotobacter vinelandii* enhanced the Physiological and biochemical assay of drought stress responses in *Solanum melongena* L. (Kiran et al. 2022).

Phytohormones, such as ABA, jasmonic acid (JA), and salicylic acid (SA), orchestrate multifaceted responses to abiotic stress, modulating physiological and molecular processes (Zhao et al. 2021). ABA, predominantly synthesized in roots, regulates stomatal aperture, antioxidant enzymes root architecture, and the expression of stress-responsive genes. JA induces tolerance through mechanisms like ROS scavenging, stomatal closure, and root development. SA contributes to plant defense against stress,

impacting antioxidant capacity and gene expression involved in secondary metabolite biosynthesis, chaperones, and heat shock proteins. Indole-3-acetic acid (IAA), recognized for its role in plant growth and development, influences cell elongation, vascular tissue development, and apical dominance. It coordinates growth during water deficit and salt stress, inducing gene expression for root meristem initiation, promoting root branching, and enhancing stress tolerance through triggering physiological changes such as stomatal closure and influencing the overall adaptation of the plant to drought conditions. The AMF (*Glomus etunicatum*, *Glomus intraradices*, and *Glomus mosseae*) inoculation decreased the ABA and increased the JA and SA content in salt-affected *Cucumis sativus* plants (Hashem et al. 2018).

Increased photosynthesis rates in microbial-inoculated plants are a result of higher chlorophyll content and leaf area. As light-absorbing pigments, chlorophylls and carotenoids are crucial to photosynthesis. Chlorophyll and carotenoid contents are decreased by drought (Khazaei et al. 2020) and salinity stress (Khan et al. 2023). Water deficit-induced reduction in chlorophyll content is associated with various physiological changes, including the loss of chloroplast membranes, excessive swelling, and distortion of lamellae vesiculation. Microbial inoculation reduces the impact of abiotic stress in plants. AMF substantially increased the chlorophyll and carotenoid content during drought stress (Aalipour et al. 2023). Maize plants colonized with PGPB had more chlorophyll and carotenoid content during abiotic stress (Vishnupradeep et al. 2022, Solórzano-Acosta et al. 2023) and *Colocasia esculenta* colonized with AMF contained a higher level of chlorophyll and carotenoids during salinity stress (Baltazar-Bernal 2022). Higher photosynthetic pigments in microbial-inoculated plants are a consequence of antioxidant accumulation, protecting the chloroplast structure, and increasing the solubilization and bioavailability of organic minerals (Khan et al. 2019, Parihar et al. 2020). *Bacillus* strains increase chlorophyll levels in maize due to increases in nutrient balance (Moreno-Galván et al. 2020). Similarly, AMF increases chlorophyll levels in maize and fenugreek through improved uptake of nutrients (Evelin et al. 2012, Parihar et al. 2020).

Relative Water Content (RWC) serves as a vital indicator of plant water status. RWC declines during drought stress, affecting water potential, transpiration rate, and stomatal

conductance (Yan et al. 2016). Stomatal opening and closing are strongly influenced, impacting water-use efficiency. Leaf turgor maintenance under drought involves osmotic adjustment through the accumulation of solutes like proline, sucrose, soluble carbohydrates, and glycine betaine. Proline contributes to stress tolerance by stabilizing cellular structures and scavenging free radicals. Moreover, osmolyte accumulation in AMF-colonized plants reduces structural damage to the photosynthetic apparatus (Puccio et al. 2023) during salinity stress. The glycine betaine and sugar content were enhanced in mustard plants inoculated with the PGPB under salinity stress (khan et al. 2023). *Acinetobacter*, *Calcoaceticus*, and *Penicillium* sp. effectively ameliorated drought-induced stress in foxtail millet by increasing glycine betaine, proline, and sugar accumulation (Kour et al. 2020) by maintaining cell turgidity and membrane integrity. According to Liu et al. 2022, rhizobium symbiosis enhances drought tolerance in *Astragalus sinicus* through the production of putrescine, spermidine, and spermine. AMF effectively ameliorated salinity-induced stress in *Colocasia esculenta* by increasing glycine betaine, proline, and sugar accumulation (Baltazar-Bernal et al. 2022) by maintaining cell turgidity and membrane integrity. According to Yaghoubian et al. 2022, *P. indica* and azotobacter symbiosis enhance salinity tolerance in *Silybum marianum* through the production of glycine betaine, proline, and sugar accumulation. The antioxidant property of carotenoids protects the photosynthetic apparatus against free radical damage, inhibits lipid peroxidation, stabilizes cell membranes, and plays a critical role in light-harvesting complex assembly (Farooq et al. 2009, Gómez-Sagasti et al. 2023).

2.5.4 Biochemical changes

2.5.4.1 Photosynthetic pigments

As light-absorbing pigments, chlorophylls and carotenoids are crucial to photosynthesis. Chlorophyll and carotenoid contents are decreased by drought (Khazaei et al. 2020) and salinity stress (Khan et al. 2023). Microbial inoculation reduces the impact of abiotic stress in plants. AMF substantially increased the chlorophyll and carotenoid content during drought stress (Aalipour et al. 2023). Maize plants colonized with PGPB had more chlorophyll and carotenoid content during abiotic stress (Vishnupradeep et al. 2022, Solórzano-Acosta et al. 2023) and *Colocasia esculenta* colonized with AMF

contained a higher level of chlorophyll and carotenoids during salinity stress (Baltazar-Bernal 2022). Higher photosynthetic pigments in microbial-inoculated plants are a consequence of antioxidant accumulation, protecting the chloroplast structure, and increasing the solubilization and bioavailability of organic minerals (Khan et al. 2019, Parihar et al. 2020). *Bacillus* strains increase chlorophyll levels in maize due to increases in nutrient balance (Moreno-Galván et al. 2020). Similarly, AMF increases chlorophyll levels in maize and fenugreek through improved uptake of nutrients (Evelin et al. 2012, Parihar et al. 2020).

2.5.4.2 Osmolytes

Abiotic stress is associated with a significant accumulation of osmolytes, including carbohydrates (glucose and trehalose), amino acids (proline), quaternary ammonium compounds (glycine betaine), and polyamines (putrescine, spermidine, and spermine), which play a critical role in maintaining cell turgor pressure and stabilizing membranes, enzymes, and proteins (Sharma et al. 2019, Patel et al. 2020). Furthermore, osmolytes control ROS levels, provide energy for stress management, help repair processes, and support growth (Silva et al. 2018, Bashir et al. 2021). As a highly stable molecule, trehalose is resistant to high temperatures and acids; it can prevent protein aggregation and degradation during drought (Jalili et al. 2009) and salinity (Roy et al. 2021) stress. Proline is an important secondary amino acid that has a major role in osmotic regulation and scavenging ROS under drought stress (Noreen et al. 2019, Adejumo et al. 2021). Microbial colonization increases the osmolyte accumulation in plants during abiotic stress. Proline and sugar content significantly increased in maize inoculated with the microbial consortium under drought stress (Hashem et al. 2016). The glycine betaine, and sugar content were enhanced in mustard plants inoculated with the PGPB under salinity stress (khan et al. 2023). Enhanced drought tolerance in maize plants colonized by AMF (*Glomus versiforme*), is attributed to the increased synthesis of compatible osmolytes such as proline, glycine betaine, and sugars. Maize plants demonstrating elevated osmolyte accumulation manifest superior stress resilience and growth performance by preserving tissue water content, protein structure, and functionality (Begum et al. 2019). AMF improved the growth, osmolyte content, and yield of *Nicotiana tabacum* under drought-stress conditions (Begum et al. 2021). Furthermore, AMF exerts influence over

the expression of stress-responsive genes in plants, particularly those involved in the biosynthesis of osmolytes, thereby fostering their augmented production in response to stress conditions (Puccio et al. 2023). Additionally, AMF contributes to increased photosynthetic efficiency, even under stressful conditions, thereby furnishing requisite substrates for osmolytic synthesis. The regulatory role of AMF in managing reactive oxygen species levels, generated in response to stress, serves to mitigate oxidative stress, indirectly supporting the plant in its osmolyte production endeavors. Notably, AMF imparts regulatory influence upon the hormonal equilibrium of the plant, including key elements such as ABA and cytokinins, pivotal in managing stress responses and facilitating osmolyte accumulation.

Under drought stress conditions, *Solanum melongena* inoculated with *Azotobacter chroococum* and *Azotobacter vinelandii* exhibited increased proline content (Kiran et al. 2022). Under drought conditions, *Enterobacter bugandensis*, exhibiting drought tolerance, induced systemic tolerance in *Triticum aestivum* L. through enhanced osmolyte production. PGPB plays a pivotal role in augmenting the production of osmolytes in plants confronted with drought and salinity stress, employing diverse physiological mechanisms. PGPB exerts its influence by enhancing the availability and uptake of essential nutrients, thereby facilitating the synthesis of osmolytes. Additionally, PGPB enhances the production of plant growth hormones, specifically auxins and cytokinins, which intricately regulate plant growth and stress responses (Ullah et al. 2013), thereby influencing osmolyte production. Furthermore, PGPB exhibits the potential to induce the expression of genes associated with osmolyte biosynthesis, thereby contributing to an augmented production of osmolytes under conditions of stress (Yasin et al. 2018). The regulatory role of PGPB extends to the management of ROS levels, effectively mitigating oxidative stress and establishing a conducive environment for osmolyte synthesis. Additionally, PGPB demonstrates the capacity to stimulate the activity of antioxidant enzymes within plants, thereby effectively counteracting oxidative stress and bolstering the accumulation of osmolytes. Moreover, by fostering enhanced nutrient availability and promoting overall plant health, PGPB contributes significantly to increased photosynthetic efficiency, consequently providing a well-endowed substrate pool for osmolyte synthesis.

Acinetobacter, *Calcoaceticus*, and *Penicillium* sp. effectively ameliorated drought-induced stress in foxtail millet by increasing glycine betaine, proline, and sugar accumulation (Kour et al. 2020) by maintaining cell turgidity and membrane integrity. According to Liu et al. 2022, rhizobium symbiosis enhances drought tolerance in *Astragalus sinicus* through the production of putrescine, spermidine, and spermine. AMF effectively ameliorated salinity-induced stress in *Colocasia esculenta* by increasing glycine betaine, proline, and sugar accumulation (Baltazar-Bernal et al. 2022) by maintaining cell turgidity and membrane integrity. According to Yaghoubian et al. 2022, *P. indica* and azotobacter symbiosis enhance salinity tolerance in *Silybum marianum* through the production of glycine betaine, proline, and sugar accumulation.

2.5.4.3 Protein

Proteins are organic molecules that form the structural and functional units of a cell. These compounds improve chlorophyll content, protect chloroplast structure, activate ROS scavenging, and enhance osmolyte synthesis (Riyazuddin et al. 2022). Plants inoculated with microbes have higher protein content. In microbial-inoculated plants, higher protein content is associated with higher photosynthetic rates, chlorophyll contents, membrane integrity, and osmolyte accumulation. Inoculation with AMF and supplementation with phosphorus have been demonstrated to ameliorate drought stress in *Nicotiana tabacum* by augmenting the accumulation of proteins (Begum et al. 2020). The simultaneous inoculation with rhizobia and mycorrhizal fungi has been shown to enhance both the yield and crude protein content of *Vigna unguiculata* under conditions of drought stress (Pereira et al. 2020). AMF establishes a symbiotic association with plant roots, giving rise to specialized fungal structures known as arbuscules within the confines of root cells. This morphological adaptation significantly amplifies the surface area available for nutrient exchange. The mycorrhizal network, facilitated by AMF, serves to enhance the absorption of pivotal nutrients, notably nitrogen and phosphorus, critical components for the biosynthesis of proteins. Under conditions of drought stress, AMF contributes to osmotic adjustment by augmenting the water-absorbing capacity of the plant. This mycorrhizal interaction thereby fortifies the plant's aptitude to contend with water scarcity, sustaining essential metabolic processes, including the synthesis of proteins. Concurrently, the symbiotic association with AMF has been correlated with

the activation of stress responsive proteins within plants. These proteins assume a vital role in alleviating the repercussions of salinity and drought stress by preserving cellular homeostasis and conferring protection against oxidative damage. Colonization by *P. indica* has been associated with increased vitamin C, protein, and soluble sugar content in cabbage, rapeseed, and cotton, along with a reduction in nitrate levels, contributing to the maintenance of higher plant metabolism under waterlogging conditions (Yang et al. 2014). Proteomic analysis of *P. indica* colonization in barley under waterlogging conditions revealed elevated levels of proteins involved in photosynthesis, antioxidant defense systems, and energy transfer (Ghabooli et al. 2013). The positive effects of *P. indica* in mitigating drought stress include the prevention of thylakoid and chlorophyll protein degradation, thereby reducing the impact on photosynthetic efficiency (Saddique et al. 2018). Boutasknit et al. (2020) found that drought stress could be mitigated by AMF-colonized carob plants with higher levels of protein.

PGPB employs diverse mechanisms to facilitate the solubilization of essential nutrients, notably phosphorus and nitrogen, thereby augmenting their accessibility for plant uptake. The increased availability of these nutrients serves to enhance protein synthesis in plants. Colonization by PGPB elicits the induction of stress-responsive genes within plants. These genes actively participate in the intricate orchestration of protein synthesis, assuming a critical role in alleviating the repercussions of salinity and drought stress. This involvement includes the maintenance of cellular functions and the provision of defense mechanisms against oxidative damage. PGPB can enhance the water use efficiency of plants, allowing them to thrive under limited water availability. Improved water use efficiency contributes to the maintenance of metabolic processes, including protein synthesis, even in the presence of drought stress. Co-inoculation with *Azotobacter chroococcum* and *Alcaligenes faecalis* has been observed to increase sugar and protein content in canola plants grown in saline soil (Abdel Latef et al. 2021). Additionally, PGPB has demonstrated the capacity to enhance protein content in soybean plants during salinity stress (Khan et al. 2021). Solórzano-Acosta et al. (2023) found that salinity stress could be mitigated by PGPB and AMF-colonized *Persea americana* plants with higher levels of protein.

2.5.4.4 α -tocopherol

Since α -tocopherol is an antioxidant, it inhibits ROS generation during photosynthesis (mainly $^1\text{O}_2$ and $\text{OH}\cdot$) and reduces lipid peroxidation (Kim et al. 2019). Plants inoculated with microbes have a high level of α -tocopherol, which indicates that α -tocopherol is a free radical scavenger as well as a modulator of lipid peroxidation during abiotic stress (Kim et al. 2019). Higher levels of α -tocopherol in AMF colonized mitigated drought stress *Trifolium repens* plants (Fresno et al. 2023) and salinity stress pistachio plants (Afshar and Abbaspour 2023). Microbial colonization in plants enhances drought and salinity tolerance by regulating α -tocopherol content, thus maintaining a high level of photosynthetic efficiency and chlorophyll content and reducing ion leakage. In the presence of arsenic toxicity, AMF exhibited an enhancement in the concentrations of antioxidant molecules, specifically carotenoids, and α -tocopherol, within wheat plants as a response to oxidative stress (Sharma et al. 2017). Furthermore, the co-application of olive solid waste and AMF resulted in a notable elevation in the α -tocopherol by 93% relative to conditions characterized by arsenic-induced stress in wheat plants (Albqmi et al. in 2023). Additionally, investigations conducted by Fresno et al. (2023) demonstrated that *Trifolium repens* plants subjected to water stress, with colonization by the mycorrhizal species *Rhizoglosum irregulare*, manifested a transient increase in α -tocopherol content specifically within the roots, with no concurrent elevation observed in the leaves.

Wheat plants of the HD 2967 variety, inoculated with PGPB and *P. indica*, exhibited increased levels of proline, α -tocopherol, and carotenoids in comparison to wheat plants that were not subjected to inoculation under conditions of salinity stress (Singh and Tiwari 2021). The application of PGPB, belonging to the *Haloferax* genus, demonstrated mitigation of the phytotoxic effects of cobalt on maize plants, leading to an augmentation in α -tocopherol accumulation and subsequently enhancing growth and production (Selim et al. 2022). Furthermore, the endophytic presence of *Micrococcus luteus* and *Enterobacter cloacae* was found to alleviate the adverse impacts of cadmium and nickel toxicity on *Lycopersicon esculentum*, facilitating phyto-stimulation through the augmentation of non-enzymatic antioxidant defenses, such as an increased accumulation of α -tocopherol content (Badawy et al. 2022).

2.5.4.5 Membrane damage

A key physiological indicator of abiotic stress in plants is malondialdehyde, a by-product of membrane lipid peroxidation (Yasmeen et al. 2022, Xu et al. 2022). Lipid peroxidation alters cell membrane ultrastructure and permeability. Additionally, the LOX enzyme converts lipids into hydroperoxyl fatty acids and releases free radicals that damage cells. Prevention of lipid peroxidation and relative permeability are key factors in drought (Kour et al. 2020) and salinity tolerance (Afshar et al. 2023). The amplitude of the increase in lipid peroxidation and electrolyte leakage in microbial-inoculated plants is lower than that of uninoculated plants during drought (Begum et al. 2022) and salinity stress (Afshar et al. 2023). However, the co-inoculation of AMF and PGPB increased LOX activity in tobacco plants (Begum et al. 2022). The microbial inoculated plant maintains a lower MDA concentration and relative membrane permeability by regulating adaptive responses such as the synthesis of non-enzymatic molecules (carotenoids, proline, and α -tocopherol) and enzymatic antioxidants (CAT, APX, and SOD). PGPB-inoculated plants showed improved drought stress tolerance by maintaining a high level of carotenoids, proline, and tocopherol (Timmusk et al. 2014), as well as CAT, APX, and SOD activity (Chiappero et al. 2019), thereby reducing ROS accumulation. Khan et al. (2023) that PGPB-inoculated plants were able to reduce ROS formation by maintaining their carotenoids and proline content, and CAT, APX, and SOD activities during salinity stress.

2.5.5 Antioxidant response

During unfavorable conditions, antioxidant enzymes (CAT, SOD, and APX) actively protect the plant cell from oxidative bursts and assist in scavenging ROS during adverse environmental conditions (Ru et al. 2023). The SOD enzyme plays a critical role in protecting the plant against oxidative stress by converting $O_2^{\cdot-}$ to H_2O_2 . To detoxify H_2O_2 , it must be converted into H_2O . SOD produces H_2O_2 that is scavenged by CAT and attenuates oxidative stress on the cell. Microbial inoculation improved plant growth because of the enhanced activity of the ROS-scavenging antioxidant enzyme under drought stress (Tyagi et al. 2023) and salinity stress (Solórzano-Acosta et al. 2023). Thus, microbial inoculation reduces lipid peroxidation and membrane permeability in

plants by increasing antioxidant enzyme activity (Tyagi et al. 2023). Microbial consortium-treated maize exhibited increased antioxidant enzyme activity in stress conditions (Abbas et al. 2020). *P. indica* colonized tomato exhibited increased antioxidant enzyme activity under salinity stress conditions (Xu et al. 2022). According to Zhang et al. (2022b), the use of *P. indica* increases salinity tolerance in soybeans through the enhancement of antioxidant enzyme activity. Tyagi et al. (2023) reported that deploying a microbial consortium of *Serendipita indica*, *Rhizophagus intraradices*, and *Azotobacter chroococcum* boosts drought tolerance in maize by enhancing the activity of antioxidant enzymes. However, PGPB reduced CAT activity during drought stress (Siddique et al. 2022). A study conducted by Samaddar et al. (2019) reported a significant decrease in APX activity during salinity stress in plants inoculated with PGPB.

2.5.6 Ultrastructural changes

2.5.6.1 Chloroplast

The chloroplast is the most susceptible cell organelle to drought (Gunasekera and Ratnasekera 2023) and salinity stress (Liu et al. 2022). The ultrastructure of both microbe-inoculated and uninoculated plants was affected by abiotic stress. Under low drought stress, the thylakoid membrane contracted thereby reducing the thylakoid lumen. Salinity also reduces the thylakoid lumen by contracting the membrane which may be a consequence of osmotic collapse (Semenova 2002). When stress levels increase, thylakoid swelling causes destacking of grana (Kotula et al. 2019). Several studies have shown that osmotic stress causes thylakoid swelling and membrane damage (Aldesuquy et al. 2018, Goussi et al. 2018). Microbes-inoculated plants, however, have less chloroplast damage than uninoculated plants. This may be attributed to more accumulation of osmolytes (glycine betaine, polyamines, and sugars) in microbial-inoculated plants. More accumulation of osmolytes prevents osmotic collapse, while polyamines inhibit membrane degradation by interacting with negatively charged membrane sites in granal and stromal thylakoids (Ali et al. 2020). Microbial-inoculation in plants shows reduced chloroplast membrane damage, possibly due to larger plastoglobule sizes that prevents oxidative damage. It has been reported that plastoglobules increase in size during drought (Zhu et al. 2021) and salinity

stress (Sheteiwy et al. 2022). Plastoglobules are lipoprotein bodies present in the chloroplast and function as sites for α -tocopherol synthesis under oxidative stress (Brehelin et al. 2007). Microbial-inoculated plants have higher levels of α -tocopherols than uninoculated plants. α -tocopherols have been reported to protect membrane lipids and photosystem II against photooxidation. In microbial-inoculated plants, plastoglobule accumulation may be attributed to higher polyamine levels (Shu et al. 2015). Thus, microbial-inoculated plants have less damage to the ultrastructure of chloroplast due to high levels of osmolytes and large plastoglobules that prevent oxidative damage.

Glomus intraradices alleviated salt-induced ultrastructural changes in *Trigonella foenum-graecum* plants, as evidenced by reduced disorganization of grana in chloroplasts, reduced swelling, and an increase in the number of thylakoids, less disintegration of the chloroplast membrane, and accumulation of plastoglobules, which were more abundant and larger. The observed decrease in lipid peroxidation and electrolyte leakage in AMF plants implies a reduction in membrane damage, potentially linked to elevated concentrations of osmolytes (glycine betaine, sugars) and polyamines, as well as increased levels of α -tocopherol in AMF plants compared to uninoculated plants (Evelin et al. 2013). *Lycium barbarum* plants inoculated with *Diversispora versiformis* demonstrated intact chloroplast structure with well-organized grana, exhibiting a reduced gap between the chloroplast membrane and plasma membrane when exposed to 200 mmol/L NaCl (Liu et al. 2021). *Zea mays* plants inoculated with AMF, PGPB, and *P. indica* displayed diminished thylakoid membrane contraction and grana destacking during abiotic stress. This effect was attributed to osmolyte accumulation preventing osmotic collapse. Additionally, higher polyamine content inhibited thylakoid membrane degradation and contributed to plastoglobule accumulation, protecting chloroplast membranes. The increased number of plastoglobules in microbe-colonized plants safeguarded chloroplast metabolism, while elevated α -tocopherol synthesis acted as antioxidants, mitigating chloroplast oxidative damage. The overall microbial colonization reduced oxidative stress by maintaining high osmolyte levels and plastoglobule abundance (Singh et al. 2023a, Singh et al. 2023b).

AMF symbiosis with *Funneliformis mosseae* demonstrated amelioration of salt stress effects on maize. The chloroplasts in AMF plants exposed to 100 mM NaCl exhibited

mild inflation with some swollen thylakoids, reduction in plastoglobules, maintenance of intact grana stacks, and a compacted arrangement of thylakoids however, there was a notable increase in starch grain formation (Wang et al. 2021). Li et al. (2023) reported that the symbiotic association between the endangered tree species *Heptacodium miconioides* and AMF mitigated chloroplast structural damage induced by drought stress. Notably, AMF inoculation resulted in an augmentation of starch grain size and an increased abundance of plastoglobules within the chloroplast ultrastructure. The chloroplasts observed in the foliar tissues of *Arabidopsis thaliana* plants subjected to inoculation with *Rhizobium* sp. and *Bradyrhizobium* sp. strains exhibited a compact stroma characterized by a well-elaborated thylakoid system. Additionally, these chloroplasts manifested abundant formation of grana, hosting numerous starch grains and small plastoglobules (Sujkowska-Rybkowska et al. 2022).

2.5.6.2 Plasma membrane

Cell membrane stability in plants is considered a reliable indicator of drought tolerance (Abobatta 2019) and salinity tolerance (Pathiana et al. 2023). Plasma membranes disintegrated under drought and salinity stress. With increasing drought levels, the distance between the plasma membrane and cell wall increases in *Ormosia hosiei* seedlings (Liu and Wei 2021). In response to increasing salinity levels, the space between the plasma membrane and cell wall increases in *Halodule wrightii* (Ferreira et al. 2017). The disintegration of membranes occurs due to the peroxidation of polyunsaturated fatty acids by free radicals (Zhang et al. 2021) and the shrinkage of protoplasm during drought conditions. Compared to control plants, however, microbial-inoculated plants show less membrane damage and protoplasm shrinkage. This statement is further strengthened by the accumulation of osmolytes (glucose, trehalose, proline, glycine betaine, and polyamines), which maintain cell turgor pressure and stabilize membranes, enzymes, and proteins (Sharma et al. 2019). The lower levels of electrolyte leakage, lipid peroxidation, and LOX activity in microbial-inoculated plants, play a significant role in membrane protection. This is because microbial-inoculated plants acquire more non-enzymatic antioxidant molecules (proline, α -tocopherols, and polyamines), enzymatic antioxidants (SOD, CAT, APX), and nutrients (calcium and potassium) compared with uninoculated plants. Proline is an effective ROS scavenger,

protecting cells from damage by oxidative stress under a stressful environment (Ashraf and Foolad 2007). Moreover, α -tocopherol protects host tissue from lipid peroxidation and maintains the integrity of their membrane by scavenging free radicals (Niki 2021). Polyamines provide stability to membranes (Wang et al. 2022). In adverse environmental conditions, antioxidant enzymes (CAT, SOD, and APX) scavenge ROS and protect membranes from oxidative stress (Ru et al. 2023). By forming intermolecular links, calcium maintains the structural integrity of plant membranes, stabilizes cell walls, and regulates ion transport and selectivity (de Bang et al. 2021). Potassium plays a vital role in plants, including osmoregulation and membrane potential (Sardans and Peñuelas, 2021). So, *Serendipita indica*, *Rhizophagus intraradices*, and *Azotobacter chroococcum* inoculation reduce membrane damage through osmolyte, nutrient, and antioxidant-mediated reductions of lipid peroxidation in maize plants (Tyagi et al. 2023). Vesicle formation was more in microbial inoculated as compared to uninoculated plants. Vesicle formation increases under stress conditions (Karim et al. 2014). They increase the membrane surface area, which facilitates ion transport and storage, thus protecting the cells from ionic imbalance. *Glomus intraradices* mitigated salt-induced ultrastructural alterations in *Trigonella foenum-graecum* plants, evidenced by diminished protoplasmic shrinkage and apoplastic space widening. The observed lower lipid peroxidation and electrolyte leakage in AMF plants suggest reduced membrane damage, possibly attributed to higher concentrations of osmolytes (glycine betaine, sugars) and polyamines, as well as increased α -tocopherol levels in AMF plants relative to non-mycorrhizal plants (Evelin et al. 2013). *Zea mays* plants inoculated with an AMF consortium (*Scutellospora* sp, *G. mosseae*, and *G. intraradices*), PGPB consortium (*Lactococcus lactis*, *Enterobacter asburiae*, and *A. chroococcum*), and *P. indica* exhibited reduced cell wall-plasma membrane gap when subjected to salinity stress. The microbial inoculation exerted a protective effect by mitigating ultrastructural damage through the augmentation of osmolytes, nutrients, and antioxidants. Concurrently, vesicle formation was promoted, facilitating ion transport and storage, thereby alleviating ionic imbalances and preventing membrane damage under salinity stress conditions (Singh et al. 2023a).

Lycium barbarum inoculated with *Diversispora versiformis* exhibited decreased plasmolysis in mesophyll cells compared to the control under 200 mmol/L NaCl. This

reduction was attributed to enhanced osmotic adjustment, facilitated by elevated soluble and reduced sugar content, reinforcing arbuscular mycorrhiza-mediated efficiency in osmotic regulation and alleviating physiological drought-induced plasmolysis in mesophyll cells (Liu et al. 2021). AMF consortium (*Scutellospora* sp, *G. mosseae*, and *G. intraradices*), PGPB consortium (*Lactococcus lactis*, *Enterobacter asburiae*, and *Azotobacter chroococcum*), and *P. indica*-inoculated maize plants showed reduced plasma membrane detachment, narrowing the cell wall-plasma membrane gap during drought stress. Microbial inoculation lessened ultrastructural damage by enhancing osmolyte, nutrient, and antioxidant accumulation, while promoting vesicle formation in maize plants, thereby aiding ion transport and storage to mitigate ionic imbalances and prevent membrane damage (Singh et al. 2023b).

3. MATERIALS AND METHODS

3.1 Plant materials

HD-2967 wheat (*Triticum aestivum* var. Pusa Borlaug), a double dwarf variety, was procured from the Department of Genetics, Indian Agricultural Research Institute (IARI), New Delhi, India. The hybrid maize seeds Maharaja BIO-9637 (*Zea mays*) were obtained from National Seeds Corporation Limited, Beej Bhawan, Pusa Complex, New Delhi, India. The classifications are given below:

Kingdom: Plantae	Kingdom: Plantae
Order: Poales	Order: Poales
Family: Poaceae	Family: Poaceae
Subfamily: Pooideae	Subfamily: Panicoideae
Genus: <i>Triticum</i>	Genus: <i>Zea</i>
Species: <i>Triticum aestivum</i>	Species: <i>Zea mays</i>

3.1.1 Habit

HD-2967 wheat cultivar (*Triticum aestivum* var. Pusa Borlaug), is a double dwarf variety belonging to the family Poaceae. Wheat is an annual herb having adventitious and fibrous roots. The stem is erect, cylindrical, and branched, branching is only at the basal region of the stem and is known as tillering. Leaves are alternate, simple, sessile, leaf distinguished into a linear leaf blade and a leaf sheath, and at the junction of these two a small membranous ligule is present, lamina lanceolate, entire, acute, minutely hairy. Inflorescence is a spike of spikelets. Each spikelet consists of a pair of glumes at the base, lemma, and palea. Flowers are sessile, complete, hermaphrodite and fruit is a caryopsis. Perianth is represented by two rudimentary tepals known as lodicules. Stamens are three in number, polyandrous, filaments, ditheous, and versatile. Monocarpellary superior ovary, with one marginal ovule, is present. Style absent and stigma are two in number and feathery.

Maize (*Zea mays* L.), a key cereal crop in the Poaceae family, is grown primarily during the summer monsoon season (July to October). The maize hybrid “Maharaja” (BIO-

9637), is a medium-maturity single-cross hybrid developed by Bioseed Research Ltd. in 1999 (Hari et al. 2008). Classified as a medium-maturing hybrid (A Saga of Progress 2012), it exhibits sensitivity to drought (Kumar et al. 2015b), resilience to waterlogging (Jyoti et al. 2013), and resistance to common rust (Akhilesh et al. 2000). The hybrid maize variety exhibits a growth cycle of 110-115 days. This variety displays resistance and tolerance to prevalent diseases and pests. With a moderate plant height, it offers a high-yielding capacity. Notably, its kernels boast a productive profile characterized by an orange-yellow coloration and a semi-flint grain texture. It has potential tolerance to drought stress (Srinivasa et al. 2016). Maize is an annual large monoecious herb having an adventitious and fibrous root system. It possesses specific traits, including tall stature, medium ear placement, broad leaves, medium-sized tassel, thick ears, semi-dent to flint yellow grains, and white cobs. Stem is cylindrical, erect having distinct nodes and internodes. Leaves are simple, sessile having leaf sheath, leaf blade, and collar. The maize plant is monoecious, bearing male flowers in the tassel and female flowers on the lateral ear shoots of the same plant. The male spikelets are arranged in a terminal panicle. Male spikelets have two flowers one of which is pedicellate and the other is sessile. The calyx and corolla are converted into lodicules. The female spikelets are arranged in a row on a thickened rachis envelope by many green spathes like bract. Each spikelet has two flowers covered by palea. The female flower is zygomorphic, hypogynous, and irregular. The calyx and corolla are converted into lodicules which look like a scale. The gynoecium has one carpel, basal placentation, superior ovary, long silky style, and feathery stigma.

3.1.2 Economic importance

Wheat (*Triticum aestivum* L.) is considered to be the first-ever domesticated plant (Raza et al. 2019). The plant is used to make bread, macaroni cake, cookies, and flour. Moreover, wheat is used in the production of starch, malt, dextrose, gluten, alcohol, and other products. The productivity of wheat is adversely affected by drought stress and by mid-century, climate change is expected to reduce world wheat production by 1.9% (Pequeno et al. 2021). Maize (*Zea mays* L.) is used for the manufacture of starch, glucose, and cornflakes (Sinha et al. 2019). The productivity of maize is adversely affected by abiotic stress (Ali et al. 2022).

3.2 Microbial inoculum

AMF consortium (*Glomus intraradices*, *Glomus mosseae*, and *Scutellospora* sp.) and PGPB consortium (*Azotobacter chroococcum*, *Enterobacter asburiae*, and *Lactococcus lactis*) were sourced from the Department of Microbiology, IARI, New Delhi, India, and *P. indica* inoculum were acquired from Amity University, Noida, India. *Azotobacter chroococcum* is a gram-negative bacterium, with spores white, cream, or yellow-brown, rod-shaped, fixed nitrogen under aerobic conditions. *Lactococcus lactis* gram-positive bacteria, round in shape nonsporulating, and non-motile. *Enterobacter asburiae* is gram-negative, facultative anaerobic, rod-shaped, and nonsporulating (**Figure 3.1**).

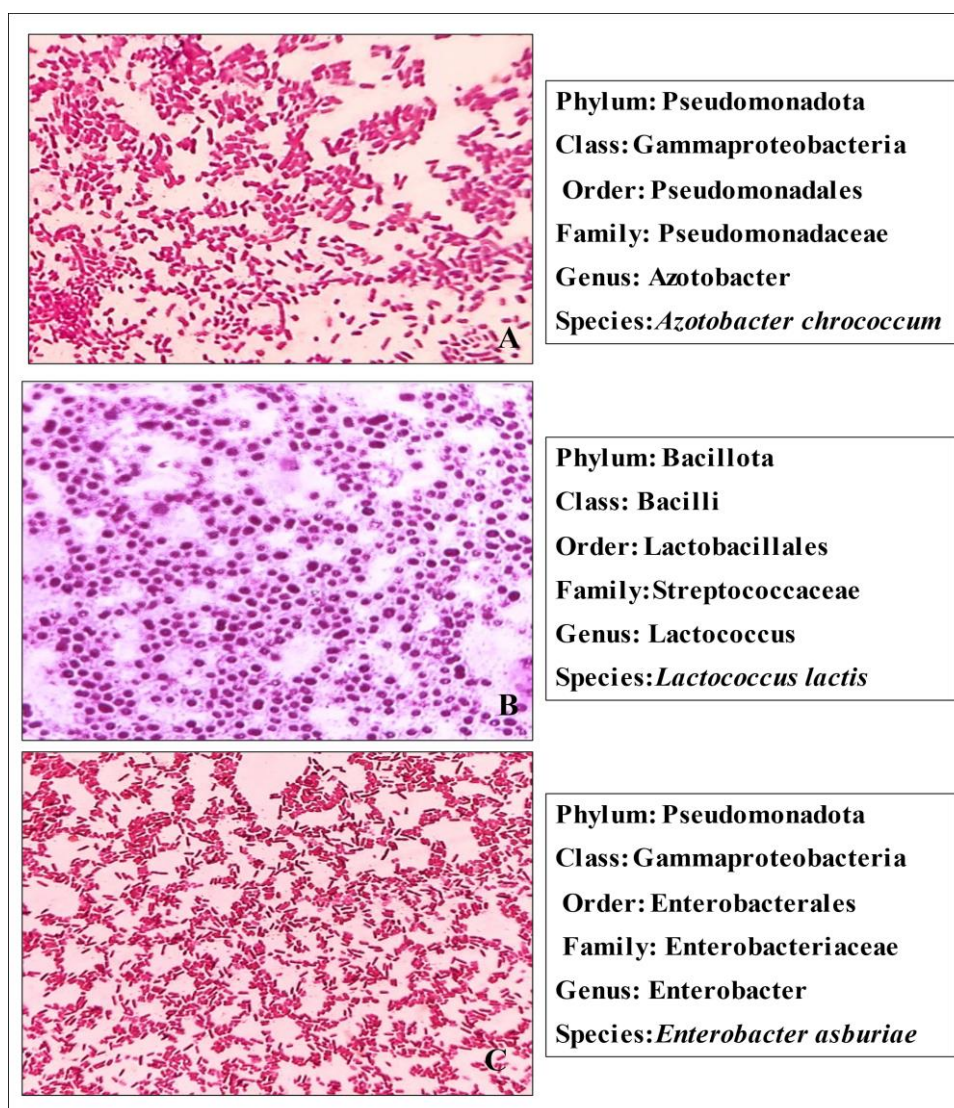


Figure 3.1: Microscopic view of (A) *Azotobacter chroococcum* (B) *Lactococcus lactis* (C) *Enterobacter asburiae* bacteria after gram staining.

The AMF is a filamentous fungus from the phylum “Glomeromycotina” (Giovannini et al. 2020) that forms mutualistic associations with more than 80% of land plants (van der Heijden et al. 2015, Chourasiya et al. 2021). AMF hyphal network proliferates several meters beyond the depletion zone and acquires soil nutrients and water (Tyagi et al. 2021), thereby boosting the nutrient uptake capacity of their partners (**Figure 3.2 A-E**). *P. indica* is an endophytic root-colonizing species, discovered from orchid plants in the Thar desert in Rajasthan, India by Prof. Ajit Verma and group, School of Life Sciences, Jawaharlal Nehru University, New Delhi. The fungus has typical pear-shaped chlamydospores. *P. indica* can be easily grown on various substrates (**Figure 3.2 F**). The classification of AMF and *P. indica* is given below in **Table 3.1**.

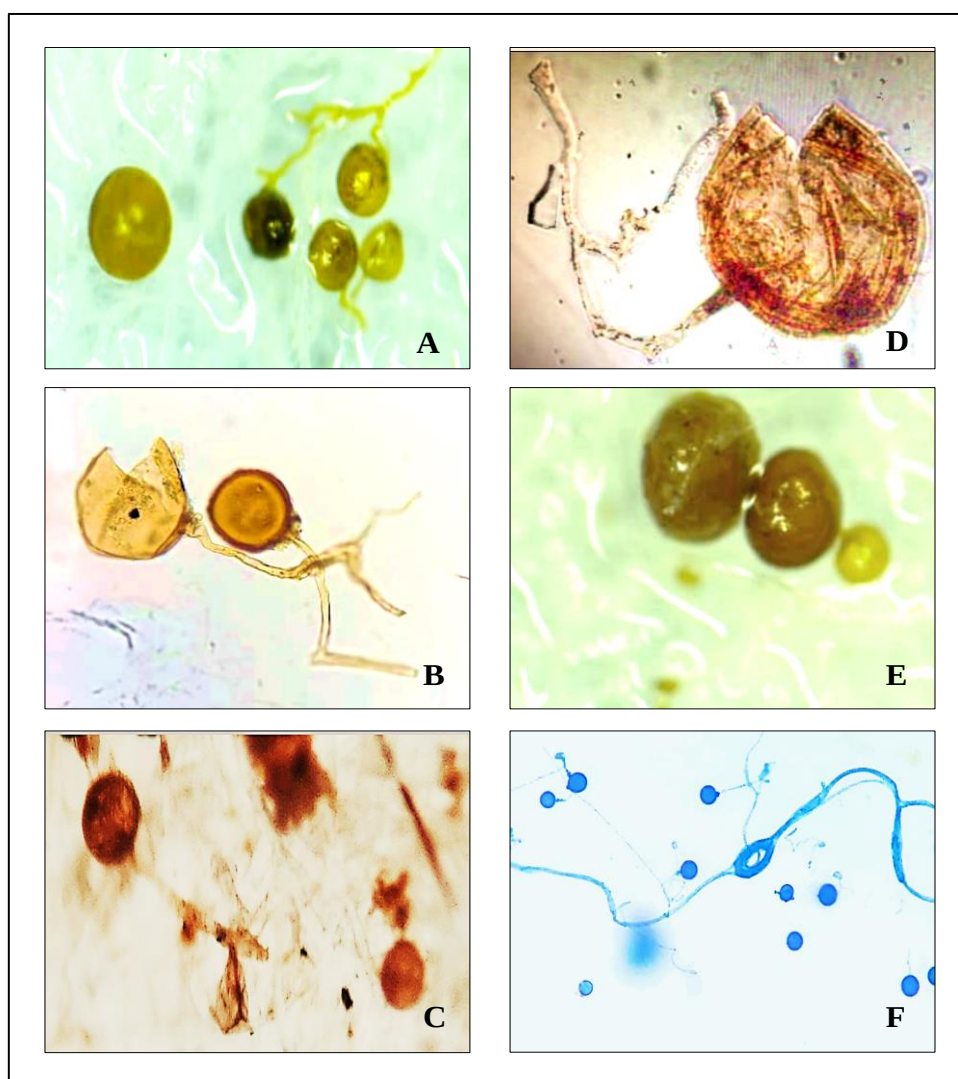


Figure 3.2: Arbuscular mycorrhizal fungi (A-E) and *P. indica* (F) spores as seen under a microscope.

Table 3.1: Classification of AMF and *P. indica*

Microbe	Division	Class	Order	Family	Genus	Species
AMF	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Glomus	<i>Glomus intraradices</i>
AMF	Glomeromycota	Glomeromycetes	Diversisporales	Gigasporaceae	Scutellospora	<i>Scutellospora sp</i>
AMF	Glomeromycota	Glomeromycetes	Glomerales	Glomeraceae	Glomus	<i>Glomus intraradices</i>
<i>P. indica</i>	Basidiomycota	Agaricomycetes	Sebacinales	Serendipitaceae	Piriformospora	<i>Piriformospora indica</i>

3.3 Experimental design, growth condition, and soil

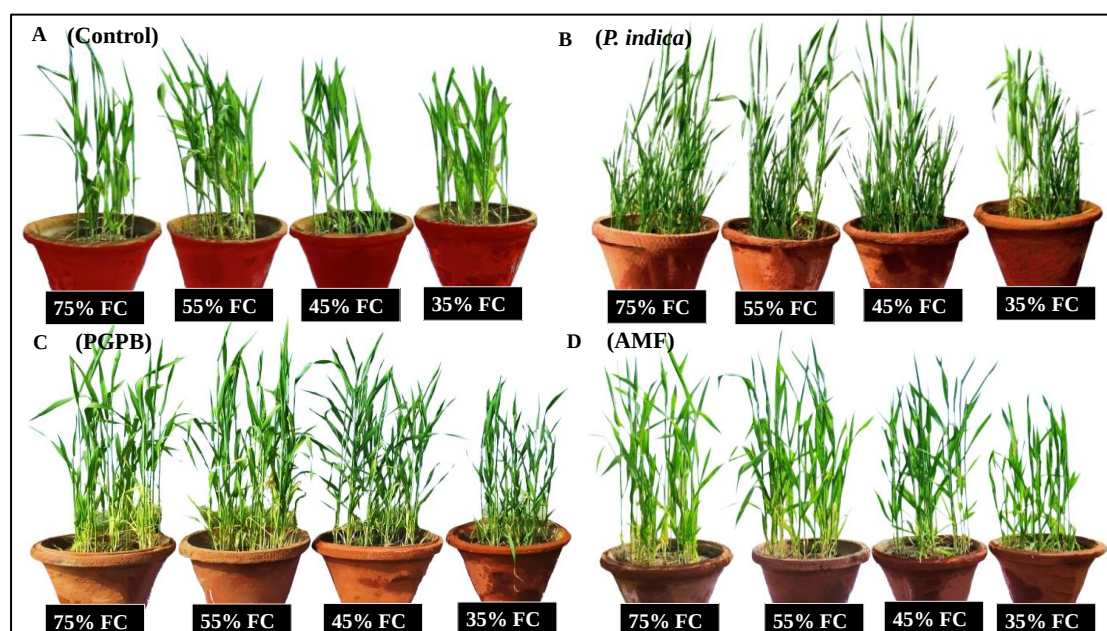
3.3.1 Wheat plants subjected to drought stress treatment

A pot experiment was conducted in the botanical garden of Swami Shraddhanand College, University of Delhi, India to investigate the role of microbes in alleviating drought stress in wheat. During the experimental period, the temperature ranged between 23 and 30°C and relative humidity between 55 and 65%. The experiment was laid out in a completely randomized block design with two factors: i) microbe status (non-microbial ie. control, inoculation with *P. indica*, inoculation with PGPB, and inoculation with AMF) and ii) four levels of water stress (75%, 55%, 45%, and 35% of soil field capacity). Therefore, 16 treatments (4 x 4) with three replicates each were used. The wheat seeds were surface sterilized by treating them with 4% sodium hypochlorite for 2 minutes and then washed with distilled water. Five wheat seeds were sown at a depth of 3 cm in pots containing 4 kg of sterile soil. In the inoculation process, precise quantities of microorganisms, namely, *P. indica* (2 g), which contained 70-80 spores per g of talc, PGPB (20 g) with a density of 10^7 - 10^8 Colony Forming Units per g of the charcoal carrier, and AMF (50 g) with a population of 70-80 spores per g of soil were added to the soil at a depth of 3 cm at the time of seeding in different treatments. The same amount of sterile sand was mixed with soil in control. For the pot experiment the recommended dose of fertilizer was viz: nitrogen (N) as urea: 187.5 kg ha⁻¹, diammonium phosphate (P): 75 kg ha⁻¹, and muriate of potash as (K): 75 kg ha⁻¹. The soil used in the experiment was collected from the fields of Alipur village, New Delhi, and was autoclaved at 121°C and 15 psi for 30 minutes. The soil properties were determined by the Department of Soil Sciences and Agricultural Chemistry, IARI, Delhi, India, and are given in **Table 3.2**.

Table 3.2: Physio-chemical properties of soil used for growing wheat plants under drought stress.

Parameter	Properties /content
Soil type	Sandy loam
pH	7.8
Organic matter	1.5 %
Available N	196 kg ha ⁻¹
Available P	49.8 kg ha ⁻¹
Available K ⁺	492 kg ha ⁻¹
Mg ²⁺	150 mg kg ⁻¹
Zn ²⁺	2.32 mg kg ⁻¹
Fe ³⁺	7.88 mg kg ⁻¹
Cu ²⁺	2.08 mg kg ⁻¹
Mn ²⁺	12.8 mg kg ⁻¹

Before the water stress treatment, plants were allowed to grow for 20 days to allow microbes to colonize. The plants were irrigated with different water regimes (75%, 55%, 45%, and 35% of soil field capacity) twice a week for two months. After 80 days of sowing, the entire plant was uprooted manually (**Figure 3.3**).

**Figure 3.3:** Experimental set-up showing the influence of drought stress on (A) un-inoculated wheat plants (control); (B) *P. indica*-inoculated wheat plants; and (C) PGPB-inoculated wheat plants (D) AMF-inoculated wheat plants stress after 80 days of sowing

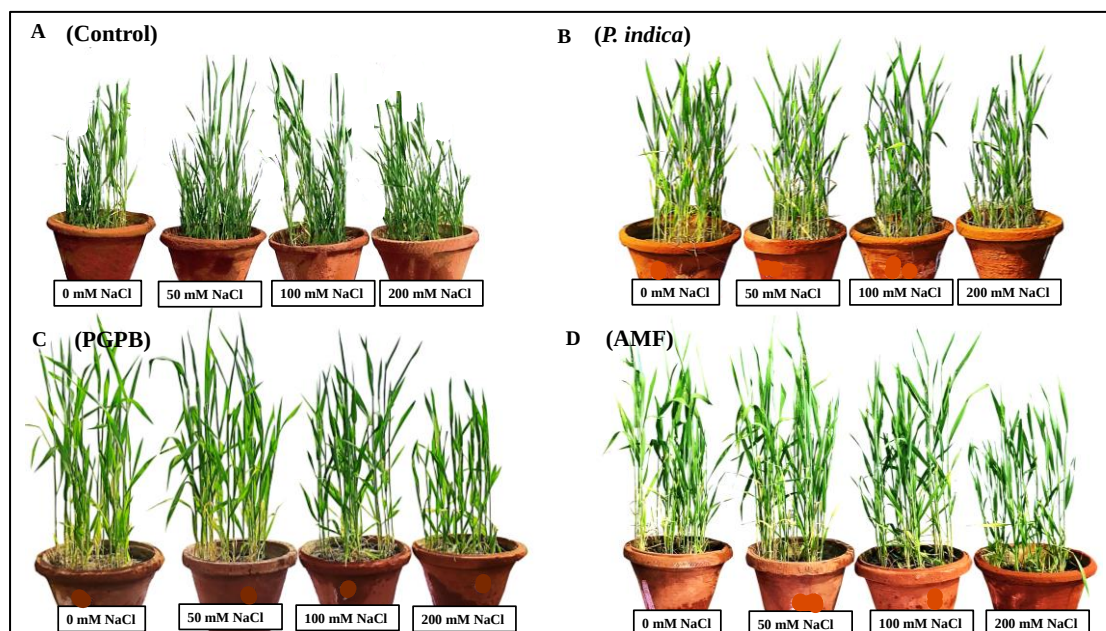
3.3.2 Wheat plants subjected to salinity stress treatment

A pot experiment was conducted in the botanical garden of Swami Shraddhanand College, University of Delhi, India to investigate the role of microbes in alleviating salinity stress in wheat. During the experimental period, the temperature ranged between 23 and 30°C and relative humidity between 55 and 65 %. This experiment was set up in a completely randomized block design with 2 factors: i) microbe status (uninoculated plants ie. control, *P. indica* inoculated plants, PGPB-inoculated plants, and AMF-inoculated plants) and ii) four levels of salinity (0, 50 100, and 200 mM NaCl). A total of 16 treatments (4 x 4) with three replicates each were used. The wheat seeds were surface sterilized for two minutes by using 4% sodium hypochlorite and then washed with distilled water. A total of five seeds were sown in pots containing 4 kg of sterile soil at a depth of 3 cm. In the inoculation process, precise quantities of microorganisms, namely, *P. indica* (2 g), which contained 70-80 spores per g of talc, PGPB (20 g) with a density of 10^7 - 10^8 Colony Forming Units per g of the charcoal carrier, and AMF (50 g) with a population of 70-80 spores per g of soil were added to the soil at a depth of 3 cm at the time of seeding in different treatments. In the control, sterile sand was mixed with soil in the same amount. The recommended dose of fertilizer, namely urea (187.5 kg ha^{-1}), diammonium phosphate (75 kg ha^{-1}), and muriate of potash (75 kg ha^{-1}) were added. For the experiment, the soil was collected from Alipur village, New Delhi, and autoclaved for 30 minutes at 121°C and 15 psi. A soil property analysis was conducted by the Department of Soil Sciences and Agricultural Chemistry at the Indian Agricultural Research Institute in Delhi. and are given in **Table 3.3**.

Salinity stress treatment was administered after plants had grown for 20 days to allow microbes to colonize. In this experiment, plants were treated with different salinity levels once every week for 60 days with 50 ml of each NaCl solution per pot. Soil electrical conductivity (EC) was measured by a conductivity meter (HACH analyzer, HQ440d multi) and it increased to 0.21, 5.16, 9.23, 18.54 mS cm^{-1} at the 0, 50, 100, and 200 mM NaCl salinity levels, respectively. Plants were manually uprooted after 80 days of sowing (**Figure 3.4**).

Table 3.3: Physioco-chemical properties of soil used for growing wheat plants under salinity stress.

Parameter	Properties /content
Soil type	Sandy loam
pH	7.8
Organic matter	1.5 %
Available N	196 kg ha ⁻¹
Available P	49.8 kg ha ⁻¹
Available K ⁺	492 kg ha ⁻¹
Mg ²⁺	150 mg kg ⁻¹
Zn ²⁺	2.32 mg kg ⁻¹
Fe ³⁺	7.88 mg kg ⁻¹
Cu ²⁺	2.08 mg kg ⁻¹
Mn ²⁺	12.8 mg kg ⁻¹

**Figure 3.4:** Experimental set-up showing influence of salinity stress on (A) un-inoculated wheat plants (control); (B) *P. indica*-inoculated wheat plants; and (C) PGPB-inoculated wheat plants (D) AMF-inoculated wheat plants stress after 80 days of sowing.

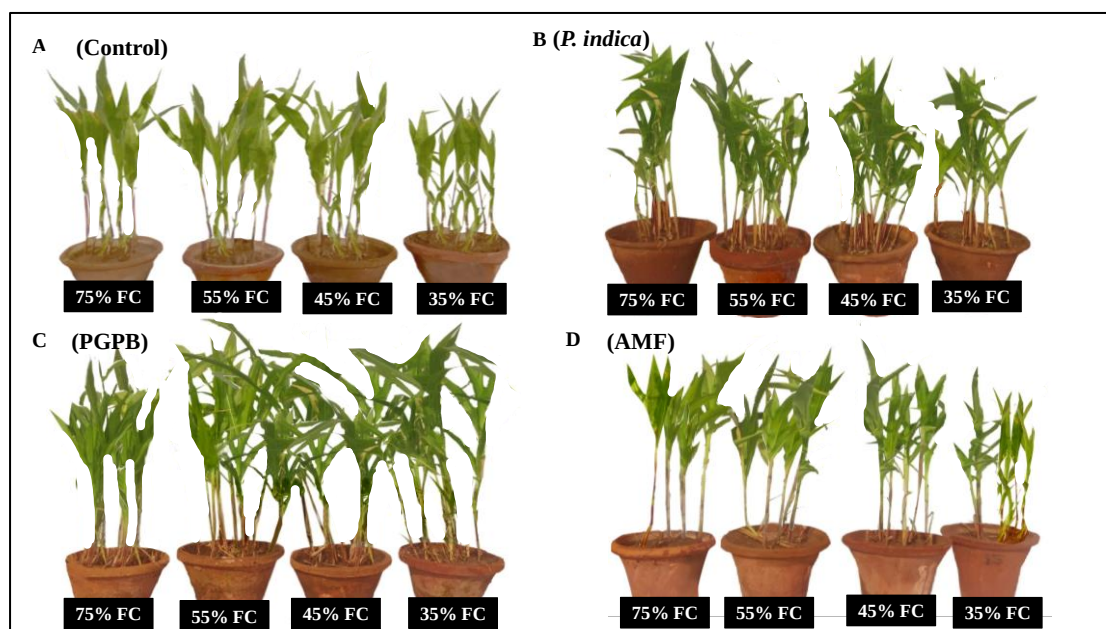
3.3.3 Maize plants subjected to drought stress treatment

A pot experiment was conducted in the botanical garden of Swami Shraddhanand College, University of Delhi, India to investigate the role of microbes in alleviating drought stress in maize plants. During the experimental period, the temperature ranged between 28 and 35°C and relative humidity between 66 and 75%. The experiment was laid out in a completely randomized block design with two factors: i) microbe status (non-microbial ie. control, inoculation with *P. indica*, inoculation with PGPB, and inoculation with AMF) and ii) four levels of water stress (75%, 55%, 45%, and 35% of soil field capacity). Therefore, 16 treatments (4 x 4) with three replicates each were used. The maize seeds were surface sterilized by treating them with 4% sodium hypochlorite for 2 minutes and then washed with distilled water. Five maize seeds were sown at a depth of 3 cm in pots containing 4 kg of sterile soil. In the inoculation process, precise quantities of microorganisms, namely, *P. indica* (2 g), which contained 70-80 spores per g of talc, PGPB (20 g) with a density of 10^7 - 10^8 Colony Forming Units per g of the charcoal carrier, and AMF (50 g) with a population of 70-80 spores per g of soil were added to the soil at a depth of 3 cm at the time of seeding in different treatments. The same amount of sterile sand was mixed with soil in control. For the pot experiment the recommended dose of fertilizer was viz: nitrogen (N) as urea: 150 kg ha⁻¹, diammonium phosphate (P): 75 kg ha⁻¹, and muriate of potash as (K): 50 kg ha⁻¹. The soil used in the experiment was collected from the fields of Alipur village, New Delhi, and was autoclaved at 121°C and 15 psi for 30 minutes. The soil properties were determined by the Department of Soil Sciences and Agricultural Chemistry, IARI, Delhi, India, and are given in **Table 3.4**.

Before the water stress treatment, plants were allowed to grow for 20 days to allow microbes to colonize. The plants were irrigated with different water regimes (75%, 55%, 45%, and 35% of soil field capacity) twice a week for two months. After 80 days of sowing, the entire plant was uprooted manually (**Figure 3.5**).

Table 3.4: Physio-chemical properties of soil used for growing maize plants under drought stress.

Parameter	Properties /content
Soil type	Sandy loam
pH	7.3
Organic matter	1.3 %
Available N	172 kg ha ⁻¹
Available P	45.1 kg ha ⁻¹
Available K ⁺	476 kg ha ⁻¹
Mg ²⁺	132 mg kg ⁻¹
Zn ²⁺	1.97 mg kg ⁻¹
Fe ³⁺	6.78 mg kg ⁻¹
Cu ²⁺	2.00 mg kg ⁻¹
Mn ²⁺	11.62 mg kg ⁻¹

**Figure 3.5:** Experimental set-up showing the influence of drought stress on (A) un-inoculated maize plants (control); (B) *P. indica*-inoculated maize plants; and (C) PGPB-inoculated maize plants (D) AMF-inoculated maize plants stress after 80 days of sowing.

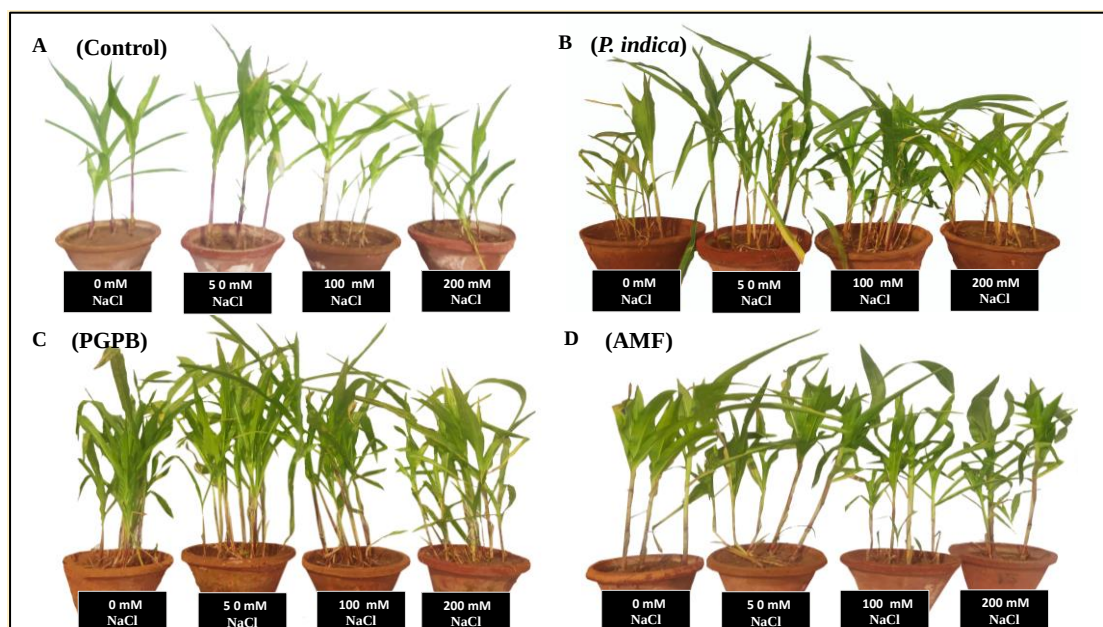
3.3.4 Maize plants subjected to salinity stress treatment

A pot experiment was conducted in the botanical garden of Swami Shraddhanand College, University of Delhi, India to investigate the role of microbes in alleviating salinity stress in maize plants. At the time of the experiment, the temperature ranged between 28 and 35 °C, and the relative humidity was between 66% and 75%. This experiment was set up in a completely randomized block design with two factors: i) microbe status (uninoculated plants ie. control, *P. indica* inoculated plants, PGPB-inoculated plants, and AMF-inoculated plants) and ii) four levels of salinity (0, 50 100, and 200 mM NaCl). A total of 16 treatments (4 x 4) with three replicates each were used. The maize seeds were surface sterilized for two minutes by using 4% sodium hypochlorite and then washed with distilled water. A total of five seeds were sown in pots containing 4 kg of sterile soil at a depth of 3 cm. In the inoculation process, precise quantities of microorganisms, namely, *P. indica* (2 g), which contained 70-80 spores per g of talc, PGPB (20 g) with a density of 10^7 - 10^8 Colony Forming Units per g of the charcoal carrier, and AMF (50 g) with a population of 70-80 spores per g of soil were added to the soil at a depth of 3 cm at the time of seeding in different treatments. In the control, sterile sand was mixed with soil in the same amount. The recommended dose of fertilizer, namely urea (150 kg ha^{-1}), diammonium phosphate (75 kg ha^{-1}), and muriate of potash (50 kg ha^{-1}) were added. For the experiment, the soil was collected from Alipur village, New Delhi, and autoclaved for 30 minutes at 121°C and 15 psi. A soil property analysis was conducted by the Department of Soil Sciences and Agricultural Chemistry at the Indian Agricultural Research Institute in Delhi. and are given in **Table 3.5**.

Salinity stress treatment was administered after plants had grown for 20 days to allow microbes to colonize. In this experiment, plants were treated with different salinity levels once every week for 60 days with 50 ml of each NaCl solution per pot. Soil electrical conductivity (EC) was measured by a conductivity meter (HACH analyzer, HQ440d multi) and it increased to 0.21, 5.16, 9.23, 18.54 mS cm^{-1} at the 0, 50, 100, and 200 mM NaCl salinity levels, respectively. Plants were manually uprooted after 80 days of sowing (**Figure 3.6**).

Table 3.5: Physio-chemical properties of soil used for growing maize plants under salinity stress.

Parameter	Properties /content
Soil type	Sandy loam
pH	7.3
Organic matter	1.3 %
Available N	172 kg ha ⁻¹
Available P	45.1 kg ha ⁻¹
Available K ⁺	476 kg ha ⁻¹
Mg ²⁺	132 mg kg ⁻¹
Zn ²⁺	1.97 mg kg ⁻¹
Fe ³⁺	6.78 mg kg ⁻¹
Cu ²⁺	2.00 mg kg ⁻¹
Mn ²⁺	11.62 mg kg ⁻¹ .

**Figure 3.6:** Experimental set-up showing the influence of salinity stress on (A) un-inoculated maize plants (control); (B) *P. indica*-inoculated maize plants; and (C) PGPB-inoculated maize plants (D) AMF-inoculated maize plants stress after 80 days of sowing.

3.4 Measurements

3.4.1 Objective 1: To study the role of soil microbes on growth, changes in nutritional, biochemical, and physiological parameters of wheat and maize plants under salt and drought stresses

3.4.1.1 Estimation of AMF and *P. indica* colonisation

The roots were rinsed using distilled water. To assess colonization, root samples were softened in a 10% KOH solution, followed by immersion in 1% hydrochloric acid for 5 min. The samples were stained with 0.05% trypan blue for 10 min (Phillips and Hayman 1970). Microbial colonization was assessed employing the gridline intersect technique (Giovannetti and Mosse 1980). Intersections at which fungal structures or hyphae intersected the grid lines were enumerated using an electronic binocular compound microscope (Nikon, ECLIPSE E100).

$$\% \text{ Root colonization} = \frac{\text{Total count of intersections with fungal structure}}{\text{Total count of intersections}} \times 100$$

3.4.1.2 Estimation of plant growth parameters

The lengths of shoots and roots were measured using a graduated scale. Shoot and root biomass was determined by oven-drying the samples at 75°C for 3 days until a constant weight was achieved, and biomass was calculated based on the dried sample weight. The measurement of leaf area was carried out using a leaf area meter (laser area meter, CI-202, CID Bio-Science, USA).

3.4.1.3 Elemental analysis of tissue

The dried leaf powder sample (100 mg) was digested with 3 ml nitric acid and 5 ml hydrogen peroxide in a microwave digestion unit (Microwave Reaction system, Multiwave PRO) (Alhagri and Albeshry 2023). After cooling, the digested sample was transferred volumetric flask and the final volume was made to 25 ml with Milli Q water. The element standards were prepared in a 2 % HNO₃ solution. The standard concentrations ranged from 0.1-10 ppm in each element. The standards and samples were run through an Inductively coupled plasma mass spectrometry (ICP-MS)

instrument (Agilent Technologies 7700 series ICPMS, United States), and the data were analyzed with Mass Hunter workstation software.

3.4.1.4 Estimation of physiological changes

Measurements of leaf physiological parameters viz. photosynthetic rate, transpiration rate, stomatal conductance, and internal CO₂ were done using LI-COR, LI-6400XT Portable photosynthesis system between 8:00 am to 12:00 pm. All measurements were taken using a 2 cm² leaf chamber having a red-blue light-emitting diode light source at 1200 µmol m⁻²s⁻¹ photosynthetic active radiation and 400 ppm CO₂. A light curve was prepared to determine the light saturation rate (Bidalía et al. 2018).

3.4.1.5 Estimation of biochemical changes

3.4.1.5.1 Photosynthetic pigments

The content of photosynthetic pigments in plant leaves was estimated according to the protocol of Hiscox and Isradtom (1979). Leaves (100 mg) were cut into small pieces and placed in a vial containing 7 ml dimethyl sulfoxide. The tubes were incubated at 65°C till the tissue was decolorized. The total volume was made to 10 ml with dimethyl sulfoxide. The absorbance was read at 480, 510, 645, and 663 nm. The chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content were measured using the formula given by Arnon (1949) and expressed as mg g⁻¹fw.

$$\text{Chlorophyll a} = (12.7 \times D_{663} - 2.69 \times D_{645} \times V) / 1000 \times \text{Weight of sample}$$

$$\text{Chlorophyll b} = (22.9 \times D_{645} - 4.68 \times D_{663} \times V) / 1000 \times \text{Weight of sample}$$

$$\text{Total Chlorophyll} = (20.2 \times D_{645} + 8.02 \times D_{663} \times V) / 1000 \times \text{weight of sample}$$

$$\text{Carotenoids} = (7.6 \times D_{480} - 1.49 \times D_{510} \times V) / (1000 \times \text{Weight of Sample})$$

3.4.1.5.2 Osmolytes

To determine glycine betaine content, a 100 mg leaf sample was crushed in 1000 µl Milli Q water. The homogenate was centrifuged for 20 minutes at 20°C at 12,000 rpm (Eppendorf, Centrifuge 5810 R). To the extract 2 N sulphuric acid was added in the ratio of 1:1. Aliquot (500 µl) was cooled for 1 h, added cold 200 µl KI-I₂ reagent and

stored at 4°C for 16 h. The mixture was centrifuged at 10,000 rpm for 15 min at 2°C and the supernatant was discarded to isolate the periodide crystals. To dissolve the periodide crystals, 9 ml of 1,2-dichloroethane was added and then vortexed. An absorbance measurement at 365 nm was performed after 2 h (Eppendorf UV-vis Spectrophotometer, BioSpectrometer basic model) (Grieve and Grattan 1983).

The Anthrone method was used to estimate leaf total sugar content (Sadasivam and Manickam 2008). The leaf samples (100 mg) were hydrolyzed with 2.5 N HCl for 3 h in a boiling water bath. After cooling to room temperature, the mixture was neutralized with solid Na₂CO₃. The volume was made up to 100 ml and centrifuged at 5000 rpm for 15 minutes. Anthrone reagent (4 ml) was added to 500 µl of supernatant. The mixture was boiled for 8 minutes in a water bath. After cooling, the absorbance was read at 630 nm. A standard curve was prepared with glucose to determine sugar content.

Trehalose extract was prepared according to the method of Ferreira et al. (1997) with slight modifications. The leaves (40 mg) were crushed in liquid nitrogen with the help of a mortar and pestle. The sample was boiled in 2 ml of ethanol in a water bath for a few minutes. Ethanol was then evaporated using nitrogen gas and the residue was dissolved in 5 ml of the mobile phase (acetonitrile: H₂O (70:30)) of the High-performance liquid chromatography (HPLC). The content was centrifuged at 10,000 rpm for 10 min and filtered through a 0.2 µm syringe filter. The filtrate was heated in boiling water for 60 minutes. The pH was adjusted to 7.0. The solution was evaporated and the residue was dissolved in the mobile phase. The extract was analyzed by HPLC system (Agilent 1200 infinity series), using a monosaccharide column (C-8, 150 mm × 4.6 mm × 5 µm) at a flow rate of 0.5 ml/min, 20 µl injection volume, and the elute was detected by refractory index detector (Hewlett-Packard 1037A). By comparing the chromatogram with a standard trehalose concentration, the trehalose content was calculated.

Proline content was determined by homogenizing 0.2 g leaf tissue in 3% aqueous sulfosalicylic acid (5 ml) (Bates et al. 1973). At 4°C, the homogenate was centrifuged for 10 minutes at 12,000 rpm. To 2 ml aliquot, 2 ml acid ninhydrin and 2 ml anhydrous acetic acid were added. After heating for 1h, the mixture was cooled immediately. To

the mixture, 4 ml of toluene was added and vortexed for 20 seconds. Absorbance was read at 520 nm. Proline content was determined using a standard curve.

The concentrations of polyamines viz. putrescine, spermidine, and spermine were measured in dried leaf samples according to the method of Marcé et al. (1995) with some modifications. A dried leaf sample (100 mg) was extracted in 4 ml 5 % cold perchloric acid (Minocha et al. 1994). The extract was centrifuged at 20,000 g for 30 minutes and the supernatant was stored at -20°C until dansylation. For dansylation, 200 μl perchloric acid extract was mixed with 40 μl 0.05 M diamino heptane (internal standard). Next 200 μl of Na_2CO_3 and 400 μl of dansyl chloride were added. The resultant mixture was vortexed and incubated in the dark overnight. Then 100 μl of proline solution (100 mg ml^{-1} water) was added and incubated in the dark for 30 minutes. To the dansylated polyamines 500 μl toluene was added and vortexed for a few seconds. The 400 μl of the organic phase was aspirated, evaporated by nitrogen gas, and dissolved in 800 μl acetonitrile. The sample was filtered using a 0.22 μm syringe filter and 50 μl was injected into the HPLC system (Agilent 1200 infinity series) at a flow rate of 1 ml/min. Polyamines were separated on an ORO Chem C-18, 150 mm $\times$ 4.6 mm $\times$ 5 μm column and detected by Hewlett Packard 1046A Programmable Fluorescence detector (excitation at 340 nm, emission at 500 nm). The polyamines were eluted with acetonitrile and 10 mM 1-Heptanesulphonic acid.

3.4.1.5.3 Protein

The analytical Kjeldahl method (EN ISO 20483:2006, AN 300) for determining protein was conducted by estimating total nitrogen using a FOSS nitrogen analyzer (FOSS Kjeltex 8200). To 1 g of dried leaf sample 7 g K_2SO_4 , 0.8 g $\text{CuSO}_4 \times 5\text{H}_2\text{O}$, and 12 ml concentrated H_2SO_4 were added. The mixture was digested at 420°C for 60 minutes with a digester (FOSS LabtecTM Line DT208 Digester) attached to a scrubber (Labquest Borosil KSC 01) to neutralize harmful fumes. The solution was left to cool for 15 minutes and then diluted cooled digest with 80 ml H_2O . To the diluted digest 50 ml of 40 % NaOH was added and to the receiver flask, 30 ml of 4% boric acid having methyl red and bromocresol green dye was added. The content was distilled for 5 minutes with FOSS fully automated distillation unit. A reagent blank was performed before each batch of

samples. The solution in the receiving conical flask was titrated with 0.1 M of HCl, the titre was measured and the percentage of nitrogen content was calculated.

$$\% \text{ Nitrogen} = \frac{(T-B) \times N \times 14.007}{\text{Weight of sample (mg)}} \times 100$$

T = Sample titration, B = Blank titration, N = Normality of titrant

% Protein = % Nitrogen $\times$ F (Conversion Factor)

F = 6.25 for cereals

3.4.1.5.4 α -tocopherol

The 100 mg of the sample was crushed in liquid nitrogen and homogenized in 0.1 N H_2SO_4 (Sadasivam and Manickam 2008). Homogenate was centrifuged at 10,000 rpm for 10 minutes at room temperature. To the supernatant, ethanol was added in equal parts and centrifuged at 10000 rpm for 5 minutes. An equal volume of xylene was added to the mixture and centrifuged at 10000 rpm temperature for 5 minutes at room. To the upper phase (1 ml), 1 ml of 2,2'-bipyridyl was added. Incubated the mixture for 15 minutes at room temperature with 0.33 ml of 0.12% (w/v) FeCl_3 . Absorbance was measured at 520 nm (Eppendorf UV-vis Spectrophotometer, BioSpectrometer basic model).

$$\alpha\text{-tocopherol } (\mu\text{g g}^{-1}) = \frac{\text{Sample OD}_{520} - \text{Sample OD}_{460}}{\text{Standard OD}_{520}} \times 0.29 \times 15 \times \frac{\text{Total Volume of homogenate}}{\text{Volume used} \times \text{weight of tissue}}$$

3.4.1.5.5 Membrane damage

The malondialdehyde content (MDA) of leaves was used to determine the level of lipid peroxidation (Heath and Packer). The leaf tissue (0.5 g) was crushed in 10 ml of 0.1% trichloroacetic acid (TCA). Homogenate was centrifuged at 12,000 rpm for 15 minutes (Eppendorf, Centrifuge 5810 R). A solution of 0.5% thiobarbituric acid (TBA) prepared in 20% thiobarbituric acid (TCA) was added to 2 ml supernatant. After heating for 30 minutes at 95°C, the mixture was cooled. The mixture was centrifuged at 10000 rpm for 10 minutes. Supernatant absorbance was measured at 532 nm (Eppendorf UV-vis Spectrophotometer, BioSpectrometer basic model). The calculation of MDA content was done using its extinction coefficient.

To determine relative permeability twenty-five leaf discs with a diameter of 5 mm were cut out and placed in a test tube containing 25 ml Milli Q water. The electrical conductivity of the solution was measured after one hour of incubation using a conductivity meter (HACH analyzer, HQ440d multi). After boiling the leaf discs for 30 minutes, the solutions were cooled and conductivity was measured again (Zwiazek and Blake 1991).

To determine LOX activity 500 mg leaf samples were homogenized in ice-cold phosphate buffer (7.5) having 0.5 mM EDTA. Homogenate was centrifuged at 12,000 rpm for 15 minutes at 4°C. The extract (50 µl) was added to 2.95 ml substrate, which was prepared by adding 35 µl of linoleic acid to 5 mL milli Q water containing 50 µl tween 20, and the final volume was made to 100 ml with 0.1 M phosphate buffer. Absorbance reading was recorded at 234 nm (Doderer et al. 1992).

$$\text{LOX activity} = \frac{\text{Volume of reaction mixture} \times \text{Absorbance difference per min}}{\text{Molar extinction coefficient of hydroperoxides} \times \text{volume of enzyme}}$$

3.4.2 Objective 2: To study the role of soil microbes on cell ultrastructural changes in wheat and maize plants under salt and drought stresses

3.4.2.1 Determination of ultrastructural changes

The leaf samples were cut into 1 mm size and fixed in Karnovsky's fixative (Karnovsky 1965) containing paraformaldehyde (8 %), glutaraldehyde (12.5%), and phosphate buffer (0.1 M, pH 7). The samples were rinsed thrice in 0.1 M phosphate buffer (pH 7.0) to remove excess fixatives. The secondary fixation of samples was done with 2 % osmium tetroxide followed by rinsing with buffer. The samples were passed through ascending concentrations of ethanol and propylene oxide for dehydration. Then infiltration of samples was done by passing the dehydrated samples through a series of epon–araldite mixtures with propylene oxide. Leaf samples were embedded in resin. The sections were cut with the help of ultramicrotome. The sections were mounted on copper grids and stained with uranyl acetate. Stained sections were washed with 50% alcohol, followed by distilled water. Finally, the sections were stained using lead citrate, washed with 1 N NaOH, and dried. The stained sections were observed in the

transmission electron microscope (Thermoscientific, Talos). Mesophyll cells were analyzed to study the ultrastructural changes in the plasma membrane and chloroplast (Bozzola and Russell 1999).

3.4.3 Objective 3: To study the role of soil microbes during salt and drought stresses on the antioxidant accumulation in wheat and maize

3.4.3.1 Enzymatic antioxidants

Leaf tissue (500 mg) was crushed in 0.1 M phosphate buffer (pH 7.5) solution containing 0.5 mM EDTA. The homogenate was centrifuged at 12,000 rpm for 15 min at 4°C and the extract was used to measure enzyme activity. SOD activity was measured following the protocol of Dhindsa et al. (1981). The reaction mixture contained 1.5 M sodium carbonate (100 µl), 2 mM L-methionine (200 µl), 2.25 mM nitro-blue tetrazolium (100 µl), 3 mM EDTA (100 µl), 100 mM potassium phosphate buffer (1500 µl), Milli Q water (1000 µl) and 50 µl of the enzyme extract. The reaction was initiated with 0.1 ml of 60 µM riboflavin, and the tubes were illuminated for 15 minutes with 15W fluorescent lamps. A black cloth was used to stop the reaction, and the absorbance was measured at 560 nm. CAT activity was determined by the protocol of Teranishi et al. (1974). The reaction mixture containing 6 mM H₂O₂ (1000 µl), 1900 µl 0.1 M phosphate buffer (pH 7.0), and 100 µl enzyme extract was used for the enzyme assay. The reaction was stopped with a titanium reagent (4 ml). After centrifuging at 10,000 rpm for 10 minutes, absorbance (415 nm) was measured. APX activity was determined using a reaction mixture of 1500 µl potassium phosphate buffer (100 mM), 500 µl ascorbic acid (3 mM), 100 µl EDTA (3 mM), 200 µl H₂O₂ (6 mM), 610 µl Milli Q water, and 1000 µl enzyme extract. A change in absorbance was measured at 290 nm (Nakano and Asada 1981).

Statistical analysis

Analysis of the data was performed using IBM SPSS Statistics 21. The Multivariate Analysis of Variance (MANOVA), Tukey HSD, and descriptive statistics were used to analyze the independent effect of salinity and microbes and their interactions on various plant growth parameters. The means were compared using one-way ANOVA post-hoc Duncan's test ($P < 0.05$).

4. RESULTS AND DISCUSSION

4.1 PART I

To study the role of soil microbes on growth, changes in nutritional, biochemical, and physiological parameters, ultrastructural changes, and antioxidant accumulation in wheat under drought stress.

To accomplish the objective, the percentage of AMF and *P. indica* colonization, plant growth and biomass, macronutrients and micronutrients content, rate of physiological processes, photosynthetic pigments content, osmolytes content, lipid peroxidation, relative permeability, LOX activity, protein content, α -tocopherol content, ultrastructural changes, and antioxidant accumulation was estimated in microbial inoculated and uninoculated wheat plants under drought stress.

4.1.1 Results

4.1.1.1 Microbial colonization

AMF and *P. indica* successfully colonized the roots of wheat plants at all levels of drought stress (**Figure 4.1**). However, the percentage of colonization was higher in AMF-inoculated wheat roots than in *P. indica*-inoculated roots during drought stress. Plant roots not inoculated with AMF and *P. indica* did not show microbial colonization. Different levels of water regime significantly affected the percent root colonization. A gradual decline in microbial colonization was observed with increasing drought stress levels (**Table 4.1**).

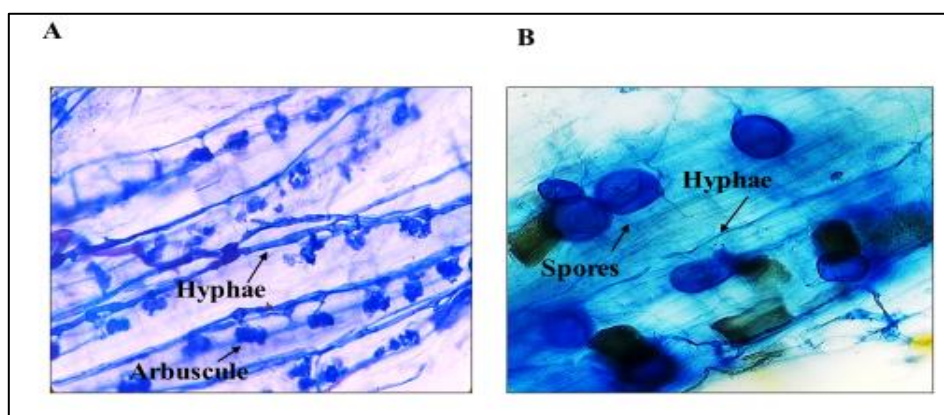


Figure 4.1: Microscopic observation of root colonization in (A) AMF-inoculated wheat plants and (B) *P. indica*-inoculated wheat plants under drought stress.

Table 4.1: Influence of drought stress and microbe inoculations on percentage microbe colonization, length, and biomass of shoot and root in wheat plants.

FC (%)	Microbe	Shoot length (cm)	Root length (cm)	Shoot biomass (g)	Root biomass (g)	Leaf area (cm ²)	Root colonization (%)
75	Control	60.32±0.83gh	25.70±1.45fg	3.51±0.52i	2.14±0.13i	17.69±0.87g	-
	<i>P. indica</i>	68.03±2.05cde	27.13±2.85cde	4.55±0.39 fg	3.58±0.39fgh	19.79±0.68e	70
	PGPB	70.12±1.30b	30.06±3.49a	6.64±0.49de	4.75±0.07cde	22.68±0.30a	-
	AMF	69.13±1.03bc	30.33±3.11bc	5.87±0.33de	3.43±0.41gh	22.14±0.23bc	75
55	Control	61.10±1.75fg	26.20±1.75def	3.22±0.26g	2.31±0.40h	18.34±0.81f	64
	<i>P. indica</i>	69.96±2.05bcd	27.47±2.78bcd	5.07±0.67d	3.38±0.48efg	18.95±0.73cd	-
	PGPB	71.70±2.05a	33.16±2.06a	6.25±0.31a	4.22±0.11ab	25.21±0.38a	62
	AMF	71.44±1.25 bc	32.77±2.37b	5.36±0.46ab	3.59±0.33def	25.34±1.13b	-
45	Control	58.70±1.95h	24.33±1.91gh	3.11±0.11ef	2.05±0.09gh	16.59±0.54h	58
	<i>P. indica</i>	66.10±1.88de	26.00±3.51fg	4.11±0.19bc	3.11±0.18cde	17.96±0.27e	-
	PGPB	68.70±1.00bcd	30.13±2.53bc	5.42±0.15ab	4.08±0.02a	22.08±0.07bc	53
	AMF	66.90±2.05cde	28.37±2.67cde	4.48±0.20abc	3.24±0.07bcd	20.14±0.39d	-
35	Control	56.80±2.36i	21.46±2.05h	2.18±0.28j	1.63±0.11i	14.30±1.06i	-
	<i>P. indica</i>	62.22±1.00gh	25.33±1.77g	3.61±0.36h	2.81±0.12h	21.33±0.77g	35
	PGPB	66.30±0.89cde	28.51±1.57def	7.03±0.08ab	3.41±0.08abc	23.58±0.55d	-
	AMF	64.22±2.42ef	27.19±2.55ef	6.28±0.07cd	3.03±0.03cde	22.53±0.56e	40
Significance							
Drought		***	**	***	**	***	***
<i>P. indica</i>		***	**	***	***	***	***
Drought × <i>P. indica</i>		ns	ns	ns	ns	ns	***
PGPB		***	***	***	***	***	-
Drought × PGPB		ns	ns	ns	**	ns	-
AMF		***	***	***	***	***	***
Drought × AMF		ns	ns	ns	ns	ns	***

Values represent averages across replicates. Letters within a column represent significant differences at $P < 0.05$. ns: not significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

4.1.1.2 Growth parameters

Growth parameters of microbial inoculated and uninoculated wheat plants at four different water regimes are shown in **Table 4.1**. At all levels of water regimes, the microbial-inoculated wheat plants performed significantly better in terms of shoot length, root length, shoot biomass, root biomass, and leaf area compared to uninoculated plants. Under severe drought conditions (35% FC), PGPB inoculation increased the shoot and root length by 16.73% and 32.85%, respectively, followed by AMF inoculation which improved it by 13.06% and 26.69% respectively, while *P. indica* inoculation increased it by 9.54% and 18.03%, respectively, compared to uninoculated plants. A 35% FC PGPB-inoculated plant showed an increase in the biomass of its shoots and roots of 146.18% and 109.20%, respectively. This was preceded by AMF inoculation which raised it by 96.33% and 109.20% each, and *P. indica* inoculation enhanced it by 65.60% and 72.19%, respectively, as opposed to uninoculated plants. The leaf area of wheat plants was significantly improved with PGPB inoculation (41.56%) followed by AMF (31.93%) and *P. indica* (20.75%) in comparison to non-inoculated wheat plants. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, and AMF on plant growth, biomass, and leaf area (**Table 4.1**). On comparing the potential of microbes to increase growth parameters, PGPB-inoculated plants showed higher drought resistance as compared to AMF and *P. indica*-inoculated plants.

4.1.1.3 Mineral nutrients content

Table 4.2 shows the macronutrient and micronutrient content of wheat plants with respect to water stress and microbial inoculation. Microbial inoculation and water stress had significant effects on leaf macronutrient (Ca^{2+} , Mg^{2+} , K^+ , Na^+) and micronutrient (Zn^{2+} , Fe, Mn^{2+} , and Cu) contents. With increasing water stress levels, Ca^{2+} levels decreased linearly in PGPB-inoculated, *P. indica*-inoculated, and uninoculated plants. At all levels of water stress, however, the presence of microbes led to higher Ca^{2+} levels than in uninoculated plants. Inoculated plants with AMF exhibited higher Ca^{2+} levels up to 45% FC and then began to decline at 35% FC. Plants inoculated with PGPB, *P.*

indica, and AMF had 238.28%, 77.75%, and 362.29% more Ca^{2+} than uninoculated plants at 35% FC, respectively.

Microbe inoculation and water stress influenced the uptake of Mg^{2+} in leaves. A rise in water stress led to a decrease in Mg^{2+} content in wheat plants. Plants inoculated with microbes, however, maintained higher levels of Mg^{2+} than uninoculated plants at the same water stress level (**Table 4.2**). Uninoculated plants responded inconsistently to water stress in terms of Mg^{2+} uptake. Leaf Mg^{2+} uptake increased up to 55% FC, then decreased to 45% and 35% FC in uninoculated plants. However, there was a gradual decline in Mg^{2+} content in microbial-inoculated plants with increasing levels of drought. The Mg^{2+} content of inoculated plants with PGPB, *P. indica*, and AMF was 289.98%, 260.73%, and 272.53% higher than that of uninoculated plants during severe drought conditions. PGPB-inoculated plants had a higher Mg^{2+} content in leaves than *P. indica* and AMF-inoculated plants.

In wheat plants, increased levels of water stress led to a decline in K^+ content. However, plants inoculated with microbes maintained higher K^+ levels than uninoculated plants at the same water stress level (**Table 4.2**). There were inconsistent responses of K^+ uptake to water stress in both uninoculated and *P. indica* inoculated plants. The K^+ uptake in leaves increased to 55% FC, then decreased to 45% and 35% FC in *P. indica* and uninoculated plants. PGPB-inoculated plants contained higher K^+ levels up to 45% FC and then began to decline at 35% FC. At 35% FC, plants inoculated with PGPB, *P. indica*, and AMF had 124.85%, 41.52%, and 25.49% more K^+ than uninoculated plants. Microbial inoculated plants continued to have higher K^+ content in leaves than uninoculated plants in all treatments.

Sodium content in leaves of both microbial-inoculated and uninoculated plants showed a linear increase with increasing water stress levels (**Table 4.2**). However, microbial-inoculated plants possessed significantly less Na^+ as compared to uninoculated plants under all water regimes. Additionally, Na^+ content was lower in PGPB-inoculated plants than in AMF and *P. indica* under all levels of drought stress. At 35% FC, plants

inoculated with PGPB, *P. indica*, and AMF had 124.85%, 25.49%, and 41.52% less Na^+ than uninoculated plants. Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF-inoculated plants.

There was a negative effect of drought on the accumulation of Zn^{2+} in wheat plants (**Table 4.2**). Plants under water stress showed a significant decrease in Zn^{2+} levels in their leaves. In contrast, plants that were inoculated with microbes maintained higher levels of Zn^{2+} at the same level of water stress. The Zn^{2+} content of PGPB, *P. indica*, and AMF-inoculated plants increased by 3.9, 3.8, and 1.0-fold at 35% FC, respectively as compared to uninoculated plants. Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF-inoculated plants.

Plants under water stress showed a significant decrease in Fe levels in their leaves. In contrast, plants that were inoculated with microbes maintained higher levels of Fe at the same level of water stress (**Table 4.2**). The Fe content of *P. indica*, PGPB, and AMF-inoculated plants increased by 180.27%, 149.77%, and 287.55% at 35% FC, respectively in comparison to uninoculated plants. AMF has a greater ability to alleviate drought stress than *P. indica* and PGPB-inoculated plants. Mn^{2+} content significantly decreased under severe drought conditions (35% FC) in both microbial-inoculated and uninoculated plants. In contrast, plants that were inoculated with microbes maintained higher levels of Mn^{2+} at the same level of water stress (**Table 4.2**). The Mn^{2+} content of PGPB, *P. indica*, and AMF-inoculated plants increased by 3.4, 4.5, and 6.0-fold at 35% FC, respectively compared to uninoculated plants. AMF has a greater ability to alleviate drought stress than *P. indica* and PGPB-inoculated plants in terms of Mn^{2+} content. There was a negative effect of drought on the accumulation of Cu in wheat plants (**Table 4.2**). Microbial inoculated and uninoculated plants under water stress showed a significant decrease in Cu levels in their leaves. In contrast, plants that were inoculated with microbes maintained higher levels of Cu at the same level of water stress. The Cu content of PGPB, *P. indica*, and AMF-inoculated plants decreased 0.9, 1.9, and 0.7-fold at 35% FC, respectively. While in uninoculated plants, there was almost a 1.5-fold decrease in Cu^+ content in comparison to watered plants.

Table 4.2: Influence of water stress and microbial inoculation on the content of macro-nutrients and micronutrients in leaves of the wheat plants.

FC (%)	Microbe	Macronutrients (mg kg ⁻¹)				Micronutrients (mg kg ⁻¹)			
		Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	Zn ²⁺	Fe	Mn ²⁺	Cu
75	Control	375.78±7.72g	1761.51±19.18e	3052.05±16.34j	489.60±15.14g	71.84±11.69d	765.76±8.11cd	95.61±6.47a	217.70±10.90c
	<i>P. indica</i>	661.47±11.58d	2251.99± 15.20c	4659.43± 16.02b	485.99±16.76g	74.42±7.41cd	870.62±5.36a	96.093±1.02a	315.96±1.67a
	PGPB	952.76±11.29a	3679.97±18.60b	3343.49±19.25i	455.98±12.51g	101.09±7.39a	817.69±2.51abc	88.83±6.44ab	135.20±9.79e
	AMF	700.33±6.18d	4047.77±13.85a	4531.37±11.77c	484.55±11.87g	44.41±5.22c	635.54±13.98e	44.63±3.25f	319.86±12.46a
55	Control	350.85±18.49gh	3749.88±9.74b	4298.74±24.11d	663.04±19.22e	83.67±10.49cd	559.26±10.19f	83.83±4.06b	189.72±3.02d
	<i>P. indica</i>	575.54±11.18e	2024.41±12.64d	4925.62±14.11a	615.45±18.43e	97.72±8.67ab	840.10±9.49ab	95.16±4.57a	285.03±14.52b
	PGPB	851.31±8.11 b	2267.13±6.48c	3535.38±31.81g	477.61±16.50g	87.23±3.35bc	722.46±20.23d	67.09±2.82cd	103.91±11.34gh
	AMF	832.61±13.65b	2224.99±16.42c	3770.43±22.08e	550.46±14.52f	79.43±7.41cd	782.69±14.27bcd	84.13±4.15b	138.01±8.85e
45	Control	267.46±8.30i	1261.35±15.56g	3439.30±16.33h	955.98±25.51b	73.34±8.79cd	468.83±19.18g	54.43±2.24e	128.54±3.12ef
	<i>P. indica</i>	320.68±13.89h	1547.23±12.62f	2654.91±17.50k	841.85±15.48c	82.84±0.72cd	740.86±7.56d	57.14±8.65e	117.47±12.32efg
	PGPB	764.86±13.40 c	1491.41±19.29f	3674.60±2.59f	658.27±17.89e	84.58±1.64cd	646.15±17.35e	60.43±2.10de	92.63±4.96h
	AMF	981.30±10.31a	1463.3±15.64f	2051.09±18.67l	721.5±18.93d	34.66±7.89e	648.53±16.75e	67.76±3.09cd	110.21±9.23fgh
35	Control	131.28±5.31j	265.67±5.55i	1463.67±14.10n	1027.89±16.94a	20.76±3.83f	167.27±13.41j	12.37±1.00g	20.54±6.57j
	<i>P. indica</i>	233.35±4.81i	958.34±13.08h	2071.41±19.02l	883.45±14.63c	78.24±9.54cd	468.81±7.51gh	55.08±4.48e	105.16±3.79gh
	PGPB	444.09±5.80f	1036.07±20.52h	2691.08±0.88k	839.27±15.51c	80.54±7.54cd	417.79±12.78i	41.91±2.83f	45.14±2.03i
	AMF	606.89±9.53e	989.70±13.00h	1836.76±16.15m	842.25±15.51c	21.66±7.89f	608.25±2.82ij	73.82±2.38c	39.07±4.04ij
Significance									
Drought		***	***	***	***	***	***	***	***
<i>P. indica</i>		***	***	***	***	***	***	***	***
<i>P. indica</i> × Drought		***	***	***	*	***	***	***	***
PGPB		***	***	***	***	***	***	***	***
PGPB × Drought		***	***	***	*	***	***	***	***
AMF		***	***	ns	***	***	***	***	*
AMF × Drought		***	***	***	***	**	***	***	***

Values represent the mean of replicates. Different letters within a column indicate significant differences at P<0.05. ns: not significant. ***P<0.001, **P<0.01, *P<0.05.

4.1.1.4 Physiological parameters

Physiological parameters of microbial-inoculated and uninoculated wheat plants at four different water regimes are depicted in **Figure 4.2**. At all levels of water regimes, microbial-inoculated wheat plants showed substantially higher photosynthetic and transpiration rates, stomatal conductance, and internal CO₂ levels than uninoculated wheat plants. PGPB (51.29%) and AMF (38.46%) significantly increased photosynthetic rates in comparison to uninoculated plants at 35% FC stress, led by *P. indica* (20.07%). The stomatal conductance of wheat plants was significantly enhanced with inoculation with PGPB (100.00%) followed by AMF (66.67%) and *P. indica* (27.27%) in comparison to uninoculated wheat plants. The transpiration rate of wheat plants inoculated with PGPB, AMF and *P. indica* increased to 99.42%, 72.90%, and 57.10% respectively at 35% FC. Plants inoculated with PGPB had an internal CO₂ increase of 150.89% under severe drought conditions, while plants inoculated with AMF and *P. indica* had an increase of 124.07% and 83.31%, respectively, in contrast to uninoculated plants. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, and AMF physiological parameters (**Table 4.3**). Therefore, this indicates that PGPB is the most efficient microbe in alleviating drought stress at all levels of the water regime as compared to AMF and *P. indica*.

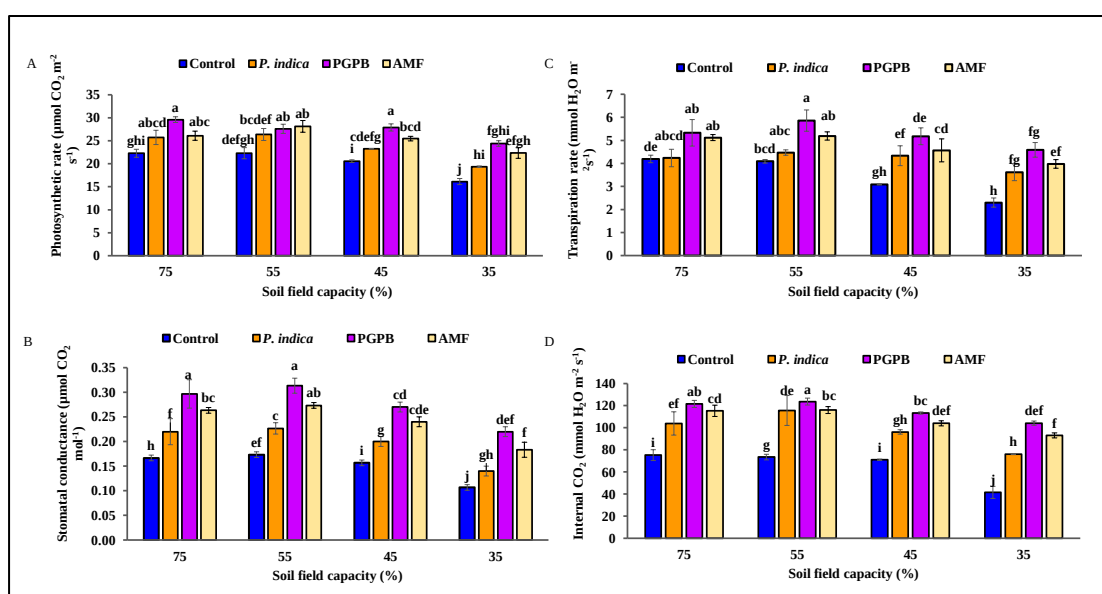


Figure 4.2: Effects of drought and microbial inoculation in wheat on (A) photosynthetic rate; (B) stomatal conductance; (C) transpiration rate; and (D) internal CO₂. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.1.1.5 Biochemical parameters

4.1.1.5.1 Photosynthetic pigment content

Photosynthetic pigments viz. chlorophyll a, chlorophyll b, total chlorophylls, and carotenoid content in microbial-inoculated wheat plants showed substantially higher levels than uninoculated wheat plants at all levels of water regimes (**Figure 4.3**).

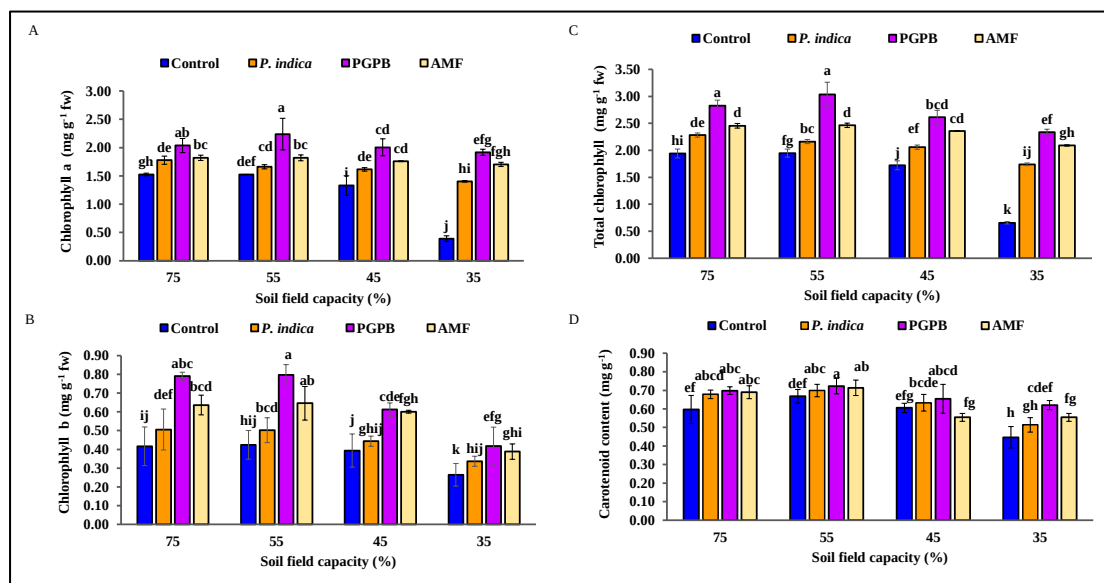


Figure 4.3: Effects of drought and microbial inoculation in wheat on the content of (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; and (D) carotenoid. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

With increasing water stress levels photosynthetic pigment content significantly decreases. At the highest level of drought (35% FC), the chlorophyll a content of wheat plants inoculated with PGPB, AMF and *P. indica* increased to 391.71%, 336.63%, and 260.19% respectively as compared to uninoculated plants (**Figure 4.3A**). Plants inoculated with PGPB had an increased chlorophyll b content of 60.70% under severe drought conditions, while plants inoculated with AMF and *P. indica* had an increase of 49.21% and 29.47%, respectively (**Figure 4.3B**). The total chlorophyll content was higher in PGPB-inoculated (253.79%) compared to AMF (216.71%) and *P. indica* (163.78%) as compared to the control at the highest level of stress (**Figure 4.3C**). At 35% FC carotenoid content was higher in PGPB (37.84%) inoculated plants, followed by AMF (23.18%) and *P. indica* (14.22%) inoculated plants in comparison to uninoculated plants (**Figure 4.3D**). Two-way ANOVA demonstrated a significant

independent effect of drought, *P. indica*, PGPB, AMF, and interaction of drought $\times$ *P. indica*, drought $\times$ PGPB, and drought $\times$ AMF on chlorophyll a and total chlorophyll content (**Table 4.3**). Therefore, this indicates that PGPB is the most efficient microbe in alleviating drought stress at all levels of the water regime as compared to AMF and *P. indica* in wheat plants.

4.1.1.5.2 Osmolyte content

With increasing water stress levels, the content of glycine betaine exhibited a linear and significant increase in leaves of microbial-inoculated and uninoculated wheat plants (**Figure 4.4A**). Comparing 35% FC and 75% FC, glycine betaine content increased by about 38.72% in PGPB-inoculated plants, 34.44% in AMF-inoculated plants, 34.09% in *P. indica* plants, and 41.11% in uninoculated plants. As compared to uninoculated plants, microbial inoculated plants had significantly higher glycine betaine content at all water regimes. At 35% FC, AMF-inoculated plants had a 40.37% higher leaf glycine betaine content than control, which was led by PGPB-inoculated plants (31.27%) and *P. indica*-inoculated plants (10.71%). Thus, AMF outperforms PGPB and *P. indica* at all levels of water regimes in alleviating drought stress. The water stress and microbial inoculation significantly affected the glycine betaine content.

In both microbial inoculated and uninoculated wheat plants, water stress increased total sugar content slowly up to 45% FC, followed by a decrease at 35% FC (**Figure 4.4 B**). At all levels of drought stress, however, microbes facilitated higher sugar accumulation in leaves than in corresponding uninoculated plant leaves. Plants inoculated with PGPB displayed 1.1, 0.7, 0.8, and 1.1-fold higher total sugar content than plants uninoculated at 75%, 55%, 45%, and 35% FC, respectively. Total sugar content in AMF-inoculated plants was 1.1, 2.5, 2.9, and 4.5-fold greater than that of uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. As a result of inoculation with *P. indica*, total sugar content increased 1.1, 1.6, 1.7, and 2.3-fold when compared with uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. Thus, at all water regimes, AMF has a greater ability to alleviate drought stress than *P. indica* and PGPB. Water stress and microbial inoculation significantly enhanced total sugar content.

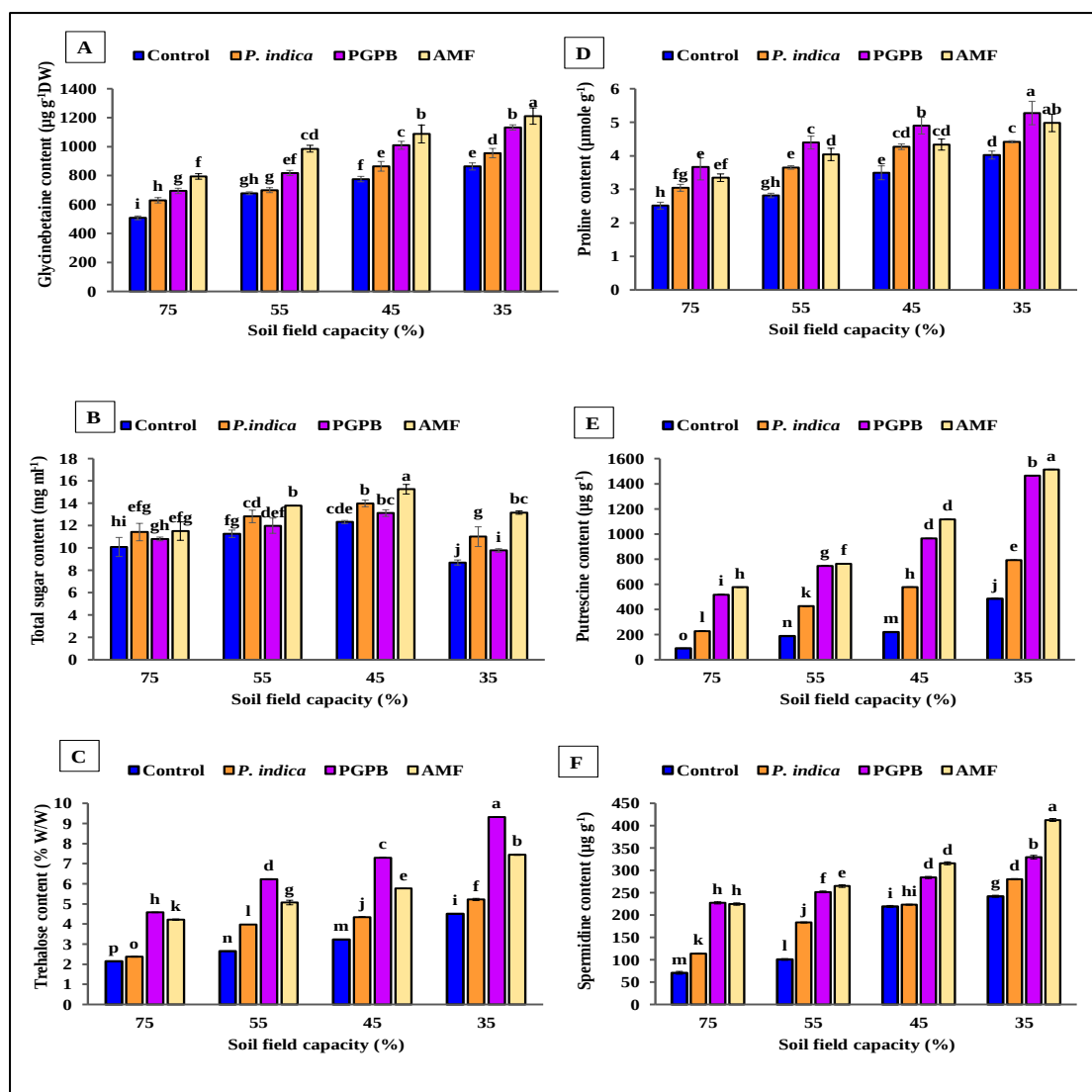


Figure 4.4: Effects of drought and microbial inoculation in wheat on the content of (A) glycine betaine; (B) total sugar; (C) trehalose; (D) proline; (E) putrescine; and (F) spermidine. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

In microbial-inoculated and uninoculated wheat plants, the amount of trehalose increased linearly with increasing levels of water stress (**Figure 4.4 C**). A significantly higher trehalose content was detected in microbial-inoculated plants compared to uninoculated plants at all water regimes. Trehalose content in PGPB-inoculated plants was 112.86%, 135.09%, 126.5%, and 106.89% higher than uninoculated plants at 75%, 55%, 45%, and 35% FC respectively. Plants inoculated with AMF had trehalose contents of 96.12%, 91.19%, 78.99%, and 65.33% higher than uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. In *P. indica* -inoculated plants, trehalose content was 10.23%, 50.19%, 34.89%, and 16% higher than in uninoculated plants at

75%, 55%, 45%, and 35% FC. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, AMF, and interaction of drought $\times$ *P. indica*, drought $\times$ PGPB, and drought $\times$ AMF on trehalose content (**Table 4.3**). Therefore, PGPB is more efficient than AMF and *P. indica* at all water regimes in alleviating drought stress. Trehalose content was significantly enhanced by water stress and microbial inoculation interaction.

Proline content in wheat leaves was significantly influenced by water stress and microbial inoculation (**Figure 4.4 D**). Both microbial inoculated and uninoculated wheat leaves exhibited an increase in proline content under water stress. However, microbial-inoculated wheat plants showed considerably better performance in terms of proline content compared to uninoculated plants at all levels of water regimes. At 35% FC, PGPB-inoculated plants had a 31.26% higher leaf proline content than control, which was led by AMF-inoculated plants (23.90%) and *P. indica*-inoculated plants (9.92%).

Water stress and microbial inoculation significantly influenced polyamine content (putrescine and spermidine). However, spermine was not detected in both microbial-inoculated and uninoculated plants at all water regimes. Increasing the level of water stress to plants showed a contrasting effect on the content of putrescine and spermidine in microbial inoculated and uninoculated plants (**Figure 4.4 E-F**). PGPB, AMF, *P. indica*, and uninoculated plants showed an increase in putrescine content by 2.8, 2.6, 3.5, and 5.4-fold respectively, at 35% FC, as compared to well-watered plants. Spermidine content recorded 70.44%, 36.08%, and 15.83% increase in AMF, PGPB, and *P. indica* inoculated plants at 35% FC respectively, over uninoculated plants. Spermidine content in PGPB-inoculated plants was 3.2, 2.5, 1.3, and 1.4-fold higher than in uninoculated plants at 75%, 55%, 45%, and 35% FC respectively. Plants inoculated with AMF had spermidine contents of 3.2, 2.6, 1.4, and 1.7-fold higher than uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. In *P. indica*-inoculated plants, spermidine content was 1.6, 1.8, 1.0, and 1.2-fold higher than in uninoculated plants at 75%, 55%, 45%, and 35% FC respectively. However, microbial-inoculated plants recorded higher putrescine and spermidine content over uninoculated plants at all water regimes. Thus, AMF outperforms PGPB and *P. indica*

at all levels of water regimes in alleviating drought stress in terms of polyamine content. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, and AMF on glycine betaine, trehalose, proline, and polyamine content (Table 4.3).

4.1.1.5.3 Protein

Water stress significantly increased protein content in both microbial inoculated and uninoculated wheat plants to 45% FC followed by a decrease at 35% FC stress. However, under water stress, microbial-inoculated plants possessed significantly higher levels of protein than uninoculated plants (Figure 4.5A). As compared with uninoculated wheat, PGPB, AMF, and *P. indica* inoculated plants produced 112.38%, 91.95%, and 47.97% more protein respectively at 35% FC. Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF-inoculated plants. Water stress and microbial inoculation significantly enhanced protein content.

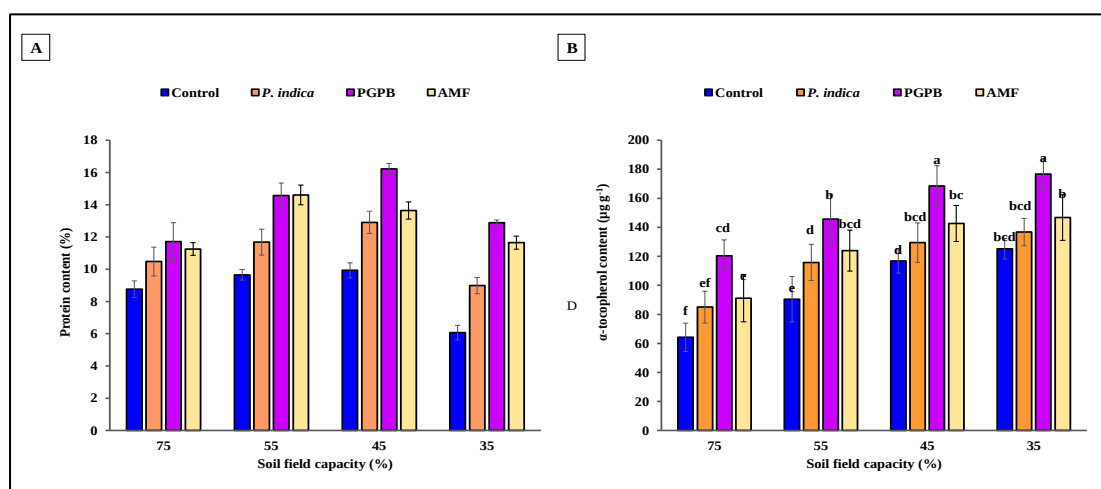


Figure 4.5: Effects of drought and microbial inoculation in wheat on the content of (A) protein; and (B) α -tocopherol. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.1.1.5.4 α -tocopherol content

Water stress significantly increased α -tocopherol content in both microbial inoculated and uninoculated wheat plants. However, under water stress, microbial-inoculated

plants possessed significantly higher levels of α -tocopherol than uninoculated plants (**Figure 4.5B**). As compared with uninoculated wheat, PGPB, AMF, and *P. indica* inoculated plants produced 41.03%, 17.15%, and 9.20% more α -tocopherol respectively at 35% FC. Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF-inoculated plants. Water stress and microbial inoculation significantly enhanced α -tocopherol content.

4.1.1.5.5 Membrane damage

In **Figure 4.6A**, the concentration of MDA is used to measure the levels of lipid peroxidation in leaves of wheat plants exposed to different levels of water stress. In both microbial inoculated and uninoculated wheat plants, exposure to water stress significantly increased MDA levels. However, uninoculated plants have a higher MDA content. At all water regimes, microbial-inoculated plants had significantly lower MDA levels. The MDA content of *P. indica*, AMF, and PGPB-inoculated plants decreased by 17.21%, 22.12%, and 29.74% over uninoculated plants at 35% FC, respectively. The relative membrane permeability of wheat plants grown under water stress was significantly affected by microbial inoculation and water stress. Electrolyte leakage increased in wheat plants in response to water stress. PGPB, AMF, and *P. indica* inoculated plants maintained lower electrolyte leakage at all water stress levels than uninoculated wheat (**Figure 4.6B**). At 35% FC, PGPB, AMF, and *P. indica* inoculated plants showed 28.61%, 12.09%, and 9.4% lower relative permeability, respectively than uninoculated plants. Water stress increased LOX activity in both microbial-inoculated and uninoculated plants; however, a significant difference in LOX activity was observed between these plants (**Figure 4.6C**). Plants inoculated with microbes displayed significantly lower LOX activity. At 35% FC, PGPB, AMF, and *P. indica* inoculated plants showed 39.83%, 29.81%, and 21.30% lower LOX activity, respectively than uninoculated plants. plants than in AMF and *P. indica*. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, AMF, and interaction of drought $\times$ *P. indica*, and drought $\times$ PGPB on membrane damage (**Table 4.3**). Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF.

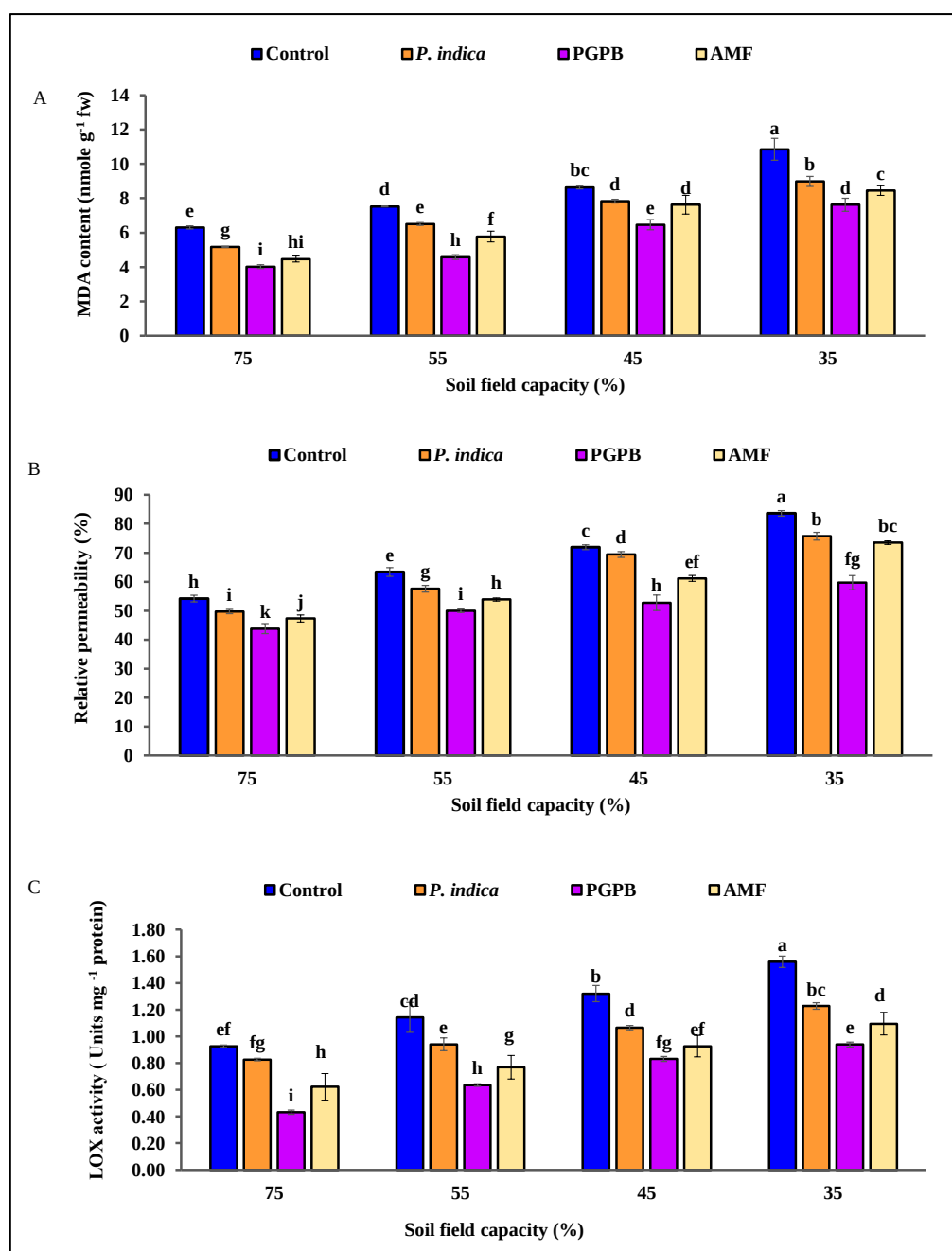


Figure 4.6: Effects of drought and microbial inoculation in wheat on (A) MDA content; and (B) relative permeability (C) LOX activity. Values represent averages across replicates. Letters within a column represent significant differences at $P < 0.055$.

4.1.1.6 Antioxidant enzyme activity

Under water stress and well-watered conditions, microbial colonization enhanced SOD activity in wheat plants (**Figure 4.7A**). Water stress increased SOD activity in microbial and uninoculated wheat plants. Under severe drought conditions (35% FC), PGPB

inoculation raised SOD activity by 8.87% followed by AMF inoculation which improved it by 6.9% while *P. indica* inoculation increased it by 3.10% compared to uninoculated wheat plants. With increasing levels of water stress, PGPB and *P. indica* inoculated wheat plants displayed increased CAT activity. Conversely, AMF-inoculated plants exhibited decreased CAT activity in response to increased water stress in comparison to the control. Plants inoculated with PGPB and *P. indica* displayed CAT activity increases of 37.20% and 21.54% as opposed to uninoculated plants respectively at 35% FC (**Figure 4.7B**).

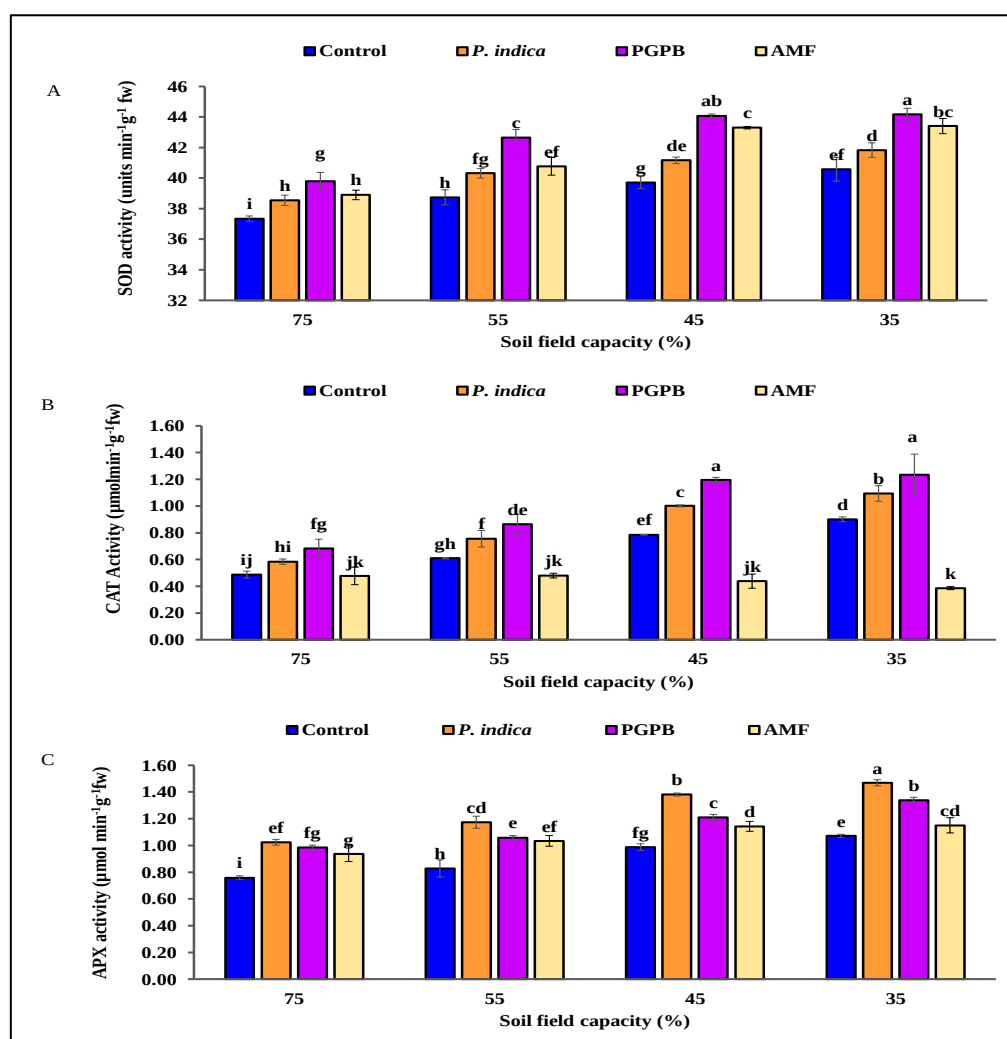


Figure 4.7: Effects of drought and microbial inoculation in wheat on the activity of (A) SOD; (B) CAT; and (C) APX. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

Microbial inoculated wheat plants displayed higher APX activity than uninoculated plants under all water regimes. At each level of water stress, *P. indica*-inoculated plants exhibited significantly higher APX activity than PGPB and AMF-inoculated plants. At all

water regimes, PGPB-inoculated wheat plants displayed 1.3-fold higher APX activity than uninoculated plants. Wheat plants inoculated with AMF had APX activities 1.2, 1.3, 1.2, and 1.0-fold higher than those uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. At all levels of water stress, *P. indica*-inoculated wheat plants showed 1.4-fold higher APX activity than uninoculated wheat plants (**Figure 4.7C**).

4.1.1.7 Cell ultrastructural studies

At all water regimes, the electron micrographs show qualitative differences between microbial inoculated and uninoculated plants.

4.1.1.7.1 Chloroplast

Figures 4.8 and **4.9** show changes in chloroplast ultrastructure in microbial-inoculated and uninoculated wheat leaves under water stress. Microbial inoculated and uninoculated wheat plants displayed chloroplasts with distinct thylakoids and grana surrounded by double-layered membranes under well-watered conditions (75% FC). However, PGPB-inoculated plants exhibited a greater number of thylakoids and granum than AMF and *P. indica*-inoculated plants. Under 55% FC water stress, uninoculated plants showed decreased numbers of thylakoids and grana, and increased numbers of plastoglobules. Under low drought stress, the thylakoid membrane contracted thereby reducing the thylakoid lumen (**Figures 4.8** and **4.9**). The chloroplast structures were almost the same in microbial-inoculated plants at 55% FC and 75% FC. A 45% water stress led to swelling in the thylakoid lumen resulting in destacking of grana. Moreover, the accumulation of smaller-size plastoglobules was more in uninoculated plants. Microbial-inoculated plants showed chloroplasts with intact thylakoids and grana and fewer but large size plastoglobules accumulation. In contrast, plants inoculated with PGPB displayed more intact thylakoids, grana, and a lesser accumulation of plastoglobules than AMF or *P. indica*. Consequently, at 35% FC, the chloroplast in uninoculated plants was characterized by disorganized thylakoids and grana and the accumulation of a large number of smaller-sized plastoglobules (**Figure 4.8D**). Contrary to this, microbial-inoculated plants contained intact thylakoids, disorganized grana, and fewer but larger-sized plastoglobules. As compared to AMF and *P. indica*, plants inoculated with PGPB showed more intact thylakoids and fewer but large-size plastoglobules at severe drought conditions.

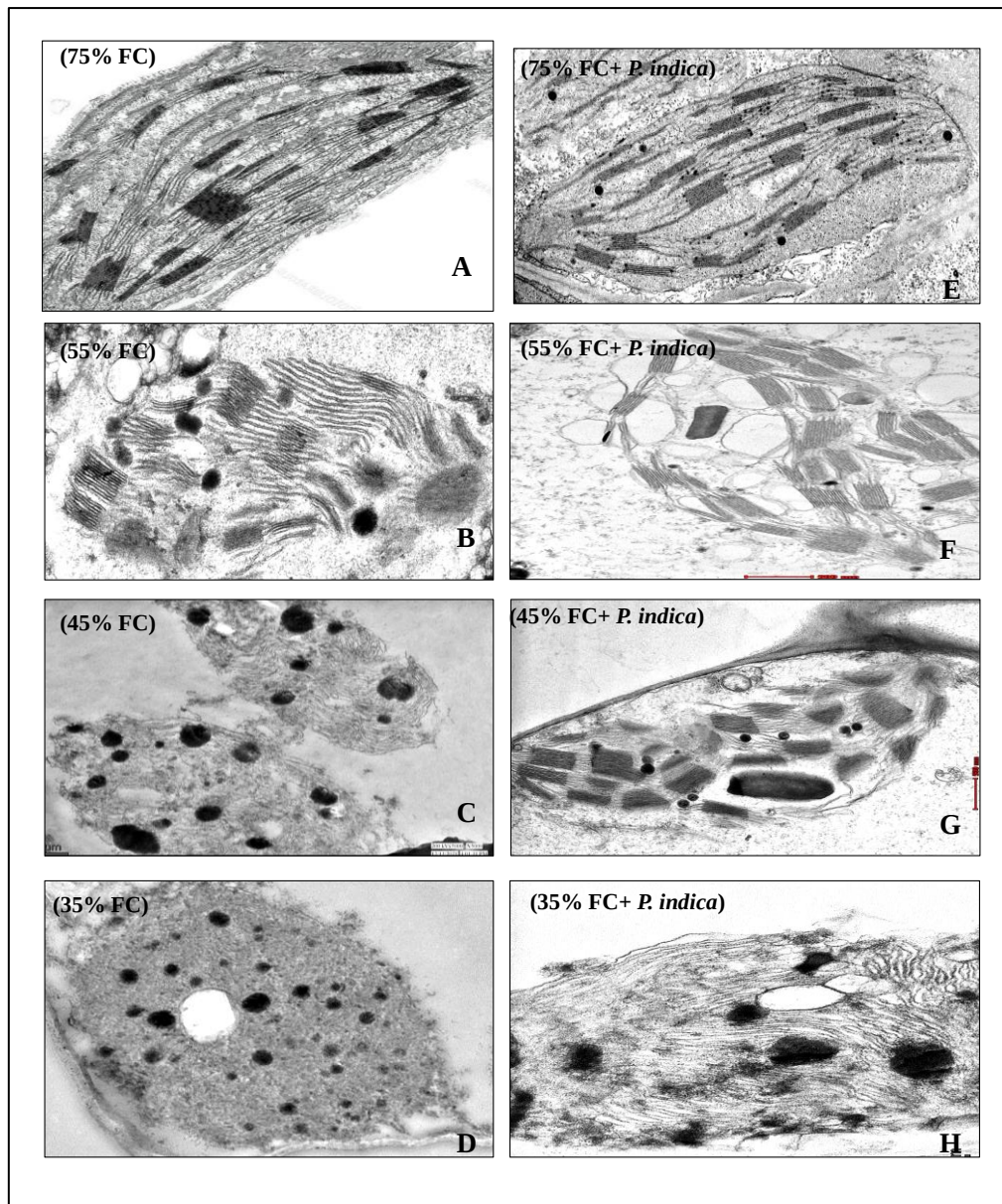


Figure 4.8: Transmission electron micrographs of chloroplast in mesophyll cells of (A) Control wheat plants not exposed to drought stress (75% FC); distinct thylakoids and grana surrounded by a well-defined double-layered membrane. (B) Control wheat plants subjected to 55% FC; with a lesser number of thylakoids and grana. Some plastoglobules are seen. (C) Control wheat plants exposed to 45% FC; disorganized thylakoids and grana. Increased accumulation of plastoglobules. (D) Control wheat plants subjected to 35% FC; disorganized thylakoids and grana. Accumulation of a large number of plastoglobules. (E) *P. indica* inoculated wheat plants not exposed to drought stress (75% FC); well-developed thylakoids and properly stacked grana surrounded by a double membrane. (F) *P. indica* inoculated wheat plants subjected to 55% FC; more grana and fewer plastoglobules. (G) *P. indica* inoculated wheat plants subjected to 45% FC; the structure of thylakoids and grana is still maintained. Fewer plastoglobules are seen. (H) *P. indica* inoculated wheat plants subjected to 35% FC; intact thylakoids and large plastoglobuli visible. Slight disintegration of grana. The values in the bracket indicate the level of drought stress imposed on plants.

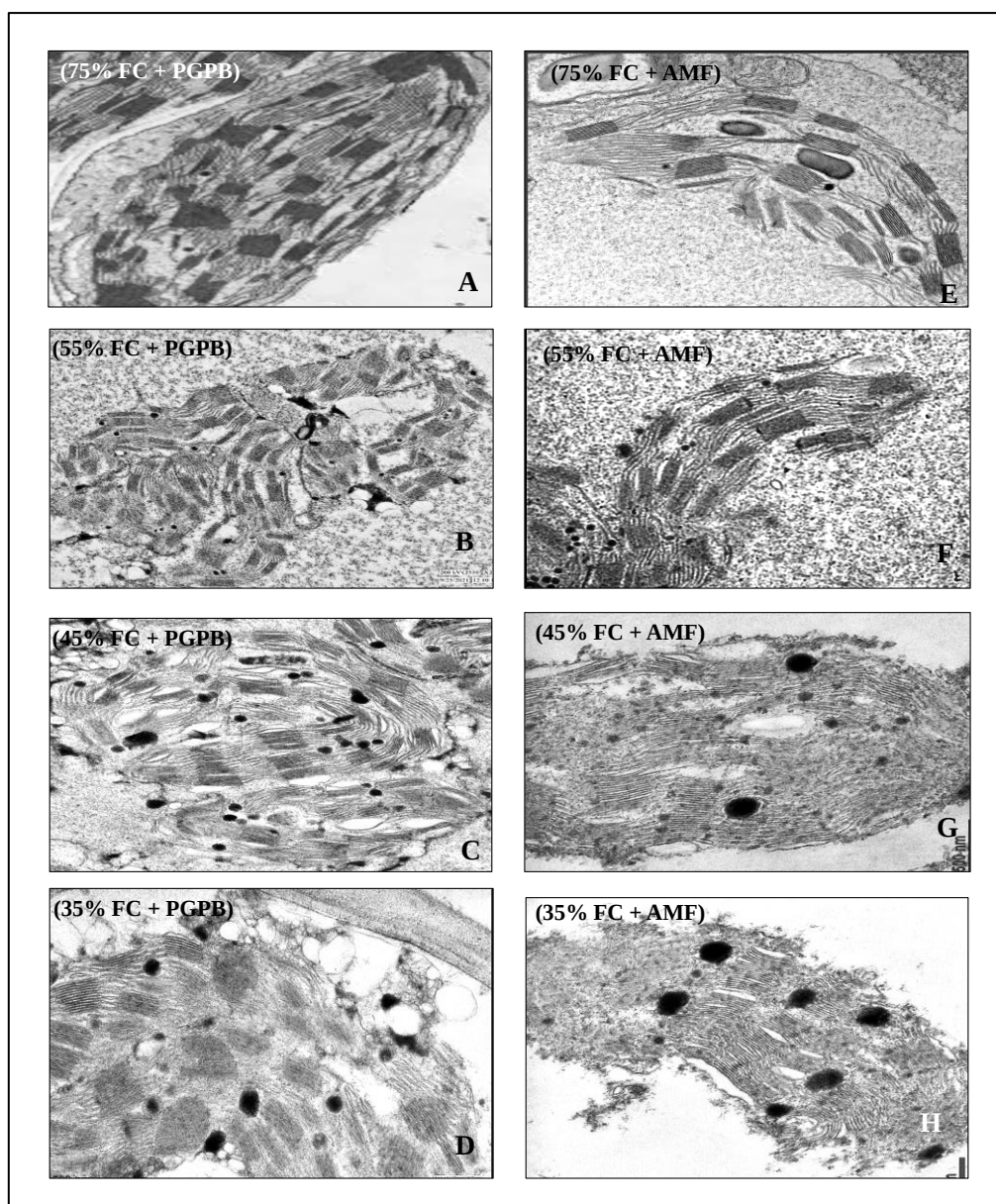


Figure 4.9: Transmission electron micrographs of chloroplast in mesophyll cells of (A) PGPB-inoculated wheat plants not exposed to drought stress (75% FC); distinct thylakoids and well-developed grana surrounded double-layered membrane. (B) PGPB-inoculated wheat plants subjected to 55% FC; intact thylakoids and grana with fewer plastoglobules. (C) PGPB-inoculated wheat plants exposed to 45% FC; grana organization still maintained and few plastoglobules. (E) AMF-inoculated wheat plants not exposed to drought stress (75% FC); properly stacked grana in the stroma (F) AMF-inoculated wheat plants subjected to 55% FC; fewer thylakoids and plastoglobules. (G) AMF-inoculated wheat plants subjected to 45% FC; the chloroplast shows intact thylakoids and fewer plastoglobules. (H) AMF-inoculated wheat plants subjected to 35% FC; fewer plastoglobules have accumulated. Intact thylakoids and disorganized grana were seen. The values in the bracket denote the level of drought stress imposed on plants.

4.1.1.7.2 Plasma membrane

Under well-watered (75% FC) conditions the plasma membrane of both microbial inoculated and uninoculated wheat plants closely adhered to the cell wall (**Figures 4.10 and 4.11**). As a result of water stress, the plasma membrane detached from the cell wall, forming an apoplastic space. In response to increased water stress, the space between the plasma membrane and the cell wall became larger. Plasma membrane detachment, however, was less pronounced in microbial-inoculated plants, resulting in a smaller cell wall-plasma membrane gap. At all levels of water stress, PGPB-inoculated plants showed comparatively less plasma membrane separation from the cell wall than AMF and *P. indica*. Furthermore, at higher water stress levels, invaginations of the plasma membrane led to the formation of vesicles in the cytoplasm. Compared with their microbial-inoculated counterparts, uninoculated plants displayed less vesicle formation.

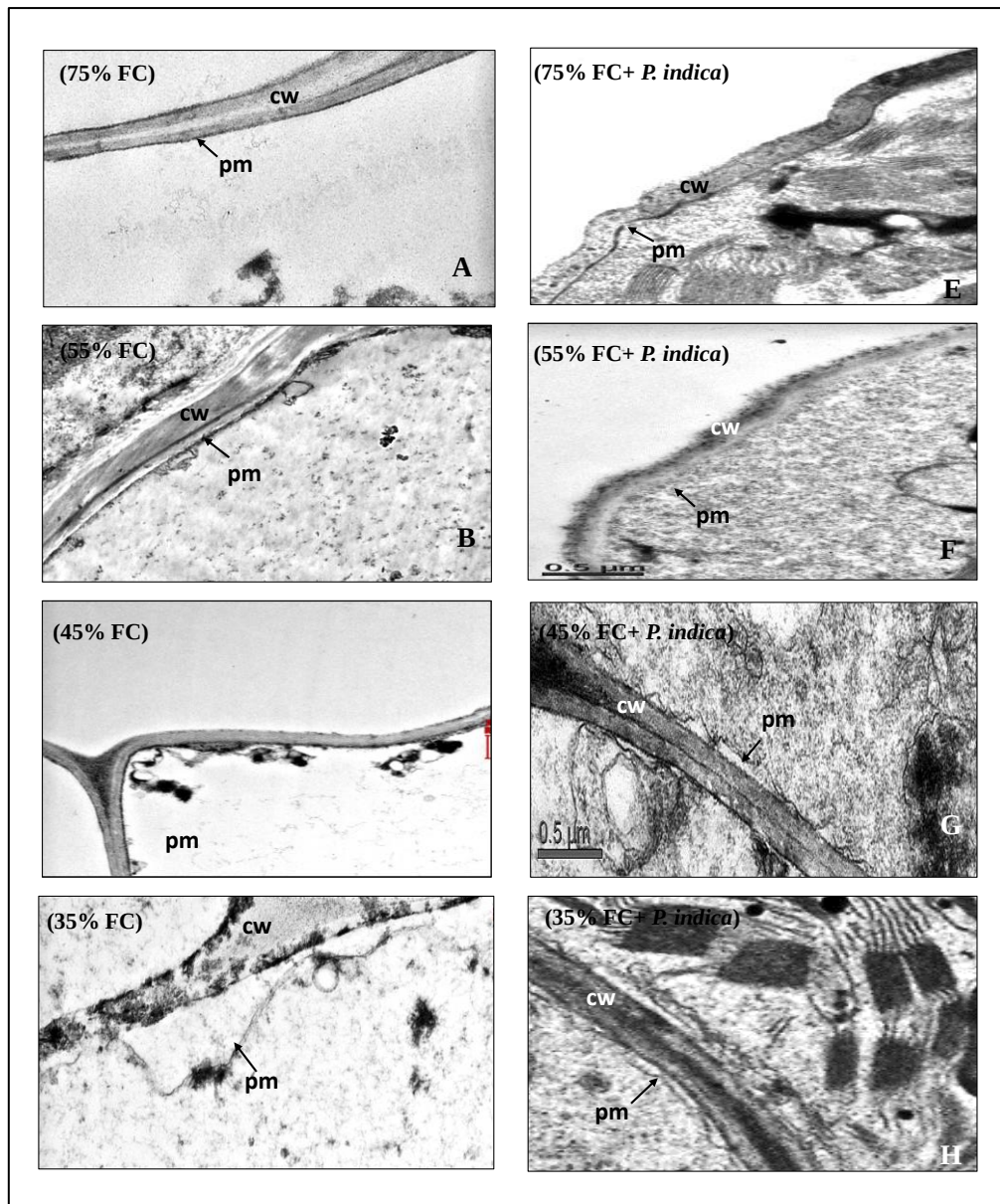


Figure 4.10: Transmission electron micrographs of the cell wall and plasma membrane in mesophyll cells of (A) Control wheat plants not exposed to drought stress (75% FC); plasma membrane closely appressed to the cell wall. (B) Control wheat plants subjected to 55% FC stress; receding plasma membrane. (C) Control wheat plants exposed to 45% FC stress; increased space between the plasma membrane and cell wall; vesicles initiated. (D) Control wheat plants subjected to 35% FC stress; tremendous increase in the apoplastic space between the plasma membrane and cell wall. (E) *P. indica* inoculated wheat plants not exposed to drought stress (75% FC); well-preserved plasma membrane in close association with the cell wall. (F) *P. indica* inoculated wheat plants subjected to 55% FC stress; plasma membrane in close association with the cell wall. (G) *P. indica* inoculated wheat plants subjected to 45% FC stress; incipient plasmolysis. (H) *P. indica* inoculated wheat plants subjected to 35% FC stress; a slight increase in the space between the cell wall and plasma membrane. The values in the bracket denote the level of water regimes imposed on plants. cw = cell wall; pm = plasma membrane; v = vesicle.

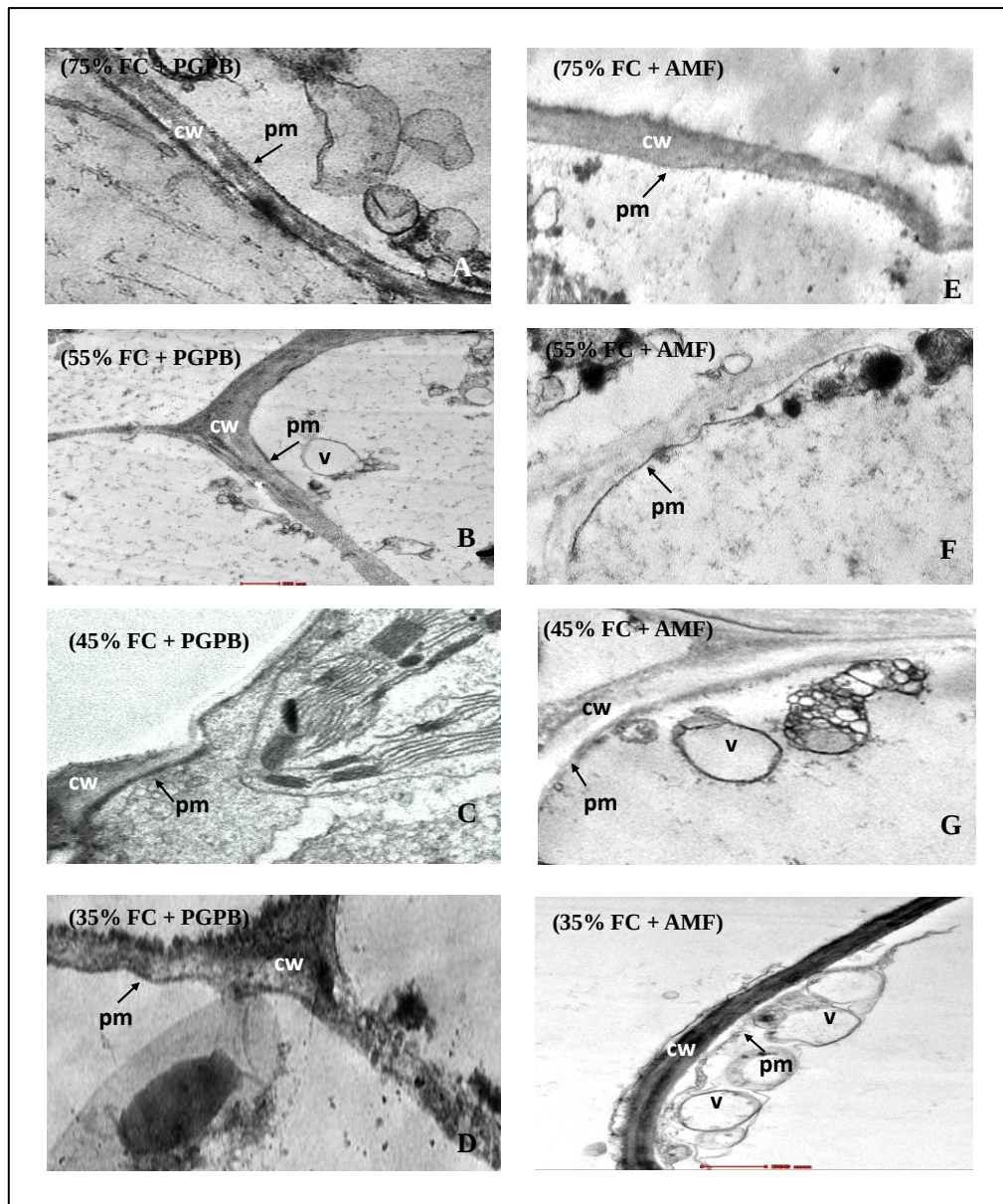


Figure 4.11: Transmission electron micrographs of the cell wall and plasma membrane in mesophyll cells of (A) PGPB-inoculated wheat plants not exposed to drought stress (75% FC); plasma membrane closely appressed to the cell wall. (B) PGPB-inoculated wheat plants subjected to 55% FC stress; plasma membrane closely appressed to the cell wall. (C) PGPB-inoculated wheat plants exposed to 45% FC; plasma membrane well preserved. (D) PGPB-inoculated wheat plants subjected to 35% FC stress; plasma membrane structure still intact. (E) AMF-inoculated wheat plants not exposed to drought stress (75% FC); well-preserved plasma membrane in close association with the cell wall. (F) AMF-inoculated wheat plants subjected to 55% FC; well-preserved plasma membrane in close association with the cell wall. (G) AMF-inoculated wheat plants subjected to 45% FC stress; plasma membrane slightly shrank from the cell wall; vesicle formation is seen. (H) AMF-inoculated wheat plants subjected to 35% FC stress; plasma membrane slightly detached from the cell wall. The values in the bracket denote the level of water regimes imposed on plants. cw = cell wall; pm = plasma membrane; v = vesicle.

Table 4.3: Effect of drought, *P. indica*, drought × *P. indica*, PGPB, drought × PGPB, AMF, drought × AMF on physiological processes, photosynthetic pigments, protein, α-tocopherol, membrane damage, osmolytes contents, and antioxidant enzyme activity in wheat plants.

Parameters	Drought	<i>P. indica</i>	Drought × <i>P. indica</i>	PGPB	Drought × PGPB	AMF	Drought × AMF
Photosynthetic rate	***	***	ns	***	*	***	ns
Transpiration	***	***	***	***	*	***	*
Stomatal conductance	***	***	ns	***	ns	***	ns
Internal CO ₂	***	***	ns	***	***	***	**
Chlorophyll a	***	***	***	***	***	***	***
Chlorophyll b	**	*	ns	***	*	***	ns
Total chlorophyll	***	***	***	***	***	***	***
Carotenoid	ns	***	*	***	ns	***	ns
Protein	***	***	ns	***	*	***	ns
α-tocopherol	***	***	ns	***	***	***	***
Lipid peroxidation	***	***	ns	***	***	***	***
Relative permeability	***	***	**	***	***	***	***
LOX	***	***	ns	***	*	***	**
Trehalose	***	***	***	***	***	***	***
Total sugars	***	***	ns	***	ns	***	**
Proline	***	***	*	***	*	***	**
Glycine betaine	***	***	ns	***	ns	***	ns
Putrescine	***	***	***	***	***	***	***
Spermidine	***	***	***	***	***	***	***
SOD	***	***	ns	***	ns	***	ns
CAT	***	***	**	***	***	***	***
APX	***	***	ns	***	***	***	***

Significant interactions at $P < 0.05$. ns: not significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. AMF, arbuscular mycorrhizal fungi; PGPB, plant growth-promoting bacteria; LOX, lipoxygenase; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase.

4.1.2 Discussion

In the present study, we observed that the ability of AMF and *P. indica* to colonize wheat roots decreased with increasing drought stress levels. The results are consistent with previous studies in which drought stress reduces hyphal growth and decreases mycorrhizal colonization (Ostadi et al. 2022). The colonization of AMF and *P. indica* in

wheat plants decreased under severe drought-stress conditions. This resulted in a decrease in shoot and root biomass, suggesting that the beneficial effects of AMF and *P. indica* are dependent on the colonization of roots.

Both microbial-inoculated and uninoculated wheat plants showed reduced plant growth under drought stress. This finding may be explained by reduced nutrient uptake and photosynthesis in the plant due to drought stress (Ullah and Farooq 2022). However, drought-stressed wheat plants inoculated with PGPB, AMF, and *P. indica* exhibited larger shoots and root growth, and greater biomass than uninoculated plants. The results of our study are in agreement with earlier reports on AMF colonization in *zea mays* (Ahmed et al. 2023), and *Vitis vinifera* (Karimi and Noori 2022). AMF was instrumental in enhancing the shoot and root elongation of *Linum usitatissimum* in response to salinity stress (Kakabouki et al. 2023). The experimental findings substantiated that the introduction of AMF resulted in a noteworthy amelioration of growth parameters in wheat, including root and shoot length, as well as the fresh and dry weight of both root and shoot tissues when exposed to salinity stress conditions (Huang et al. 2023). The application of a compound inoculum consisting of *Funneliformis mosseae*, along with two functional microorganisms, namely *P. indica* and *Agrobacterium rhizogenes*, exhibited a stimulatory effect on the root and shoot elongation of rice plants subjected to salinity stress (Zhang et al. 2023).

Based on the results of a study by Ghabooli and Kaboosi (2022), inoculating *Solanum lycopersicum* with *P. indica* under water stress conditions enhanced root and shoot biomass and root volume. We found that microbial-inoculated wheat plants had higher leaf area than uninoculated plants under drought stress. Oliveira et al. (2022) reported that AMF-inoculated soybean plants had increased leaf area as compared to uninoculated plants. The improved nutrient uptake (Rajput et al. 2022), increased osmolyte accumulation and antioxidant activity, and decreased relative permeability led to improved drought tolerance and increased plant growth in microbial-inoculated plants (Tyagi et al. 2023). On comparing the potential of microbes to increase growth parameters, PGPB-inoculated wheat plants showed higher drought resistance as compared to AMF and *P. indica*-inoculated wheat plants.

Our study showed that the nutrient contents of both microbial inoculated and uninoculated wheat plants decreased with the increased level of drought. This is because of decreased root development and less water availability during drought, plants cannot absorb essential nutrients. However, at all levels of drought stress, microbial inoculation provided better nutrient acquisition than uninoculated plants. It is believed that the increased absorption surface of extraradical hyphae is responsible for enhancing nutrient status in AMF and *P. indica* inoculated plants (Ortiz et al. 2015). In plant growth and development, nutrients play a vital role. Calcium is essential for membrane integrity, cell wall stability, and ion transport (de Bang et al. 2021). Drought induces Ca^{2+} deficiency in plants. During drought, Na^+ replaces Ca^{2+} in the membranes and cell walls, reducing cell turgidity and hydraulic conductivity. Our study showed that microbial-inoculated plants were better at absorbing Ca^{2+} ions as has also been observed by Devarajan et al. (2021).

K^+ maintains osmotic and membrane potential (Sardans and Peñuelas 2021) and regulates the synthesis of proteins, sugars, and enzyme activity (Hasanuzzaman et al. 2018). We found that drought treatment reduced K^+ levels both in inoculated and uninoculated wheat plants. As Na^+ and K^+ have similar physiological properties, they are inevitable competitors. Consequently, cytoplasmic Na^+ competes with K^+ for binding sites, inhibiting metabolic processes that rely on K^+ . However, microbial-inoculated wheat plants showed higher uptake of K^+ ions than uninoculated plants. According to our findings, higher osmolyte accumulation, protein synthesis, and antioxidant enzyme activity can be attributed to high K^+ accumulation (Hasanuzzaman et al. 2018) in microbial-inoculated plants under drought stress.

Magnesium is an essential component of chlorophyll (Tang et al. 2023), and it is required for photosynthesis and protein synthesis (Kleczkowski and Igamberdiev 2021). It was reported that drought stress reduced chlorophyll content and photosynthesis in plants, but PGPB and *P. indica* inoculation increased these parameters (Inbaraj 2021). Similarly, drought reduced Mg^{2+} uptake in wheat, but *Azospirillum* alleviated this effect. We found that drought and microbial inoculation significantly affected Mg^{2+} concentration in wheat plant tissues. This may be attributed to the low availability of Ca^{2+} under drought stress. Ca^{2+} is a strong competitor of Mg^{2+} (Pandey and Sanyal 2021) and has more affinity for plasma membrane sites than Mg^{2+} . In our study, higher

chlorophyll content and photosynthesis can also be correlated with increased Mg^{2+} content in microbial-inoculated wheat plants under drought stress.

There was a rectilinear increase in Na^+ content in wheat plants in both microbial-inoculated and uninoculated plants. However, uninoculated wheat plants had higher Na^+ contents compared to PGPB, AMF, and *P. indica* which implies that symbioses control Na^+ uptake. AMF excludes Na^+ by discriminating its uptake from soil or plant transfer. The findings indicate that microbes influence Na^+ translocation to the aerial parts. Through exopolysaccharide binding to sodium ions in the soil, PGPB can confer drought tolerance to plants by decreasing sodium absorption from the soil and increasing nutrient uptake by roots (Bhagat et al. 2021). Low Na^+ content found in AMF-treated roots can be attributed to the ability of the fungus to retain Na^+ in intraradical hyphae or compartmentalize it in root cell vacuoles. The mycorrhizal symbiosis enhanced plant growth and physiological process, thereby diluting these ions in tissues.

In this study, drought stress and microbial inoculation were investigated for their impact on micronutrient uptake in wheat plants. Unfortunately, most plant nutrient studies under drought stress have paid little attention to micronutrients. Micronutrients are essential for plant growth and development. Zn^{2+} plays a crucial role in the synthesis of proteins, chlorophyll, and enzymes (Kumar et al. 2021). Chlorophyll synthesis and chloroplast structure and function require Fe (Kumar et al. 2021). In plant cells, copper plays a vital role in photosynthesis, respiration, redox reactions, and the maintenance of cell wall structure (Kumar 2015a). In photosystem II, Mn^{2+} contributes to the structure of the water-splitting system which provides electrons for photosynthesis. In plants, drought reduces micronutrient uptake. We observed that with an increase in drought levels, the concentrations of Cu, Fe, Mn^{2+} , and Zn^{2+} in microbial-inoculated and uninoculated wheat plants decreased. This is because Cu, Fe, and Zn^{2+} are less soluble and mobile in soil solutions during drought conditions. With the increasing drought, Cu, Fe, and Zn^{2+} levels decrease, and a depletion zone forms around the roots. Because of this, uninoculated plants have limited access to these nutrients. In addition, decreases in Cu, Zn^{2+} , Mn^{2+} , and Fe in microbial-inoculated wheat plants may be due to decreased hyphal growth of AMF and *P. indica* during drought. However, at each level of drought stress, microbial inoculation exhibited better micronutrient acquisition than

uninoculated wheat plants. It is consistent with earlier studies showing an increase in Zn^{2+} , Fe, and Cu content following mycorrhizal inoculation. In our study, higher chlorophyll content and photosynthesis can also be correlated with increased micronutrient content in microbial-inoculated wheat plants under drought stress.

Photosynthesis is integral to plant growth and is, therefore, an important indicator of a plant's potential for growth and drought tolerance (Liang et al. 2019). In response to drought stress, stomatal closure decreases gas exchange and internal CO_2 levels, resulting in a decrease in photosynthetic pigment production, thereby reducing the photosynthetic rate (Liang et al. 2019), which was further confirmed in the present study. Moreover, the transpiration rate decreases during drought leading to reduced nutrient and water uptake in wheat plants. In contrast, drought-stressed wheat plants inoculated with PGPB, AMF, and *P. indica* had an increase in photosynthetic rate, stomatal conductance, internal CO_2 , and transpiration rate. Our results are in agreement with previous reports on PGPB inoculation improved plant photosynthetic efficiency by increasing the CO_2 assimilation rate, stomatal conductance, and transpiration rate (Vishnupradeep et al. 2022), and reducing structural damages to the photosynthetic apparatus by enhancing osmolyte accumulation in plants. Additionally, the results indicated that microbial inoculation significantly increased the carotenoid content in wheat plants. The antioxidant property of carotenoids protects the photosynthetic apparatus against free radical damage, inhibits lipid peroxidation, stabilizes cell membranes, and plays a critical role in light-harvesting complex assembly (Farooq et al. 2009). Increased photosynthesis rates are a result of higher chlorophyll content and leaf area in wheat plants.

As light-absorbing pigments, chlorophylls and carotenoids are crucial for photosynthesis in wheat plants. Drought decreases chlorophyll and carotenoid content (Khazaei et al. 2020), which is consistent with our findings. It was observed that PGPB, AMF, and *P. indica* inoculated wheat plants had higher chlorophyll a and chlorophyll b levels, total chlorophyll and carotenoid levels than uninoculated wheat plants. This suggests that microbial inoculation reduces the impact of drought on wheat. In a similar study, an AMF substantially increased the chlorophyll and carotenoid content of *Cupressus arizonica* (Aalipour et al. 2023). A study found that maize plants colonized

with PGPB had more chlorophyll and carotenoid content (Vishnupradeep et al. 2022). Our studies are in line with earlier studies suggesting higher photosynthetic pigments are probably a consequence of antioxidant accumulation, protecting the chloroplast structure, and increasing the solubilization and bioavailability of organic minerals, such as Mg (Khan et al. 2019). Our results coincide with the findings that *Bacillus* strains increase chlorophyll levels in maize due to increases in nutrient balance (Moreno-Galván et al. 2020).

Drought stress is associated with a significant accumulation of osmolytes, including carbohydrates (glucose and trehalose), amino acids (proline), quaternary ammonium compounds (glycine betaine), and polyamines (putrescine, spermidine, and spermine), which play a critical role in maintaining cell turgor pressure and stabilizing membranes, enzymes, and proteins (Sharma et al. 2019). Furthermore, osmolytes control ROS levels, provide energy for stress management, help repair processes, and support growth (Silva et al. 2018). As a highly stable molecule, trehalose is resistant to high temperatures and acids; it can prevent protein aggregation and degradation during droughts (Jalili et al. 2009). Proline is an important secondary amino acid that has a major role in osmotic regulation and scavenging ROS under drought stress (Adejumo et al. 2021). We observed significantly higher osmolyte accumulation in both microbial inoculated (PGPB, AMF, and *P. indica*) and uninoculated wheat plants. However, microbial-inoculated wheat plants had a greater amplitude of osmolyte increase than uninoculated plants. The results of this study are consistent with a previous study that demonstrated increased proline and sugar content in maize inoculated with the microbial consortium under drought stress (Hashem et al. 2016). *Acinetobacter*, *Calcoaceticus*, and *Penicillium* sp. effectively ameliorated drought-induced stress in foxtail millet by increasing glycine betaine, proline, and sugar accumulation (Kour et al. 2020) by maintaining cell turgidity and membrane integrity. According to Liu et al. (2022), rhizobium symbiosis enhances drought tolerance in *Astragalus sinicus* through the production of putrescine, spermidine, and spermine. Based on these results, microbial-inoculated wheat plants are more efficient at osmotic adjustment than their uninoculated counterparts. However, PGPB outpaces AMF and *P. indica* in osmotic adjustment in wheat plants under drought stress.

Proteins are organic molecules that form the structural and functional units of a cell. These compounds improve chlorophyll content, protect chloroplast structure, activate ROS scavenging, and enhance osmolyte synthesis (Riyazuddin et al. 2022). In our study, there was a remarkable difference in protein content in microbial inoculated and uninoculated wheat plants under all levels of drought stress. Wheat plants inoculated with PGPB, *P. indica*, and AMF had higher protein content. In microbial inoculated wheat plants, higher protein content is associated with higher photosynthetic rates, chlorophyll contents, membrane integrity, and osmolyte accumulation. Similarly, Boutasknit et al. (2020) found that drought stress could be mitigated by AMF-colonized carob plants with higher levels of protein. According to this study, inoculating wheat plants with PGPB, *P. indica*, or AMF enhanced drought tolerance through protein regulation.

Since α -tocopherol is an antioxidant, it inhibits ROS generation during photosynthesis (mainly $^1\text{O}_2$ and $\text{OH}\cdot$) and reduces lipid peroxidation (Kim et al. 2019). In our study, wheat plants inoculated with PGPB, *P. indica*, and AMF have a high level of α -tocopherol, which indicates that α -tocopherol is a free radical scavenger as well as a modulator of lipid peroxidation during abiotic stress (Kim et al. 2019). This study substantiates the findings of Fresno et al. (2023) that higher levels of α -tocopherol in AMF colonized *Trifolium repens* plants mitigate drought stress. Based on the current study, inoculating wheat plants with PGPB, *P. indica*, and AMF enhanced drought tolerance by regulating α -tocopherol content, thus maintaining a high level of photosynthetic efficiency and chlorophyll content and reducing ion leakage.

A key physiological indicator of drought in plants is malondialdehyde, a by-product of membrane lipid peroxidation (Yasmeen et al. 2022). Lipid peroxidation alters cell membrane ultrastructure and permeability in wheat plants. Additionally, the LOX enzyme converts lipids into hydroperoxyl fatty acids and releases free radicals that damage cells. Prevention of lipid peroxidation and relative permeability are key factors in drought tolerance (Kour et al. 2020). We observed a rectilinear increase in lipid peroxidation, relative membrane permeability, and LOX activity in wheat plants inoculated with microbes (PGPB, AMF, and *P. indica*) and uninoculated plants with increasing stress levels. However, the amplitude of the increase in lipid peroxidation and electrolyte leakage in microbial inoculated wheat plants was lower than that of

uninoculated plants, which is in accordance with earlier reports (Begum et al. 2022). The reduced LOX activity in microbial inoculated wheat plants is inconsistent with a previous report in which the co-inoculation of AMF and PGPB resulted in an increase in LOX activity in tobacco plants (Begum et al. 2022). In our study, the microbial inoculated wheat plants maintained a lower MDA concentration and relative membrane permeability by regulating adaptive responses such as the synthesis of non-enzymatic molecules (carotenoids, proline, and α -tocopherol) and enzymatic antioxidants (CAT, APX, and SOD). These findings are similar to previous findings that PGPB-inoculated plants showed improved drought stress tolerance by maintaining a high level of carotenoids, proline, and tocopherol (Timmusk et al. 2014), as well as CAT, APX, and SOD activity (Chiappero et al. 2019), thereby reducing ROS accumulation.

During unfavourable conditions, antioxidant enzymes (CAT, SOD, and APX) actively protect the plant cell from oxidative bursts and assist in scavenging ROS during adverse environmental conditions (Ru et al. 2023). The SOD enzyme plays a critical role in protecting the plant against oxidative stress by converting $O_2^{\cdot -}$ to H_2O_2 . In order to detoxify H_2O_2 , it must be converted into H_2O . SOD produces H_2O_2 that is scavenged by CAT and attenuates oxidative stress on the cell. In our study, the antioxidant enzyme activity of uninoculated and inoculated wheat plants differed significantly. This result is consistent with an earlier study demonstrating that microbial inoculation improved plant growth under drought stress because of the enhanced activity of the ROS-scavenging antioxidant enzyme (Tyagi et al. 2023). In the study conducted by Wen-Ya et al. (2022), it was observed that AMF triggers a reduced oxidative burst in drought-stressed walnut plants by stimulating the activation of antioxidant defense systems. Thus, microbial inoculation reduces lipid peroxidation and membrane permeability in plants by increasing antioxidant enzyme activity (Tyagi et al. 2023). Earlier studies have found that microbial consortium-treated maize exhibited increased antioxidant enzyme activity in stress conditions (Abbas et al., 2020), which lends weight to our study. Tyagi et al. (2023) reported that deploying a microbial consortium of *Serendipita indica*, *Rhizophagus intraradices*, and *Azotobacter chroococcum* boosts drought tolerance in maize by enhancing the activity of antioxidant enzymes. In our study, CAT activity in AMF-inoculated plants decreased with increasing levels of drought, which is consistent

with previous findings that PGPB reduced CAT activity during drought stress in maize (Siddique et al. 2022). The current study proposes the use of PGPB, AMF, and *P. indica* to enhance antioxidant defense mechanisms in wheat and reduce drought stress. Future agriculture practices may benefit from the use of microbes as a bio-inoculant for promoting plant growth. The chloroplast is the most susceptible cell organelle to drought stress (Gunasekera and Ratnasekera 2023).

The present study illustrated that the ultrastructure of both microbial-inoculated and uninoculated wheat plants was affected by drought stress. Under low drought stress, the thylakoid membrane contracted thereby reducing the thylakoid lumen. Thylakoid lumen contraction may be a consequence of osmotic collapse (Semenova 2002). As the drought level increased, the lumen swelled, resulting in the destacking of grana. Several studies have shown that osmotic stress causes thylakoid swelling and membrane damage (Aldesuquy et al. 2018). Microbial inoculated plants, however, seem to have less chloroplast damage than uninoculated plants. This may be attributed to more accumulation of osmolytes (glycine betaine, polyamines, and sugars) in microbial-inoculated plants. More accumulation of osmolytes prevents osmotic collapse, while polyamines inhibit membrane degradation by interacting with negatively charged membrane sites in grana and stroma thylakoids (Ali et al. 2020). In our study, microbial-inoculated plants showed reduced chloroplast membrane damage, possibly due to larger plastoglobule sizes that prevented oxidative damage. It has been reported that plastoglobules increase in size during drought (Zhu et al. 2021). Plastoglobules are lipoprotein bodies present in the chloroplast and function as sites for α -tocopherol synthesis under oxidative stress (Brehelin et al. 2007). In our study, microbial-inoculated plants had higher levels of α -tocopherols than uninoculated plants. α -tocopherols have been reported to protect membrane lipids and photosystem II against photooxidation. In microbial-inoculated plants, plastoglobule accumulation may be attributed to higher polyamine levels (Shu et al. 2015). Thus, in this study, microbial-inoculated plants had reduced damage to the ultrastructure of chloroplast due to high levels of osmolytes and large plastoglobules that prevented oxidative damage.

Cell membrane stability in plants is considered a reliable indicator of drought tolerance (Abobatta 2019). Electron micrographs showed that plasma membranes disintegrated

under drought stress. With increasing drought levels, the distance between the plasma membrane and cell wall increases. A similar finding was reported for *Ormosia hosiei* seedlings (Liu and Wei 2021). The disintegration of membranes occurs due to the peroxidation of polyunsaturated fatty acids by free radicals (Zhang et al. 2021) and the shrinkage of protoplasm during drought conditions. Compared to uninoculated plants, however, microbial-inoculated plants showed less membrane damage and protoplasm shrinkage. This statement is further strengthened by the accumulation of osmolytes (glucose, trehalose, proline, glycine betaine, and polyamines), which maintain cell turgor pressure and stabilize membranes, enzymes, and proteins (Sharma et al. 2019). We found lower levels of electrolyte leakage, lipid peroxidation, and LOX activity in microbial-inoculated plants, which play a significant role in membrane protection. This is because microbial-inoculated plants acquire more non-enzymatic antioxidant molecules (proline, α -tocopherols, and polyamines), enzymatic antioxidants (SOD, CAT, APX), and nutrients (calcium and potassium) compared with uninoculated plants. Proline is an effective ROS scavenger, protecting cells from damage by oxidative stress under a stressful environment (Ashraf and Foolad 2007). Moreover, α -tocopherol protects host tissue from lipid peroxidation and maintains the integrity of their membrane by scavenging free radicals (Niki 2021). Polyamines provide stability to membranes (Wang et al. 2022). In adverse environmental conditions, antioxidant enzymes (CAT, SOD, and APX) scavenge ROS and protect membranes from oxidative stress (Ru et al. 2023). By forming intermolecular links, calcium maintains the structural integrity of plant membranes, stabilizes cell walls, and regulates ion transport and selectivity (de Bang et al. 2021). Potassium plays a vital role in plants, including osmoregulation and membrane potential (Sardans and Peñuelas 2021). So, microbial inoculation reduces membrane damage through osmolyte, nutrient, and antioxidant-mediated reductions of lipid peroxidation in plants (Tyagi et al. 2023). Vesicle formation was more in microbial inoculated as compared to uninoculated plants. Vesicle formation increases under stress conditions (Karim et al. 2014). They increase the membrane surface area, which facilitates ion transport and storage, thus protecting the cells from ionic imbalance. Thus, more vesicle formation observed in this study indicates a better ability of microbial-inoculated plants to tolerate droughts.

4.2 PART II

To study the role of soil microbes on growth, changes in nutritional, biochemical and physiological parameters, ultrastructural changes, and antioxidant accumulation in wheat under salinity stress.

To accomplish the objective, the percentage of AMF and *P. indica* colonization, plant growth and biomass, macronutrients and micronutrients content, rate of physiological processes, photosynthetic pigments content, osmolytes content, lipid peroxidation, relative permeability, LOX activity, protein content, and α -tocopherol content was estimated in microbial inoculated and uninoculated wheat plants under salinity stress.

4.2.1 Results

4.2.1.1 Microbial colonization

AMF and *P. indica* successfully colonized wheat plants' roots at all salinity stress levels. However, the percentage of colonization was higher in AMF-inoculated wheat plants than in *P. indica*-inoculated plants during salinity stress. No microbial colonization was observed in roots not inoculated with AMF and *P. indica* (**Figure 4.12**). Root colonization rates were significantly affected by different levels of salinity stress. Increased salinity levels caused a gradual decline in microbial colonization (**Table 4.4**).

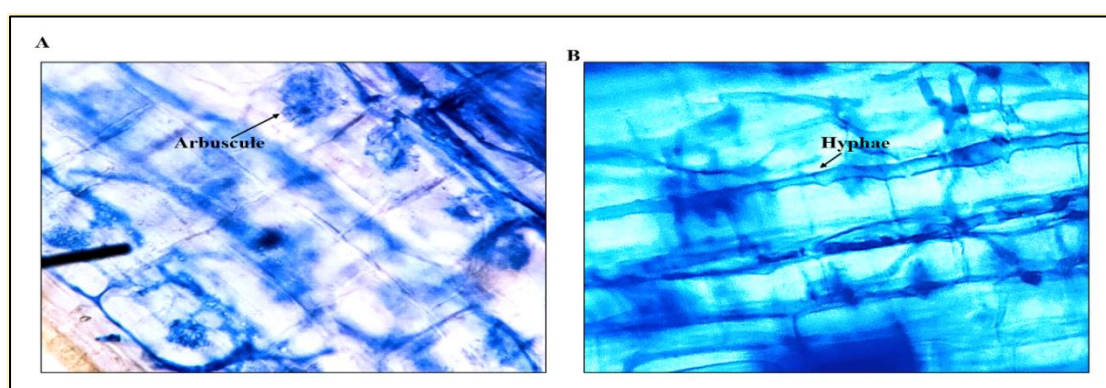


Figure 4.12: Microscopic observation of root colonization of (A) AMF-inoculated wheat plants; (B) *P. indica* inoculated wheat plants under salinity stress.

4.2.1.2 Growth parameters

Table 4.4 shows the growth parameters of microbial inoculated and uninoculated wheat plants under four different levels of salinity stress.

Table 4.4: Influence of salinity stress and microbe inoculations on percentage microbe colonization, length, and biomass of shoot and root in wheat plants

NaCl (mM)	Microbe	Shoot length (cm)	Root length (cm)	Shoot biomass (g)	Root biomass (g)	Leaf area (cm ²)	Root colonization (%)
0	Control	59.66±0.34gh	27.70±2.07cd	5.02±0.75gh	4.53±0.13h	18.95±0.63f	-
	<i>P. indica</i>	65.53±2.42bcd	31.00±1.65bcd	6.77±0.10de	5.96±0.39cde	23.18±0.24cd	70
	PGPB	69.60±0.54ab	37.29±1.51ab	7.70±1.13bc	6.80±0.42b	28.10±0.72b	-
	AMF	67.83±1.91b	33.46±0.57ab	7.60±0.11bc	5.81±0.41de	26.30±0.16b	73
50	Control	61.10±0.27g	29.70±0.61cd	6.69±0.28de	5.50±0.58ef	21.09±1.19ef	-
	<i>P. indica</i>	66.86±1.64ab	32.43±1.82bcd	7.84±0.08b	6.37±0.18bc	25.25±0.45de	62
	PGPB	74.66±1.76a	37.56±1.18a	9.14±0.74a	7.61±0.11a	28.77±0.38a	-
	AMF	68.23±0.07b	34.66±1.14a	8.84±0.14a	6.64±0.46b	26.57±0.62b	65
100	Control	57.26±0.93hi	25.43±0.22de	4.70±0.27hi	4.27±0.21hi	17.27±0.70g	-
	<i>P. indica</i>	64.03±3.83de	27.83±1.69cd	5.51±0.27g	5.22±0.14f	22.81±0.57ef	55
	PGPB	67.00±3.97bc	33.63±1.80ab	7.22±0.15cd	6.14±0.07cd	25.83±0.48b	-
	AMF	66.20±0.42cd	31.33±1.72bc	7.15±0.13cd	5.72±0.08de	24.43±0.27c	58
200	Control	52.93±0.82i	23.16±1.12e	4.39±0.25i	4.00±0.11i	13.83±0.64h	-
	<i>P. indica</i>	58.80±3.08fg	26.16±0.80cd	5.14±0.05gh	4.69±0.11gh	19.13±0.66fg	34
	PGPB	66.00±2.89de	30.27±1.87bc	6.55±0.08ef	5.90±0.12cde	24.37±0.52c	-
	AMF	63.50±0.40ef	29.47±0.61bcd	6.09±0.05f	5.13±0.02fg	22.97±0.85de	37
Significance							
NaCl		**	**	ns	ns	***	
<i>P. indica</i>		***	***	ns	ns	***	
NaCl × <i>P. indica</i>		ns	ns	ns	ns	**	
PGPB		***	***	ns	ns	***	
NaCl × PGPB		ns	*	*	**	***	
AMF		***	***	ns	ns	***	
NaCl × AMF		ns	**	ns	ns	***	

Values represent averages across replicates. Letters within a column represent significant differences at P<0.05. ns: not significant. ***P<0.001, **P<0.01, *P<0.05.

Plants inoculated with microbes performed significantly better than uninoculated plants at all salinity levels in terms of shoot and root length, shoot and root biomass, and leaf area. PGPB inoculation improved shoot and root length by 1.25 and 1.31-fold respectively under severe salinity conditions (200 mM NaCl) while AMF inoculation improved them by 1.2 and 1.3-fold, respectively. In contrast to uninoculated plants, plants inoculated with *P. indica* exhibited 1.11 and 1.13-fold increases, respectively. PGPB-inoculated plants displayed increases of 1.5-fold in shoot and root biomass at 200 mM NaCl stress. In comparison to uninoculated plants, AMF inoculation raised it by 1.4 and 1.3-fold respectively, and *P. indica* inoculation raised it by 1.2-fold. PGPB significantly increased the leaf area of wheat plants by 1.8-fold followed by AMF (1.7-fold) and *P. indica* (1.4-fold) in comparison to uninoculated wheat plants. There was a significant independent effect of salinity, *P. indica*, PGPB, and AMF on plant growth, biomass, and leaf area in the two-way ANOVA (**Table 4.4**). When comparing the ability of microbes to increase growth parameters, PGPB-inoculated plants showed higher salinity resistance as compared to AMF and *P. indica*-inoculated plants.

4.2.1.3 Mineral nutrients content

Table 4.5 shows the macronutrients and micronutrient content of wheat plants with respect to salinity stress and microbial inoculation. Microbial inoculation and salinity stress had significant effects on leaf macronutrient (Ca^{2+} , Mg^{2+} , K^+ , Na^+) and micronutrient (Zn^{2+} , Fe, Mn^{2+} , and Cu) contents. Ca^{2+} levels decreased linearly as salinity stress levels increased both in microbial-inoculated and uninoculated plants. The presence of microbes, however, led to higher Ca^{2+} levels than those in plants without microbes. The Ca^{2+} content of plants treated with PGPB, *P. indica*, and AMF was 2.55, 1.16, and 2.46-fold higher than that of uninoculated plants at 200 mM NaCl. In comparison with *P. indica* and AMF-inoculated plants, PGPB is more effective at alleviating salinity stress based on Ca^{2+} content at maximum salinity levels.

Mg²⁺ uptake in wheat leaves was affected by microbe inoculation and salinity stress. Mg²⁺ levels decreased in wheat plants as salinity levels increased. However, the Mg²⁺ levels of plants inoculated with microbes were higher than those of uninoculated plants at the same salinity level (**Table 4.5**). Mg²⁺ content decreased gradually with increasing salinity levels in both microbial-inoculated and uninoculated wheat plants. When plants were inoculated with PGPB, *P. indica*, and AMF, their Mg²⁺ contents were 2.3, 1.7, and 1.9-fold greater than those of uninoculated plants. In all treatments, plants inoculated with PGPB had higher Mg²⁺ content in leaves compared to those inoculated with *P. indica* and AMF.

The K⁺ content of wheat plants declined as salinity stress increased in both microbial-inoculated and uninoculated wheat plants. Wheat plants inoculated with microbes, however, maintained higher K⁺ levels at the same level of salinity stress (**Table 4.5**). In plants inoculated with PGPB, *P. indica*, and AMF, K⁺ content was 2.12, 1.8, and 1.9-fold higher than those without inoculation at 200 mM NaCl. All treatments showed that microbial-inoculated plants had higher leaf K⁺ content than uninoculated plants.

A linear increase in sodium content was observed in the wheat leaves of both microbial inoculated and uninoculated plants with increasing salinity stress levels (**Table 4.5**). Microbial inoculated plants, however, possess substantially less Na⁺ than uninoculated plants at all levels of salinity stress. In addition, the Na⁺ content of PGPB-inoculated plants was 26.18% lower than that of control plants, followed by AMF (21.75%) and *P. indica* (19.41%) colonized plants. As a result, PGPB has a greater ability to alleviate salinity stress than *P. indica* and AMF-inoculated plants at all levels of salinity.

Salinity negatively affected Zn²⁺ accumulation in wheat plants (**Table 4.5**). A significant decrease in Zn²⁺ levels was observed in microbial-inoculated plants as well as control plants under salinity stress. Conversely, plants inoculated with microbes maintained higher levels of Zn²⁺ under similar salinity conditions. At 200 mM NaCl,

PGPB, and *P. indica* inoculated plants had 2.6, and 1.9-fold higher Zn^{2+} content than controls. Regardless of salinity level, AMF colonization did not affect Zn^{2+} content, and its value was lower than the control. PGPB is more effective in alleviating salinity stress at all levels of salinity stress than *P. indica* inoculated plants. Salinity stress and microbial inoculation significantly enhanced Zn^{2+} content.

There was a substantial decrease in Fe levels in the leaves of both microbial inoculated and uninoculated wheat plants under salinity stress. In contrast, microbial-inoculated plants maintained higher levels of Fe in response to the same level of salinity stress than their counterparts (**Table 4.5**). At 200 mM NaCl, *P. indica*, PGPB, and AMF-inoculated plants increased their Fe content by 2.14, 2.48, and 1.9-fold, respectively. Salinity stress is alleviated more effectively by PGPB than by *P. indica* and AMF-inoculated plants.

In both microbial inoculated and uninoculated plants, Mn^{2+} content decreased significantly with increased salinity stress. PGPB, *P. indica*, and AMF-inoculated wheat plants, however, maintained higher levels of Mn^{2+} at the same salinity level than their counterparts (**Table 4.5**). Mn^{2+} content increased by 52.52% in *P. indica*-inoculated plants compared to controls at the highest salinity treatment. Plants inoculated with PGPB and AMF showed an increase in Mn^{2+} by 91.68% and 74.54%, respectively, at 200 mM NaCl. In terms of Mn^{2+} content, PGPB plants can alleviate salinity stress better than *P. indica* and AMF plants.

In wheat plants, salinity stress negatively affected Cu accumulation (**Table 4.5**). There was a significant decrease in Cu levels in leaves of both inoculated and control plants when exposed to salinity stress. Microbe-inoculated plants, however, showed higher levels of Cu at the same salinity stress level than their counterparts. Regardless of salinity level, PGPB colonization did not affect Cu content, and its value was lower than the control. In contrast to controls, *P. indica*, and AMF-inoculated plants increased their Cu concentrations 1.11 and 1.5-fold at 200 mM NaCl, respectively.

Table 4.5: Influence of salinity stress and microbial inoculation on the content of macro-nutrients and micro-nutrients in leaves of the wheat plants.

NaCl (mM)	Microbe	Macronutrients (mg kg ⁻¹)				Micronutrients (mg kg ⁻¹)			
		Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	Zn ²⁺	Fe	Mn ²⁺	Cu
0	Control	471.40±1.99g	1663.35±1.85f	4920.82±0.58d	474.79±8.63l	72.84±0.20f	639.81±0.62d	88.33±0.62d	184.77±6.02f
	<i>P. indica</i>	566.56±0.52f	1990.49±10.31d	5444.07±0.62c	467.66±0.56lm	87.77±1.42c	867.07±0.85a	93.04±0.41c	288.33±1.33b
	PGPB	1010.02±1.96a	2843.42±2.76a	6438.21±4.83a	430.61±0.56n	109.06±4.75a	885.98±0.65a	98.20±0.43a	158.63±1.42g
	AMF	656.27±3.01c	2753.43±6.87b	5733.09±7.94b	456.28±3.99m	49.80±2.15j	651.83±0.53cd	95.65±0.57b	322.75±0.62a
50	Control	366.98±0.55i	1482.10±0.56g	3136.69±3.54i	587.14±6.03i	68.52±0.62g	570.04±0.85f	80.41±0.85g	151.06±0.62gh
	<i>P. indica</i>	476.48±1.52g	1787.18±12.13e	4528.23±15.64f	514.02±0.53j	77.20±0.23e	642.99±3.24d	84.97±0.81e	200.20±0.23e
	PGPB	980.14±12.4b	2006.15±1.84d	5494.02±10.12c	469.84±9.43lm	100.64±0.67b	682.44±3.89b	89.37±1.46d	117.63±1.76ij
	AMF	653.29±4.61c	2285.49±8.82c	4673.96±9.35e	496.65±3.29k	41.34±1.33k	610.22±6.02e	83.19±0.85f	275.54±5.76c
100	Control	252.43±0.52j	1228.29±0.53i	2538.58±0.58k	793.11±12.10d	56.71±0.60i	456.22±0.62h	45.75±0.62m	126.92±6.26i
	<i>P. indica</i>	379.23±4.54i	1471.93±5.12gh	3428.95±11.01h	736.85±10.51f	64.41±5.89 h	604.30±5.12e	64.51±3.12j	140.10±0.62h
	PGPB	628.38±8.81d	1592.38±13.67f	4389.66±2.31g	643.59±8.12h	84.18±4.78d	667.63±5.19bc	77.97±1.67h	99.95±1.67k
	AMF	608.82±6.86e	1506.58±3.53g	3449.11±7.61h	717.29±7.17g	27.06±1.27l	598.63±2.74e	69.39±0.47i	212.58±4.89d
200	Control	170.48±0.52l	616.83±0.50k	1590.06±0.53i	1042.79±5.48a	27.11±2.05l	239.16±0.60i	29.94±0.60n	105.24±0.62jk
	<i>P. indica</i>	198.34±0.58k	1045.46±1.21j	2813.44±14.82j	840.41±4.58b	51.40±0.62j	512.04±4.12g	45.66±1.67m	116.79±3.42ij
	PGPB	435.51±4.76h	1398.64±20.13h	3376.56±16.32h	769.83±14.72e	69.94±3.98fg	592.49±4.67e	57.38±4.34k	80.65±2.67l
	AMF	419.48±4.67h	1169.38±26.21i	3059.57±21.2i	815.97±5.15e	21.43±2.33m	454.74±6.09h	52.25±1.47l	157.72±4.68g
Significance									
Salinity		***	***	***	***	***	***	***	***
<i>P. indica</i>		***	***	***	***	***	***	***	***
<i>P. indica</i> × Salinity		***	***	***	***	***	***	***	***
PGPB		***	***	***	***	***	***	***	***
PGPB × Salinity		***	***	***	***	***	***	***	ns
AMF		***	***	***	***	***	***	***	***
AMF × Salinity		***	***	***	***	***	***	***	***

Values represent averages across replicates. Letters within a column represent significant differences at P<0.05. ns: not significant. ***P<0.001, **P<0.01, *P<0.05.

4.2.1.4 Physiological parameters

Figure 4.13 depicts the physiological parameters of microbial-inoculated and uninoculated wheat plants under four different levels of salinity stress. The microbial-inoculated wheat plants displayed a significantly higher photosynthetic rate, transpiration rate, stomatal conductance, and internal CO₂ level than the uninoculated wheat plants at all salinity levels. Photosynthetic rates were significantly increased by PGPB, AMF, and *P. indica* by 1.23, 1.24, and 1.17-fold as compared to uninoculated plants under 200 mM NaCl stress. PGPB-inoculated wheat plants had 1.6-fold increased stomatal conductance, followed by AMF (1.5-fold), and *P. indica* (1.4-fold) as compared to uninoculated plants. In wheat plants inoculated with PGPB, AMF, and *P. indica*, transpiration rates increased to 1.3, 1.4, and 1.2-fold, respectively at 200 mM NaCl salinity stress. Under severe salinity stress conditions, PGPB-inoculated plants had an internal CO₂ increase of 3.9-fold, while AMF and *P. indica*-inoculated plants had an increase of 3.6 and 3.0-fold respectively. The results of the two-way ANOVA showed a significant independent effect of salinity on PGPB, *P. indica*, and AMF physiological parameters (**Table 4.6**). Therefore, PGPB has been found to alleviate salinity stress better than both AMF and *P. indica*.

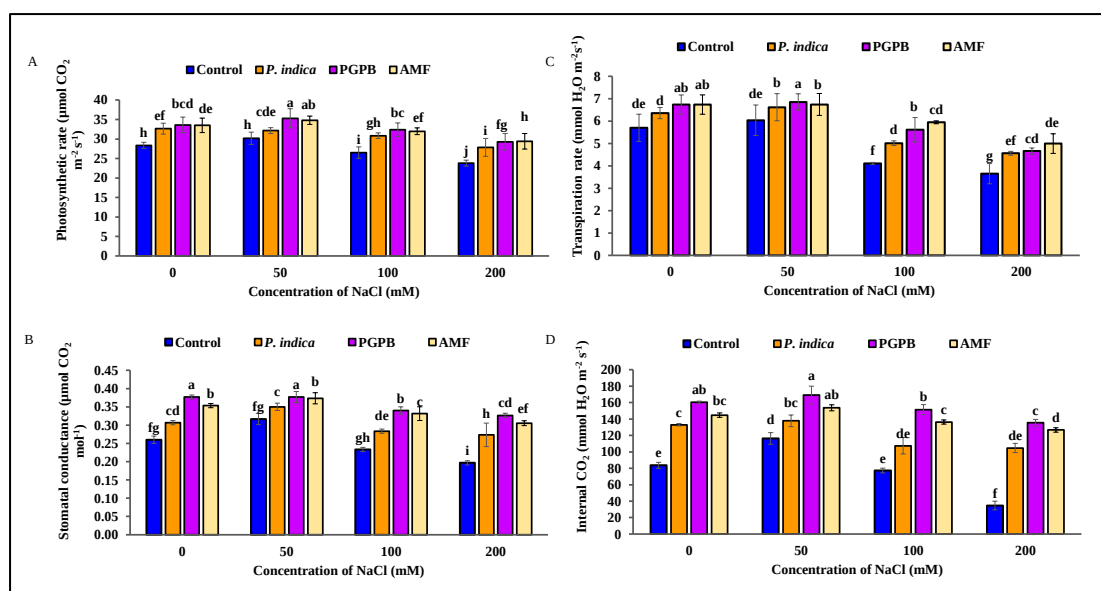


Figure 4.13: Effects of salinity and microbial inoculation in wheat on (A) photosynthetic rate; (B) stomatal conductance; (C) transpiration rate; and (D) internal CO₂. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.2.1.5 Biochemical parameters

4.2.1.5.1 Photosynthetic pigment content

Photosynthetic pigments viz. chlorophyll a, chlorophyll b, total chlorophylls, and carotenoid content in microbial-inoculated wheat plants showed substantially higher levels than uninoculated wheat plants at all levels of salinity (**Figure 4.14**).

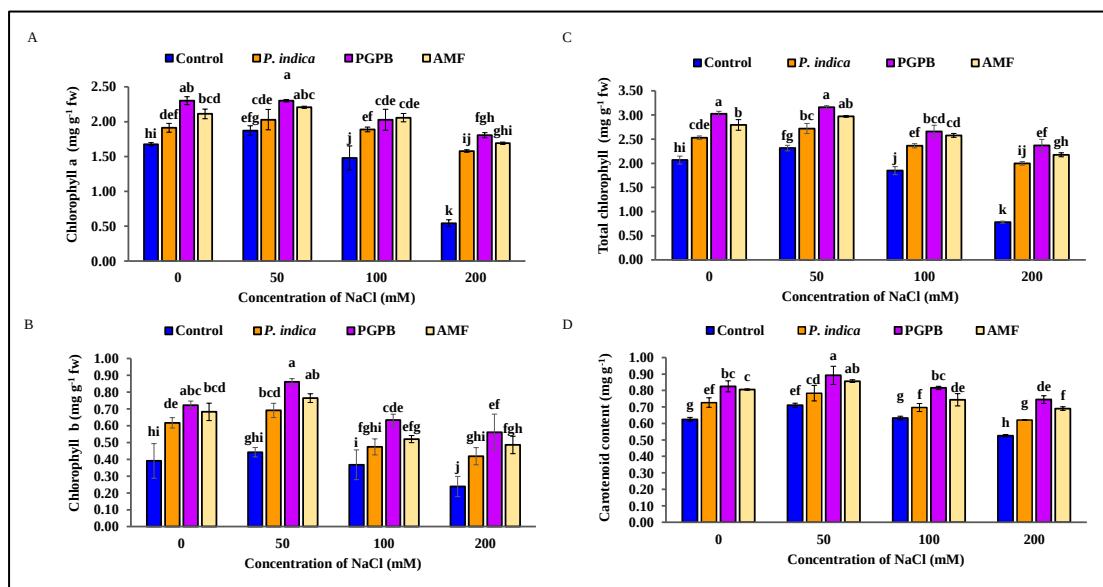


Figure 4.14: Effects of salinity and microbial inoculation in wheat on the content of (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; and (D) carotenoid. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

A substantial increase in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents was observed in microbial-inoculated wheat plants under all levels of salinity stress (**Figure 4.14**). Up to 50 mM NaCl, maize plant photosynthetic pigment content increased, while at 100 and 200 mM NaCl, it decreased significantly. Plants inoculated with PGPB, AMF, and *P. indica* increased their chlorophyll a content to 3.34, 3.13, and 2.9-fold, respectively compared to uninoculated plants, when exposed to the highest level of salinity stress (200 mM NaCl) (**Figure 4.14A**). As a result of PGPB inoculation, chlorophyll b content was increased by 2.34-fold under severe salinity conditions, whereas AMF and *P. indica* inoculation increased it by 2.02-fold and 1.74-fold, respectively (**Figure 4.14B**). At the highest stress level, the total chlorophyll content in PGPB-inoculated plants was 3.03-fold higher than in the control plants, followed by AMF (2.79-fold) and *P. indica* (2.56-fold) (**Figure 4.14C**). In comparison

with uninoculated plants, PGPB (1.4-fold) inoculated plants had higher carotenoid contents than *P. indica* (1.2-fold) and AMF (1.3-fold) inoculated plants (**Figure 4.14D**). A two-way ANOVA demonstrated that salinity, *P. indica*, PGPB, AMF, and their interactions significantly impacted chlorophyll a and total chlorophyll content (**Table 4.6**). Therefore, PGPB is more effective at alleviating salinity stress than AMF and *P. indica* in wheat plants.

4.2.1.5.2 Osmolyte content

Glycine betaine content increased linearly with till 100 mM NaCl then decreased at 200 mM NaCl stress in microbial inoculated and uninoculated wheat plants (**Figure 4.15A**). Comparing between 0 and 200 mM NaCl stress treatment glycine betaine content increased by about 1.22-fold in AMF-inoculated plants, led by PGPB (1.18-fold), *P. indica* (1.17-fold), and uninoculated (1.14-fold) plants. The glycine betaine content of microbial-inoculated plants was significantly higher at all salinity levels compared to uninoculated plants. The leaf glycine betaine content of PGPB was 1.41-fold higher than control followed by AMF-inoculated plants was 1.23-fold higher than that of control plants at 200 mM NaCl, followed by *P. indica* inoculated plants (1.14-fold). Glycine betaine content in wheat plants was significantly affected by salinity stress and microbial inoculation.

In response to salinity stress, both microbial inoculated and uninoculated wheat plants showed rectilinear increases in total sugar content (**Figure 4.15B**). The presence of microbes, however, facilitated higher sugar accumulation in leaves at all levels of salt stress. The total sugar content of plants inoculated with PGPB at 0, 50, 100, and 200 mM NaCl stress was 1.13, 1.2, 1.55, and 2.28-fold higher than that of plants uninoculated. AMF-inoculated plants had total sugar contents 1.04, 1.13, 1.48, and 2-fold higher than uninoculated plants at 0, 50, 100, and 200 mM NaCl stress. Inoculating plants with *P. indica* increased total sugar content 1.03, 1.06, 1.4, and 1.46-fold when compared with uninoculated plants under 0, 50, 100, and 200 mM NaCl stress. PGPB alleviates salinity stress more effectively than *P. indica* and AMF-inoculated plants at all salinity stress levels. In wheat plants, salinity stress and microbial inoculation increased total sugar content significantly.

A linear increase in trehalose content was observed in both microbial-inoculated and uninoculated wheat plants under salinity stress (**Figure 4.15C**). However, microbial-inoculated plants displayed significantly higher trehalose contents than uninoculated plants at all levels of salinity stress. As a result of PGPB inoculation, trehalose content was 1.7, 2.4, 2.0, and 2.0-fold higher than in uninoculated plants, respectively, at 0, 50, 100, and 200 mM NaCl stress. Upon AMF-inoculation, the trehalose contents of the plants were 1.6, 2.3, 1.1, and 1.6-fold higher than those of uninoculated plants at 0, 50, 100, and 200 mM NaCl stress. *P. indica* inoculated plants produced 1.2, 1.7, 1.2, and 1.3-fold more trehalose than uninoculated plants at 0, 50, 100, and 200 mM NaCl stress, respectively. Based on two-way ANOVA, salinity, *P. indica*, PGPB, and AMF have significant independent effects and interaction of salinity $\times$ *P. indica*, salinity $\times$ PGPB, and salinity $\times$ AMF on trehalose content (**Table 4.6**). Thus, PGPB alleviates salinity stress more efficiently than AMF or *P. indica*-inoculated plants at all salinity levels.

Microbial inoculation and salinity stress significantly influenced wheat leaf proline content (**Figure 4.15D**). Both microbial inoculated and uninoculated wheat leaves exhibited an increase in proline content under salinity stress. Microbial-inoculated wheat plants performed significantly better under all salinity stress levels compared to uninoculated plants. Leaf proline content was 2.1-fold higher in PGPB-inoculated plants compared to the control, followed by *P. indica* inoculated plants (1.3-fold) and AMF-inoculated plants (0.9-fold) at 200 mM NaCl stress.

Polyamine content (putrescine and spermidine) was significantly influenced by salinity stress and microbial inoculation. Spermine was, however, not detected in both microbial inoculated and uninoculated plants under all salinity conditions. Microbial inoculated and uninoculated plants demonstrated contrasting effects of salinity stress on putrescine and spermidine (**Figure 4.15E-F**). As compared to plants not exposed to salinity stress, PGPB, AMF, *P. indica*, and uninoculated plants displayed increases in putrescine levels by 2.8, 2.3, 5.3, and 4.6-fold, respectively at 200 mM NaCl. Plants inoculated with PGPB had spermidine levels 3.2, 2.1, 1.2, and 1.3-fold higher than uninoculated plants under 0, 50, 100, and 200 mM NaCl stress, respectively. As a result of AMF inoculation, spermidine levels of plants were 3.3, 2.2, 1.3, and 1.73-fold higher than those of uninoculated plants under 0, 50, 100, and 200 mM NaCl stress. At 0, 50, 100,

and 200 mM NaCl stress increased spermidine levels in *P. indica* inoculated plants by 1.7, 1.7, 1.1, and 1.28-fold that of uninoculated plants. Despite salinity stress, microbial-inoculated plants recorded higher putrescine and spermidine contents than uninoculated plants. At all levels of salinity stress, AMF outperforms PGPB and *P. indica* in terms of polyamine content. Two-way ANOVA demonstrated a significant independent effect of salinity, *P. indica*, PGPB, and AMF on glycine betaine, trehalose, proline, and polyamine content (**Table 4.6**).

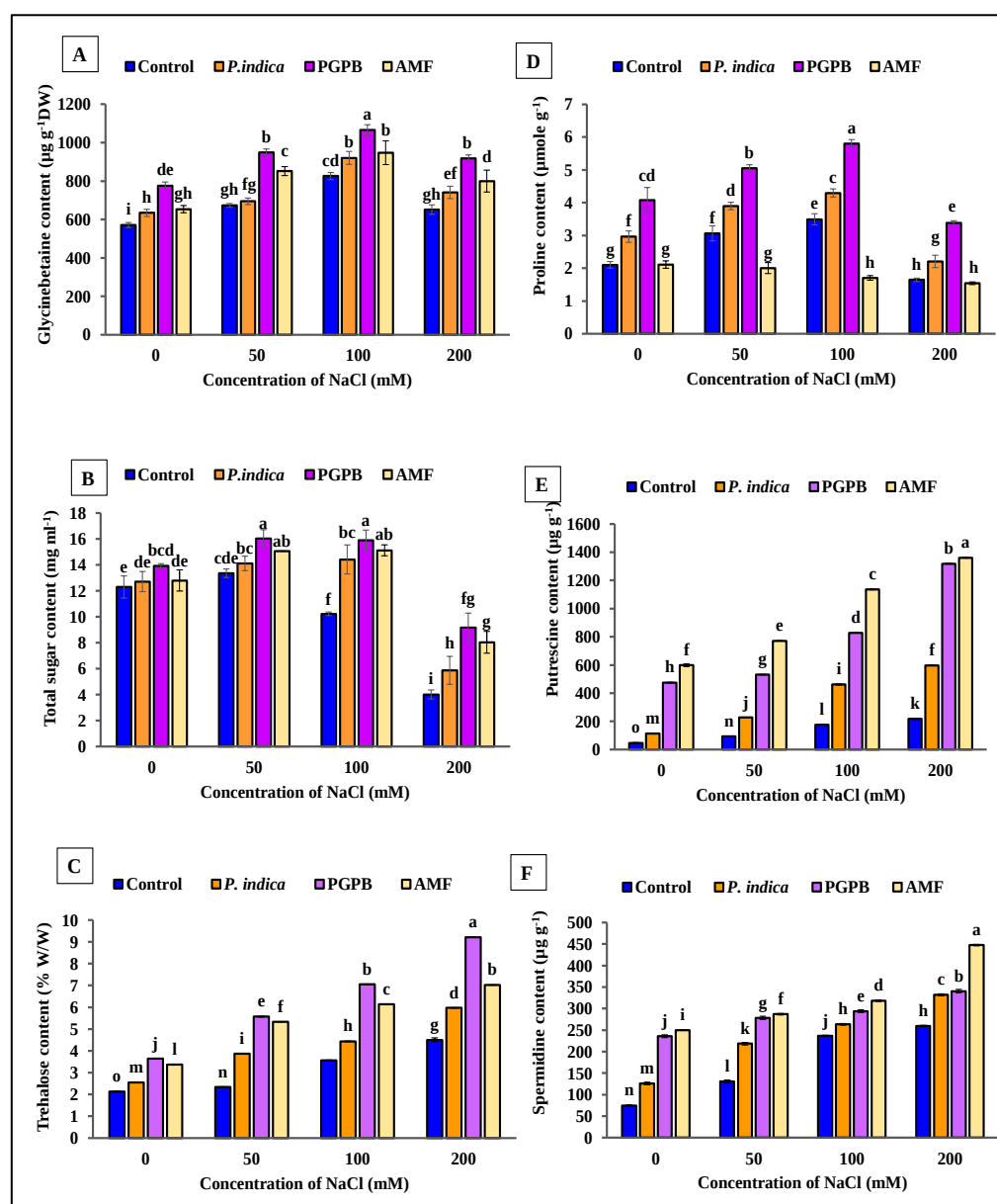


Figure 4.15: Effects of salinity and microbial inoculation in wheat on the content of (A) glycine betaine; (B) total sugar; (C) trehalose; (D) proline; (E) putrescine; and (F) spermidine. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.2.1.5.3 Protein

Both microbial-inoculated and uninoculated wheat plants exhibited significant increases in protein content till 100 mM NaCl followed by a decrease at 200 mM NaCl stress. Microbial inoculated plants, however, produced significantly more protein than uninoculated plants when exposed to salinity stress (**Figure 4.16A**). Plants inoculated with PGPB, AMF, and *P. indica* produced 2.8, 2.7, and 2.2-fold more protein than uninoculated wheat at 200 mM NaCl, respectively. As a result, PGPB has a greater ability to alleviate salinity stress than *P. indica* and AMF-inoculated plants at all salinity levels. Protein content was significantly increased by salinity stress and microbial inoculation.

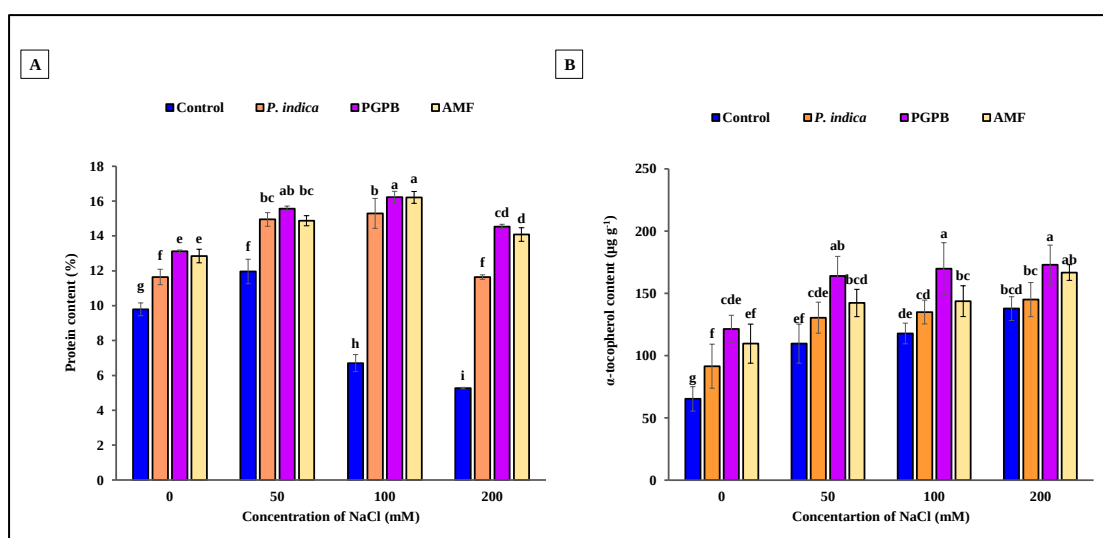


Figure 4.16: Effects of salinity and microbial inoculation in wheat on the content of (A) protein; and (B) α -tocopherol. Values represent averages across replicates. Different letters represent significant differences at $P < 0.055$.

4.2.1.5.4 α -tocopherol content

Both microbial inoculated and uninoculated wheat plants showed an increase in α -tocopherol in response to salinity stress. However, microbial-inoculated plants exhibited significantly higher levels of α -tocopherol under salinity stress (**Figure 4.16B**). Plants inoculated with PGPB, AMF, and *P. indica* produced 1.3, 1.2, and 1.1-fold more α -tocopherol than uninoculated plants at 200 mM NaCl, respectively. The PGPB-inoculated plants are, therefore, more efficient at alleviating salinity stress than

the *P. indica* and AMF-inoculated plants at all salinity levels. The α -tocopherol content of plants was significantly enhanced by salinity stress and microbial inoculation.

4.2.1.5.5 Membrane damage

The content of MDA in **Figure 4.17A** is used to measure lipid peroxidation in wheat plants exposed to different levels of salinity stress. Both microbial inoculated and uninoculated wheat plants displayed increased MDA content under salinity stress. Plants that are uninoculated, however, have higher levels of MDA. There was a significant reduction in MDA contents in plants that were microbial inoculated regardless of salinity level. *P. indica*, AMF, and PGPB-inoculated plants showed decreases in MDA content of 7.95%, 22.40%, and 30.30%, respectively over uninoculated plants, when exposed to 200 mM NaCl stress. Microbial inoculation and salinity stress significantly affected the relative membrane permeability of wheat plants. The relative permeability in wheat plants increased as a result of salinity stress. At all salinity levels, PGPB, AMF, and *P. indica* inoculated plants exhibited lower electrolyte leakage than the control (**Figure 4.17B**). Plants inoculated with PGPB, AMF, and *P. indica* exhibited 14.85%, 8.20%, and 5.88% lower relative permeability, respectively than the control at 200 mM NaCl. In both microbial inoculated and uninoculated plants, salinity stress increased LOX activity, but a significant difference was observed between them (**Figure 4.17C**). Microbe-inoculated plants had significantly lower LOX levels. Plants inoculated with PGPB (38.28%) showed lower LOX activity than those inoculated with AMF (26.26%) and *P. indica* (18.80%) as compared to control under 200 mM NaCl. Two-way ANOVA revealed a significant independent effect of salinity, *P. indica*, PGPB, AMF, and interaction of salinity $\times$ AMF, and salinity $\times$ PGPB on membrane damage (**Table 4.6**). PGPB is therefore more effective at alleviating salinity stress in terms of membrane damage at all salinity levels than *P. indica* and AMF.

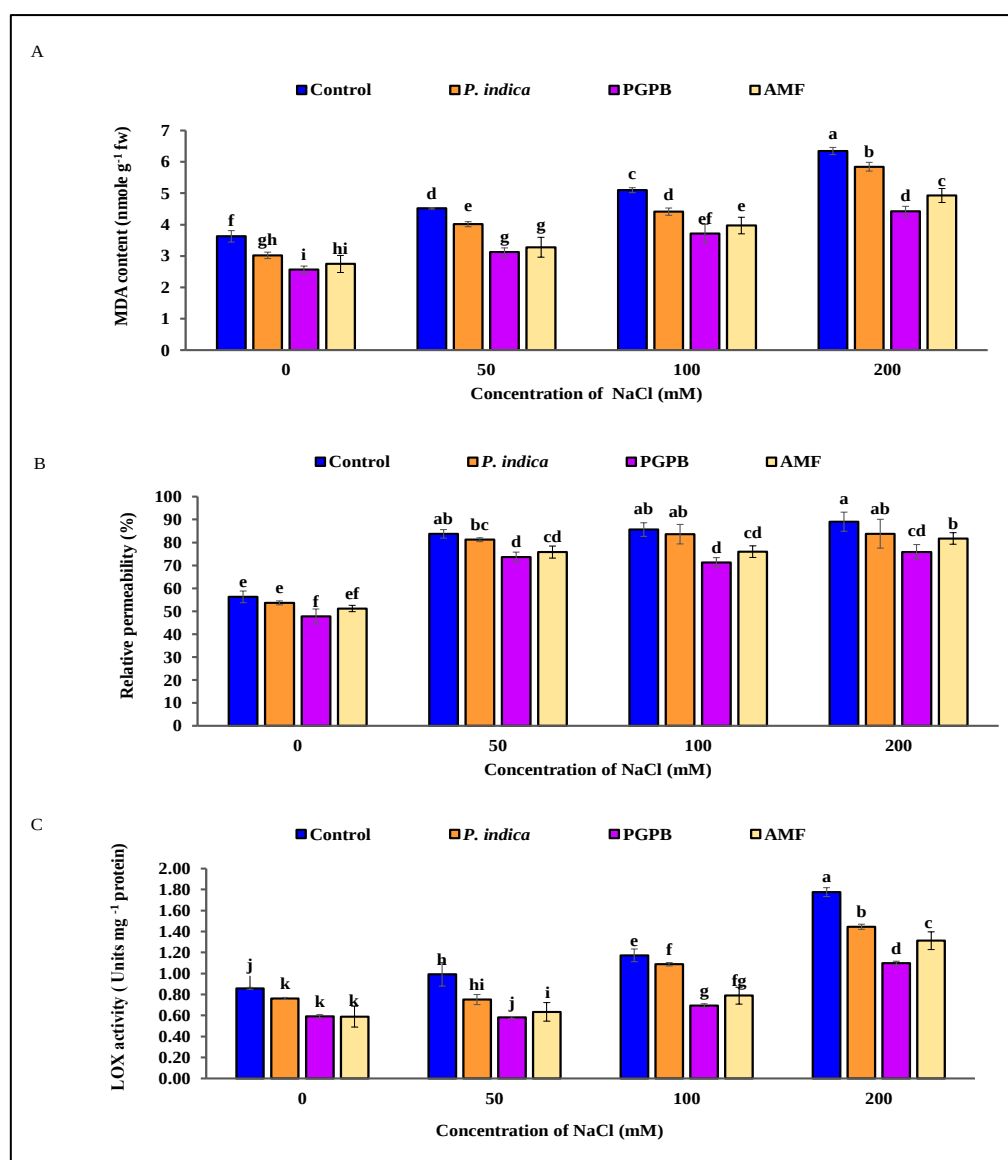


Figure 4.17: Effects of salinity and microbial inoculation in wheat on (A) MDA content; and (B) relative permeability (C) LOX activity. Values represent averages across replicates. Letters within a column represent significant differences at $P < 0.055$.

4.2.1.6 Antioxidant enzyme activity

Both microbial and uninoculated wheat plants displayed increased SOD activity with increasing salinity stress levels. However, microbial colonization increased SOD activity in wheat plants under salinity stress conditions more than in control plants (Figure 4.18A). As compared to uninoculated wheat plants at 200 mM NaCl, PGPB inoculation raised SOD activity by 1.07-fold, AMF inoculation increased it by 1.05-fold, and *P. indica* inoculation increased it by 1.03-fold.

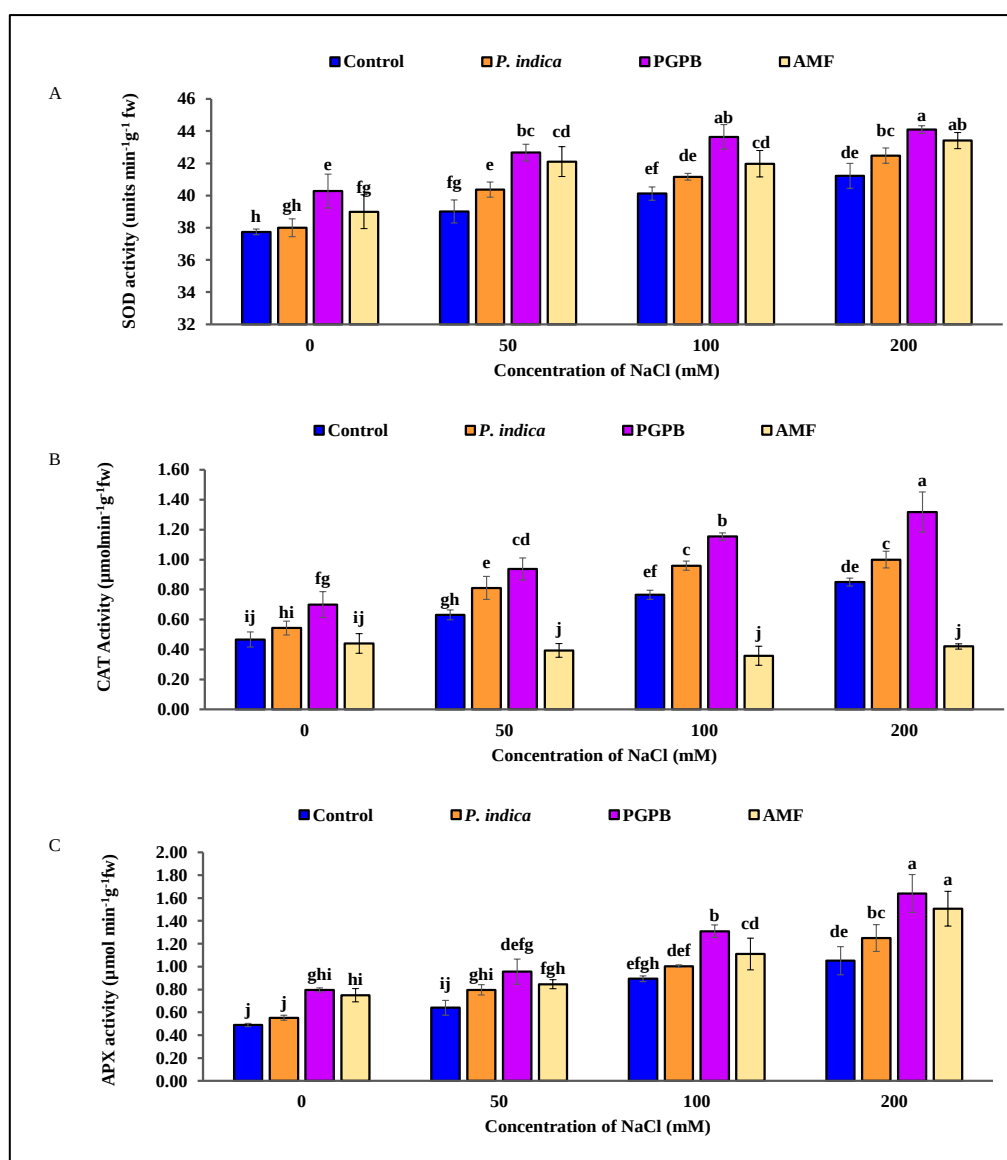


Figure 4.18: Effects of salinity and microbial inoculation in wheat on the activity of (A) SOD; (B) CAT; and (C) APX. Values represent averages across replicates. Different letters represent significant differences at $P < 0.055$.

As salinity stress increased, PGPB and *P. indica*-inoculated plants displayed increased CAT activity. The CAT activity of plants inoculated with AMF decreased with an increase in salinity stress as opposed to control plants. A 1.6-fold and 1.2-fold increase in CAT activity was observed in plants inoculated with PGPB and *P. indica* as compared to uninoculated plants at 200 mM NaCl, respectively (**Figure 4.18B**). The APX activity of PGPB, *P. indica*, and AMF-inoculated plants was higher than that of uninoculated plants at all salinity levels. APX activity was significantly higher in plants with PGPB inoculation than in wheat plants with AMF and *P. indica* inoculation at

each level of salinity stress. PGPB-inoculated wheat plants had 1.6-fold higher APX activities than their uninoculated counterparts at 200 mM NaCl stress. A 1.4 and 1.2-fold increase in APX activity was observed in AMF and *P. indica* inoculated plants at 200 mM NaCl respectively (**Figure 4.18C**).

4.2.1.7 Cell ultrastructural studies

At all levels of salinity, the electron micrographs show qualitative differences between microbial inoculated and uninoculated wheat plants.

4.2.1.7.1 Chloroplast

Figures 4.19 and **4.20** show changes in chloroplast ultrastructure in microbial-inoculated and uninoculated wheat leaves under salinity stress. Microbial inoculated and uninoculated wheat plants displayed chloroplasts with distinct thylakoids and grana surrounded by double-layered membranes under 200 mM NaCl stress. However, PGPB-inoculated plants exhibited a greater number of thylakoids and granum than AMF and *P. indica*-inoculated plants. Under 50 mM NaCl salinity stress, uninoculated plants showed decreased numbers of thylakoids and grana, and increased numbers of plastoglobules. Under low salinity stress, the thylakoid membrane contracted thereby reducing the thylakoid lumen (**Figures 4.19** and **4.20**). The chloroplast structures were almost the same in microbial-inoculated plants at 50 and 0 mM NaCl. A 100 mM NaCl salinity stress led to swelling in the thylakoid lumen resulting in destacking of grana. Moreover, the accumulation of smaller-size plastoglobules was more in uninoculated plants. Microbial-inoculated plants showed chloroplasts with intact thylakoids and grana and fewer but large size plastoglobules accumulation, though the size of the plastoglobules was large. In contrast, plants inoculated with PGPB displayed more intact thylakoids, grana, and a lesser accumulation of plastoglobules than AMF or *P. indica*. Consequently, at 200 mM NaCl, the chloroplast in uninoculated plants was characterized by disorganized thylakoids and grana and the accumulation of a large number of smaller-sized plastoglobules (**Figure 4.19D**). Contrary to this, microbial-inoculated plants contained intact thylakoids, disorganized grana, and fewer but larger-sized plastoglobules. As compared to AMF and *P. indica*, plants inoculated with PGPB showed more intact thylakoids and fewer but large-size plastoglobules at severe salinity conditions.

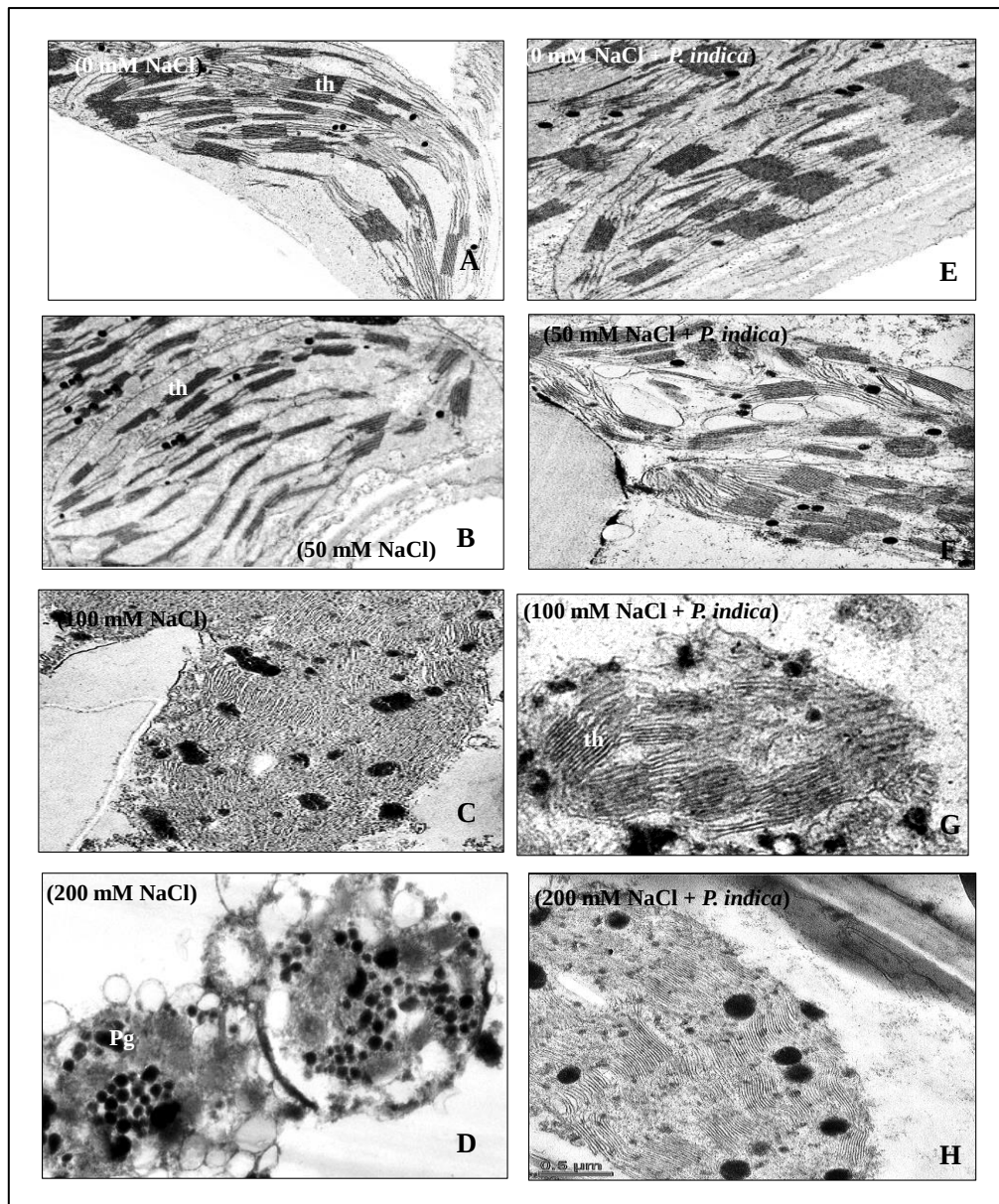


Figure 4.19: Transmission electron micrographs of chloroplast in mesophyll cells of (A) Control wheat plant not exposed to NaCl; chloroplast with intact thylakoids and grana surrounded. (B) Control wheat plant subjected to 50 mM NaCl; with a lesser number of thylakoids and some plastoglobules. (C) Control wheat plant not exposed to 100 mM NaCl; disorganized grana, and increased accumulation of plastoglobules. (D) Control wheat plant subjected to 200 mM NaCl; thylakoids and grana disorganized; a large number of plastoglobules seen (E) *P. indica* inoculated wheat plant not exposed to NaCl; Grana stacked neatly with well-developed thylakoids (F) *P. indica* inoculated wheat plant subjected to 50 mM NaCl; more thylakoids and fewer plastoglobules. (G) *P. indica* inoculated wheat plant subjected to 100 mM NaCl; structure of thylakoid and grana still maintained. (H) *P. indica* inoculated wheat plant subjected to 200 mM NaCl; fewer plastoglobules have accumulated; slight disintegration of grana and thylakoid was seen. Pg= plastoglobuli; th = thylakoids. The values in the bracket indicate the concentration of NaCl added to the soil.

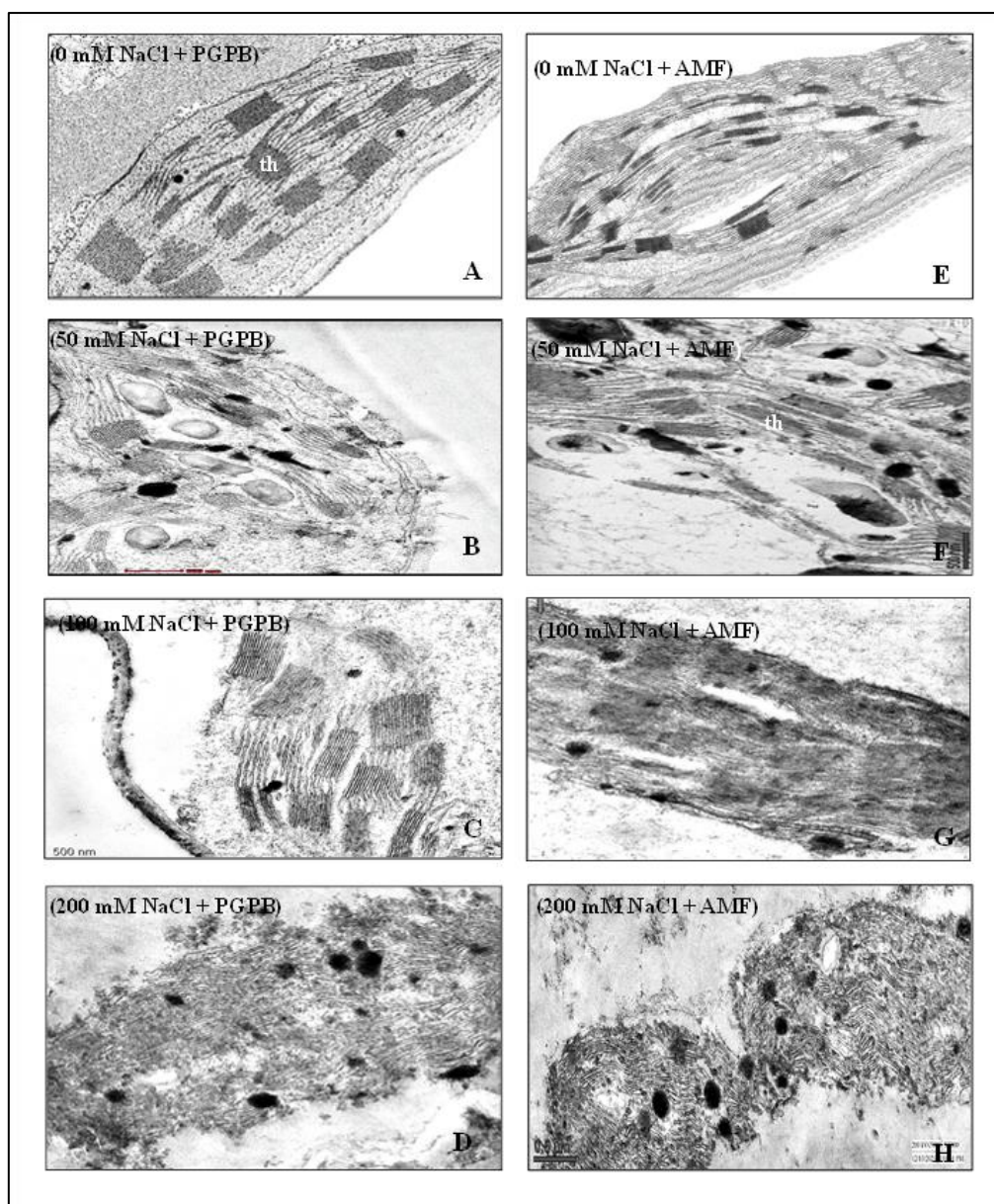


Figure 4.20: Transmission electron micrographs of chloroplast of mesophyll cells of (A) PGPB-inoculated wheat plant not exposed to NaCl; well-developed with distinct thylakoids and grana seen. (B) PGPB-inoculated wheat plant subjected to 50 mM NaCl; more thylakoids and fewer plastoglobules. (C) PGPB-inoculated wheat plant exposed to 100 mM NaCl; structure of grana is still maintained and fewer plastoglobules. (D) PGPB-inoculated wheat plant subjected to 200 mM NaCl; fewer plastoglobules have accumulated; structure of grana and thylakoid is still maintained. (E) AMF-inoculated wheat plant not exposed to NaCl; well-developed thylakoids neatly stacked into grana (F) AMF-inoculated wheat plant subjected to 50 mM NaCl; more thylakoids and fewer plastoglobules. (G) AMF-inoculated wheat plant subjected to 100 mM NaCl; the chloroplast shows a lesser number of thylakoids and fewer accumulation of plastoglobules. (H) AMF-inoculated wheat plant subjected to 200 mM NaCl; fewer plastoglobules have accumulated, and slight disintegration of thylakoid seen. Pg= plastoglobuli; th = thylakoids. The values in the bracket indicate the concentration of NaCl added to the soil.

4.2.1.7.2 Plasma membrane

Under 0 mM NaCl conditions the plasma membrane of both microbial inoculated and uninoculated wheat plants closely adhered to the cell wall (**Figures 4.21 and 4.22**). As a result of salinity stress, the plasma membrane detached from the cell wall, forming an apoplastic space. In response to increased salinity stress, the space between the plasma membrane and the cell wall became larger. Plasma membrane detachment, however, was less pronounced in microbial-inoculated plants, resulting in a smaller cell wall-plasma membrane gap. At all levels of salinity stress, PGPB-inoculated plants showed comparatively less plasma membrane separation from the cell wall than AMF and *P. indica*. Furthermore, at higher salinity stress levels, invaginations of the plasma membrane led to the formation of vesicles in the cytoplasm. Compared with their microbial-inoculated counterparts, uninoculated plants displayed less vesicle formation.

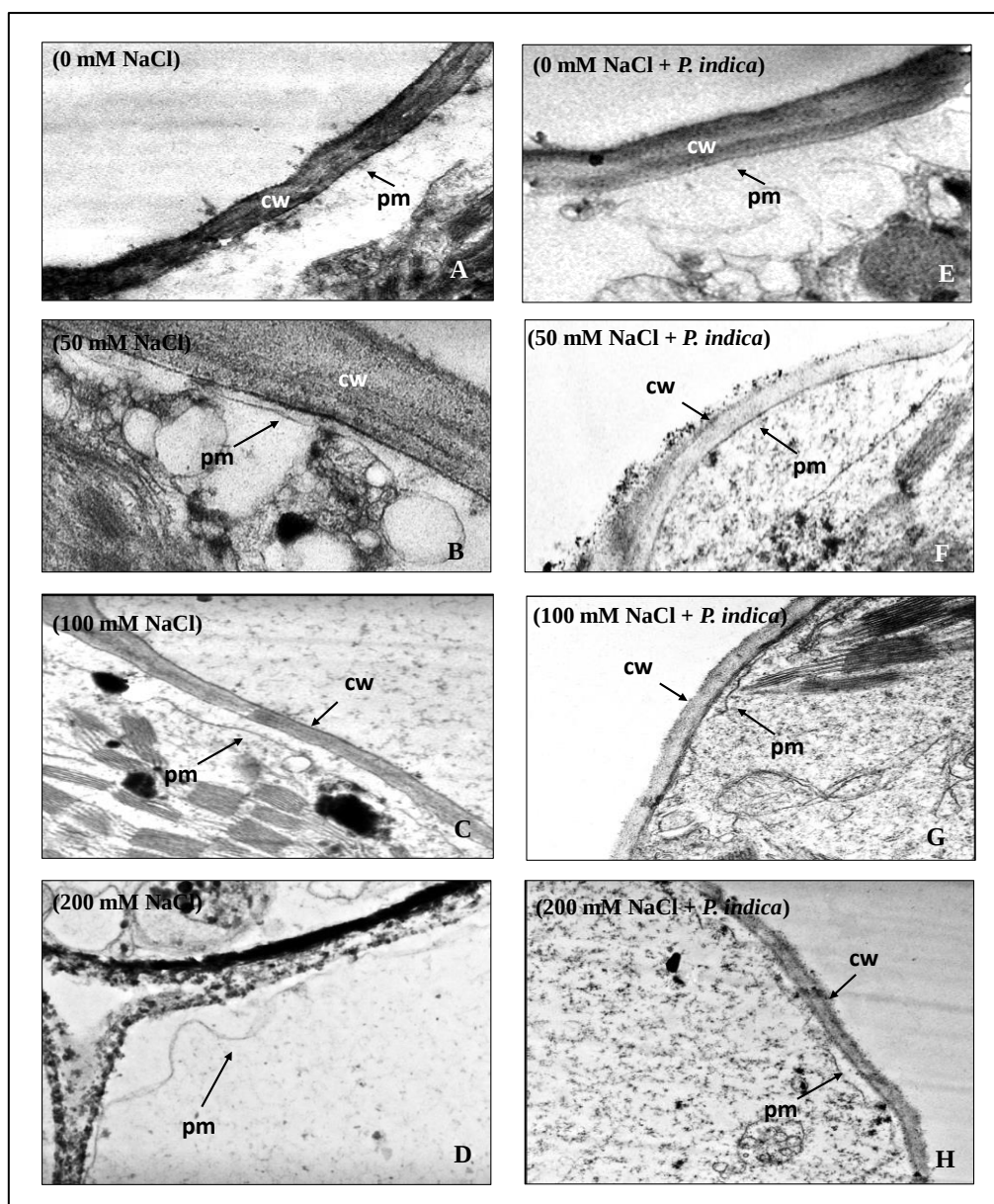


Figure 4.21: Transmission electron micrographs of the mesophyll cell wall and plasma membrane (A) Control wheat plant not exposed to NaCl; plasma membrane firmly attached to the cell wall. (B) Control wheat plant subjected to 50 mM NaCl; receding plasma membrane. (C) Control wheat plant exposed to 100 mM NaCl; the gap between the plasma membrane and cell wall increased. (D) Control wheat plant subjected to 200 mM NaCl; a marked increase in space between the plasma membrane and cell wall. (E) *P. indica* wheat inoculated plant not exposed to NaCl; plasma membrane tightly attached to the cell wall. (F) *P. indica* wheat inoculated plant subjected to 50 mM NaCl; the plasma membrane is closely bound to the cell membrane. (G) *P. indica* inoculated wheat plant subjected to 100 mM NaCl; incipient plasmolysis and vesicle formation initiated. (H) *P. indica* inoculated wheat plant subjected to 200 mM NaCl; numerous vesicles near the plasma membrane.

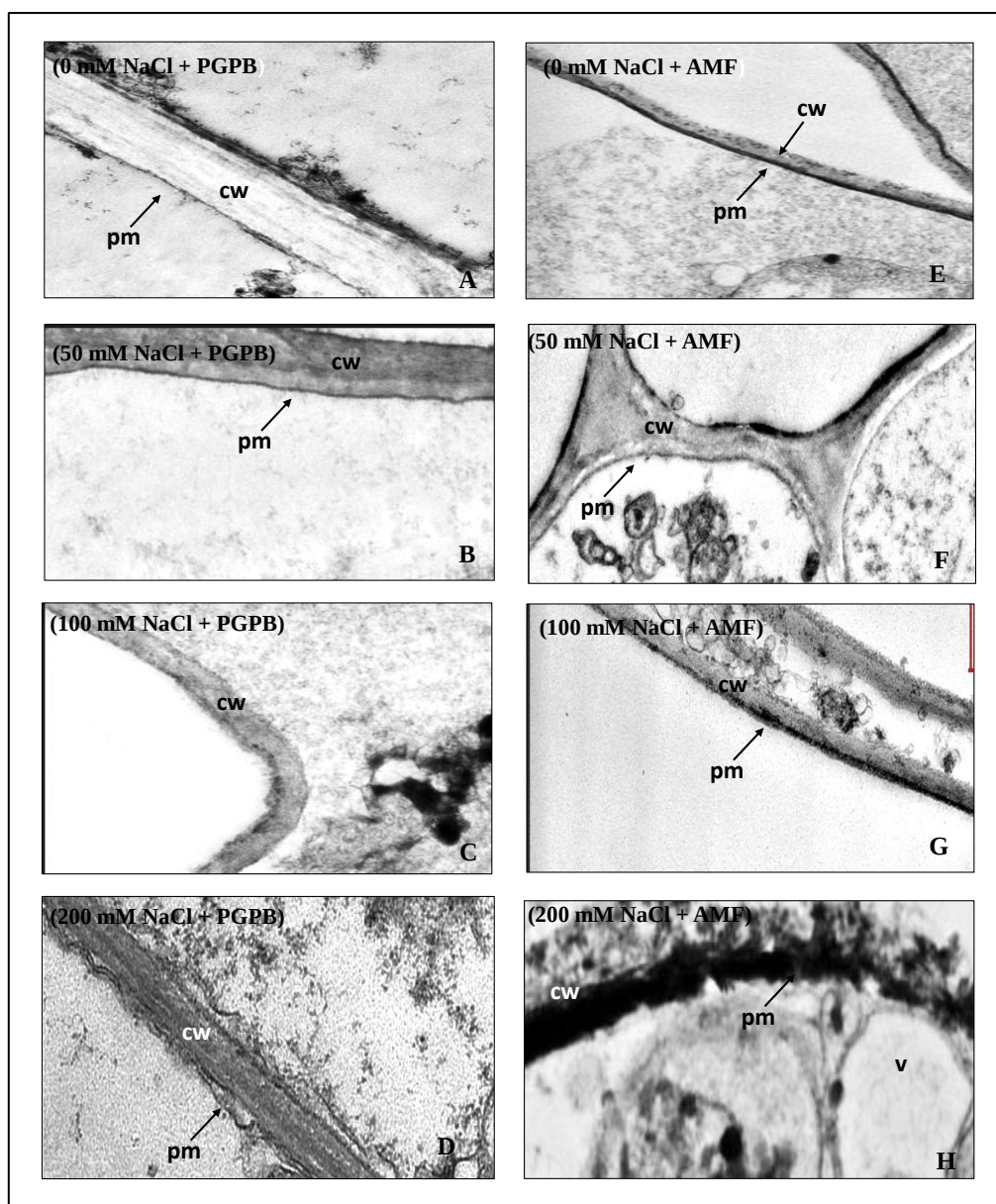


Figure 4.22: Transmission electron micrographs of the mesophyll cell wall and plasma membrane: (A) PGPB-inoculated wheat plants without NaCl treatment; plasma membrane closely associated with the cell wall. (B) PGPB-inoculated wheat plants subjected to 50 mM NaCl; plasma membrane closely attached to the cell wall. (C) PGPB-inoculated wheat plants subjected to 100 mM NaCl; slight separation between the plasma membrane and cell wall; vesicles are seen near the membrane. (D) PGPB-inoculated wheat plants subjected to 200 mM NaCl; vesicles are seen near the membrane. (E) AMF-inoculated wheat plant subjected to 0 mM NaCl; well-preserved plasma membrane firmly attached to the cell wall. (F) AMF-inoculated wheat plant subjected to 50 mM NaCl; well-preserved plasma membrane firmly attached to the cell wall. (G) AMF-inoculated wheat plants subjected to 100 mM NaCl; plasma membrane slightly detached from the cell wall; vesicles are seen near the membrane. (H) AMF-inoculated wheat plants subjected to 200 mM NaCl; numerous vesicles near the plasma membrane. The values in brackets indicate the concentration of NaCl added to the soil. cw = cell wall; pm = plasma membrane; v = vesicle.

Table 4.6: Effect of NaCl, *P. indica*, NaCl × *P. indica*, PGPB, NaCl × PGPB, AMF, NaCl × AMF on physiological processes, photosynthetic pigments, protein, α -tocopherol, membrane damage, osmolytes contents, and antioxidant enzyme activity in wheat plants.

Parameters	NaCl	<i>P. indica</i>	NaCl × <i>P. indica</i>	PGPB	NaCl × PGPB	AMF	NaCl × AMF
Photosynthetic rate	***	***	ns	***	ns	***	ns
Transpiration	***	***	ns	***	ns	***	ns
Stomatal conductance	***	***	ns	***	***	***	**
Internal CO ₂	***	***	***	***	***	***	***
Chlorophyll a	ns	ns	ns	ns	ns	***	***
Chlorophyll b	ns	ns	ns	ns	**	ns	ns
Total chlorophyll	ns	ns	ns	ns	**	ns	ns
Carotenoid	***	***	ns	***	ns	***	**
Protein	***	***	***	***	***	***	***
α -tocopherol	***	***	ns	***	ns	***	ns
Lipid peroxidation	***	***	ns	***	*	***	ns
Relative permeability	***	**	ns	***	ns	***	ns
LOX	***	***	**	***	***	***	ns
Trehalose	***	***	***	***	***	***	***
Total sugars	***	***	*	***	***	***	***
Proline	***	***	ns	***	ns	***	***
Glycine betaine	***	***	*	***	*	***	ns
Putrescine	***	***	***	***	***	***	***
Spermidine	***	***	***	***	***	***	***
SOD	***	***	ns	***	ns	***	ns
CAT	***	***	ns	***	*	***	***
APX	***	***	ns	***	*	***	ns

Significant interactions at $P < 0.05$. ns: not significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. AMF, arbuscular mycorrhizal fungi; PGPB, plant growth-promoting bacteria; LOX, lipoxygenase; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase.

4.2.2 Discussion

Our study showed that increasing salinity stress levels reduced the ability of AMF and *P. indica* to colonize wheat roots. According to previous studies, salinity stress reduces hyphal growth and mycorrhizal colonization (Saravi et al. 2022). In response, shoot and root biomass decreased, suggesting that AMF and *P. indica* benefits are dependent on root colonization.

A reduction in wheat plant growth was seen for both microbial-inoculated and uninoculated plants under salinity stress. The reduction in wheat plant growth may be attributed to decreased nutrient uptake and photosynthesis as a result of salinity stress (Azeem et al. 2023). However, AMF, PGPB, and *P. indica* inoculated wheat plants displayed longer shoots and roots, as well as higher biomass under salinity stress conditions. Our results are consistent with earlier reports on PGPB colonization in mustard (Khan et al. 2023). Studies by Ghabooli and Kaboosi 2022 found that inoculating legume plants with AMF increased shoot biomass under salinity stress conditions. The results of our study showed that microbial-inoculated wheat plants exhibited higher leaf area under salinity stress compared to uninoculated plants. Khan et al. (2023) reported that mustard plants inoculated with PGPB had increased leaf area. Plant growth was increased in microbial-inoculated plants as a result of improved nutrient uptake (Masrahi et al. 2023) and osmolyte accumulation, in addition to increased antioxidant activity and a decreased lipid peroxidation process (Khan et al. 2023). As compared to AMF and *P. indica*-inoculated wheat plants, PGPB-inoculated wheat plants showed higher salinity resistance.

Our study showed that inoculated and uninoculated wheat plants had nutrient depletion due to salinity stress. Plants are unable to absorb essential nutrients due to poor root development and a lack of water availability during salinity. However, microbial inoculation led to better nutrient acquisition than uninoculated wheat plants at every level of salinity stress. Enhanced nutrient status is attributed to the increased absorption surface of extraradical hyphae in AMF and *P. indica*-inoculated wheat plants (Ortiz et al. 2015). Nutrients play a crucial role in plant growth and development. The mineral calcium is critical for the integrity of membranes, the stability of cell walls, and ion transport (de Bang et al. 2021). Plants become deficient in Ca^{2+} when they are exposed to salinity. During salinity stress, the Ca^{2+} present in membranes and cell walls is replaced by Na^+ resulting in the reduction of cell turgidity. According to our study, wheat plants inoculated with microbes were better at absorbing Ca^{2+} ions, consistent with the findings of Ait-El-Mokhtar et al. (2020).

K^+ maintains membrane potential and osmotic pressure (Sardans and Peuelas, 2021). Moreover, it modulates protein and sugar synthesis, and enzyme activity (Hasanuzzaman et al. 2018). We found that both inoculated and uninoculated wheat plants showed a reduction in K^+ levels following salinity treatment. Because K^+ and Na^+ are inherently

competitors due to their similar physiological properties. Because of this, cytoplasmic Na^+ competes with K^+ for binding sites, inhibiting K^+ -dependent metabolic processes. In contrast, inoculated wheat plants substantially elevated K^+ content more than uninoculated plants, consistent with previous findings that PGPB inoculation enhanced K^+ uptake in *Salvia Officinalis* (Aslani et al. 2021). Higher accumulation of osmolytes, protein synthesis, and antioxidant enzyme activity was associated with higher K^+ accumulation (Hasanuzzaman et al. 2018) in plants colonized with microbes under salinity stress.

Mg^{2+} is a vital component of chlorophyll and is essential for the photosynthesis and synthesis of proteins (Kleczkowski and Igamberdiev 2021). Salinity stress reduced chlorophyll content and photosynthesis in rice plants; however, AMF inoculation increased these parameters (Tisarum et al. 2020). Similarly, Mg^{2+} uptake was also inhibited by salinity in *Pisum sativum*, but AMF mitigated this effect (Parihar et al. 2020). We found that Mg^{2+} content in wheat plant tissues was significantly affected by salinity and microbial inoculation. It may be due to a lack of Ca^{2+} availability under salinity stress. The Ca^{2+} ion competes with the Mg^{2+} ion (Pandey and Sanyal 2021) for plasma membrane sites. We found that increased levels of Mg^{2+} correlate with higher photosynthetic pigments and photosynthetic rates in microbial inoculated wheat plants under salinity stress.

During salinity stress, Na^+ content increased rectilinearly in both microbial inoculated and control wheat plants. However, uninoculated wheat plants were higher in Na^+ than PGPB, AMF, and *P. indica*, indicating that microbes play a crucial role in Na^+ uptake. Na^+ is excluded from plants by AMF because it inhibits its uptake from soil or its translocation in plants. Apparently, microbes influence Na^+ translocation to aerial parts. PGPB secrete exopolysaccharides that bind to Na^+ present in soil and restrict its absorption by plants and increase nutrient uptake by roots (Bhagat et al. 2021). AMF-colonized wheat plants have low Na^+ content, possibly due to the ability of the fungus to confine Na^+ in intraradical hyphae or root cell vacuoles. Plant growth and physiological processes are enhanced by mycorrhizal symbiosis, therefore diluting these ions in tissues.

Micronutrients play a crucial role in wheat plant growth and development. Synthesis of proteins and photosynthetic pigments requires Zn^{2+} (Kumar et al. 2021). A vital

component of chloroplast structure and function is Fe, which is necessary for chlorophyll synthesis (Kumar et al. 2021). The role of copper in plant cells includes photosynthesis, respiration, and the structure of the cell wall (Kumar 2015a). In photosystem II, Mn^{2+} contributes to the structure of the water-splitting system which provides electrons for photosynthesis. Micronutrient uptake in plants is reduced by salinity. We observed that increasing salinity levels reduced the content of Cu, Fe, Mn^{2+} , and Zn^{2+} in both microbial-inoculated and control wheat plants. This is because salinity reduces the solubility and mobility of Cu, Fe, and Zn^{2+} in soil solutions, and a depletion zone develops around them. Thus, control wheat plants are unable to access these nutrients. Furthermore, decreasing Cu, Zn^{2+} , Mn^{2+} , and Fe levels in microbial-inoculated wheat plants may be the result of decreased hyphal growth. Microbial inoculation, however, improved micronutrient acquisition at all levels of salinity stress. According to earlier studies, Zn^{2+} , Fe, and Mn^{2+} content in *Vitis vinifera* was increased after AMF and PGPB (Karimi and Noori 2022) which is in line with our results. Bacteria chelate and acidify soil to make Cu, Fe, Mn^{2+} , and Zn^{2+} more accessible to plants (Etesami et al. 2014). In our study, higher photosynthetic content and photosynthesis are also associated with higher micronutrient content in wheat plants colonized with microbes.

The ability of a plant to sustain photosynthesis determines its potential for growth and salinity tolerance (Tisarum et al. 2020). Stomatal closure reduces gas exchange and internal CO_2 levels during salinity stress, thereby reducing photosynthetic pigment production and photosynthesis (Meng et al. 2023 Mar), which was further confirmed in the present study. Additionally, during salinity, the transpiration rate decreased, resulting in reduced nutrient and water uptake in wheat plants. However, wheat plants inoculated with PGPB, AMF, and *P. indica* exhibited an increase in photosynthetic rate, stomatal conductance, internal CO_2 , and transpiration rate at all levels of salinity. The results of our study are consistent with previous studies that have found that AMF inoculation improves plant photosynthetic efficiency by improving CO_2 assimilation rate, stomatal conductance, and transpiration rate (Chandrasekaran et al. 2019). Moreover, osmolyte accumulation in AMF-colonized plants reduces structural damage to the photosynthetic apparatus (Puccio et al. 2023). Furthermore, carotenoid content was significantly increased in wheat plants by microbial inoculation in our study. As an antioxidant, carotenoids protect the chloroplast structure, inhibit MDA formation,

stabilize membranes, and play a critical role in the assembly of light-harvesting complexes (Gómez-Sagasti et al. 2023). In our study, higher chlorophyll and carotenoid levels, combined with a larger leaf area, resulted in higher photosynthesis rates in wheat plants.

A critical component of photosynthesis is chlorophyll and carotenoids, pigments that absorb light. Chlorophyll and carotenoid contents are decreased by salinity stress (Khan et al. 2023, Huang et al. 2023), which aligns with our own findings.), which is in line with our results. We found that wheat plants inoculated with PGPB, AMF, and *P. indica* had higher chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid levels than the control. Hence, microbial inoculation reduces the detrimental effects of salinity on wheat. A similar study found that PGPB colonization substantially increased the contents of chlorophyll and carotenoid in maize (Solórzano-Acosta et al. 2023). According to a study, *Colocasia esculenta* colonized with AMF contained a higher level of chlorophyll and carotenoids (Baltazar-Bernal 2022). The present study is consistent with previous studies suggesting higher photosynthetic pigments may result from antioxidant accumulation, chloroplast protection, and greater solubilization and bioavailability of nutrients (Parihar et al. 2020). Our results are in agreement with the finding that AMF increases chlorophyll levels in maize through improved uptake of nutrients (Parihar et al. 2020).

Abiotic stress is associated with a significant accumulation of osmolytes, including carbohydrates (glucose and trehalose), amino acids (proline), quaternary ammonium compounds (glycine betaine), and polyamines (putrescine, spermidine, and spermine), which play a critical role in maintaining cell turgor pressure and stabilizing membranes, enzymes, and proteins (Patel et al. 2020). Furthermore, osmolytes play a key role in quenching free radicals, inducing antioxidant machinery, and osmotic regulation (Bashir et al. 2021) during salinity stress. Trehalose is soluble, and non-reducing which maintains the osmotic balance and prevents oxidative damage thereby stabilizing membranes and macromolecules during salinity stress. As a highly stable molecule, trehalose is resistant to high temperatures and acids, it can prevent protein aggregation and degradation during salinity (Roy et al. 2021). Proline is an important secondary amino acid that has a major role in osmotic adjustment (Noreen et al. 2019), and membrane stabilization during salinity stress. We observed significantly higher osmolyte accumulation in both microbial inoculated (PGPB, AMF, and *P. indica*) and uninoculated wheat plants. However,

microbial-inoculated wheat plants had a greater amplitude of osmolyte increases than uninoculated plants. The results of this study are consistent with a previous study that demonstrated increased proline, glycine betaine, and sugar content in mustard inoculated with the PGPB under salinity stress (khan et al. 2023). Microbes enhance *Solanum melongena* adaptation to salinity stress by increasing putrescine and spermidine levels (Mokabel et al. 2022). AMF effectively ameliorated salinity-induced stress in *Colocasia esculenta* by increasing glycine betaine, proline, and sugar accumulation (Baltazar-Bernal et al. 2022) by maintaining cell turgidity and membrane integrity. According to Yaghoubian et al. 2022, *P. indica* and azotobacter symbiosis enhances salinity tolerance in *Silybum marianum* through the production of glycine betaine, proline, and sugar accumulation. Based on these results, microbial inoculated wheat plants are more efficient at osmotic adjustment than their uninoculated counterparts. However, PGPB outpaces AMF and *P. indica* in osmotic adjustment in wheat plants under salinity stress.

In our study, there was a remarkable difference in protein content in microbial inoculated and uninoculated wheat plants under all levels of salinity stress. Plants inoculated with PGPB, *P. indica*, and AMF had higher protein content. In microbial inoculated wheat plants, higher protein content is associated with higher photosynthetic rates, chlorophyll contents, membrane integrity, and osmolyte accumulation. Similarly, Solórzano-Acosta et al. (2023) found that salinity stress could be mitigated by PGPB and AMF-colonized *Persea americana* plants with higher levels of protein. According to this study, inoculating wheat plants with PGPB, *P. indica*, or AMF enhanced salinity tolerance through protein regulation.

In our study, wheat plants inoculated with PGPB, *P. indica*, and AMF have a high level of α -tocopherol, which indicates that α -tocopherol is a free radical scavenger as well as a modulator of lipid peroxidation during abiotic stress (Kim et al. 2019). This study substantiates the findings of Afshar and Abbaspour (2023) that higher levels of α -tocopherol in AMF colonized pistachio plants mitigate salinity stress. Based on the current study, inoculating wheat plants with PGPB, *P. indica*, and AMF enhanced salinity tolerance by regulating α -tocopherol content, thus maintaining a high level of photosynthetic efficiency and chlorophyll content and reducing ion leakage.

In plants, malondialdehyde, a by-product of membrane lipid peroxidation, is considered an important physiological indicator of salinity (Xu et al. 2022). Peroxidation of lipids

alters the ultrastructure and permeability of cell membranes of wheat plants during salinity stress. As a result of LOX activity, lipids are converted into hydroperoxyl fatty acids, which release free radicals that cause damage to the cells. Salinity tolerance depends on reduced lipid peroxidation and relative membrane permeability (Afshar et al. 2023). In our study, MDA content, relative membrane permeability, and LOX activity of both the inoculated plants and the control wheat plants increased rectilinearly with increasing salinity levels. In microbial inoculated wheat plants, however, MDA content, LOX activity, and electrolyte leakage were less pronounced than that in control plants, which is consistent with previous reports (Afshar et al. 2023). Microbial inoculated wheat plants have reduced LOX activity, in contrast to a previous report in which the co-inoculation of PGPB and AMF resulted in a higher LOX activity (Begum et al. 2022). Inoculated plants exhibited decreased MDA concentrations and relative membrane permeability by synthesizing non-enzymatic molecules like carotenoids, proline, and α -tocopherol, as well as enzymatic antioxidants (CAT, APX, and SOD). A similar finding was reported by Khan et al. (2023) that PGPB-inoculated plants were able to reduce ROS formation by maintaining their carotenoids and proline content, and CAT, APX, and SOD activities.

Both inoculated and uninoculated wheat plants displayed significantly different antioxidant enzyme activities in our study. The antioxidant activity of microbial-inoculated plants was higher than that of control plants. Our result is consistent with a previous study that demonstrated that microbial inoculation is effective in improving plant growth under salinity stress by enhancing the activity of ROS-scavenging antioxidant enzymes (Solórzano-Acosta et al. 2023). Therefore, microbial inoculation reduces membrane permeability and lipid peroxidation in plants by increasing antioxidant enzyme activity (Tyagi et al. 2023). It has previously been reported that *P. indica* colonized tomato exhibited increased antioxidant enzyme activity under salinity stress conditions (Xu et al., 2022) which supports our findings. According to Zhang et al. 2023, the use of *P. indica* increases salinity tolerance in soybean through the enhancement of antioxidant enzyme activity. Our study found that as salinity levels increased, CAT activity in AMF-inoculated wheat plants decreased. The findings are consistent with previous studies showing that PGPB reduces CAT activity during abiotic stress in maize (Siddique et al. 2022). In the current study, PGPB, AMF, and *P.*

indica are proposed to enhance antioxidant defense mechanisms and salinity tolerance in wheat.

Chloroplasts are highly susceptible to salinity stress (Liu et al. 2022). The present study showed that salinity stress affected the ultrastructure of chloroplasts of both microbial inoculated and control wheat plants. Salinity reduces the thylakoid lumen by contracting the membrane which may be a consequence of osmotic collapse (Semenova 2002). When salinity levels increased, thylakoid swelling caused destacking of grana (Kotula et al. 2019). In several studies, it has been demonstrated that osmotic stress damages thylakoid membranes and causes swelling (Goussi et al. 2018). The chloroplasts of microbial-inoculated plants, however, seem to be less damaged than their uninoculated counterparts in our study. This may be attributed to a better accumulation of osmolytes viz. glycine betaine, polyamines, and sugars in plants that have been inoculated with microbes. As osmolytes accumulate in plants, they prevent osmotic collapse during salinity stress. Ali et al. (2020) reported that polyamines inhibit membrane degradation in grana and stromal thylakoids through their interaction with negatively charged membrane sites. In our study, the plastoglobule sizes of microbial-inoculated wheat plants were larger, which prevented oxidative damage to the chloroplast membranes. According to Sheteiwy et al. (2022), plastoglobules grow bigger in size during salinity. Plastoglobules are lipoprotein structures that are attached to thylakoids, and they play an important role in chloroplast metabolism and stress response (Woodson 2022). In the presence of oxidative stress, they serve as sites for the synthesis of α -tocopherol. In our study, wheat plants inoculated with microbes had higher α -tocopherol levels than the control. The antioxidant activity of α -tocopherols have been linked to the protection of membrane lipids and photosystem II from photooxidation. Enhanced polyamine levels may be responsible for plastoglobule accumulation in microbially inoculated plants (Shu et al. 2015). This study showed that plants inoculated with microbes had reduced oxidative damage to chloroplasts due to their high osmolyte levels and large plastoglobule sizes. However, PGPB has a greater ability to alleviate salinity stress than *P. indica* and AMF-inoculated wheat plants at all levels of salinity in terms of maintaining the ultrastructure of the chloroplast.

Salt tolerance in plants can be predicted based on cell membrane stability (Pathiana et al. 2023). Electron micrographs showed that plasma membranes of wheat plant cells disintegrated when exposed to salinity. In response to increasing salinity levels, the

space between the plasma membrane and cell wall increases. Similar findings also were found for *Halodule wrightii* (Ferreira et al. 2017). The membrane disintegrates due to the peroxidation of polyunsaturated fatty acids by free radicals (Zhang et al. 2021) as well as salt-induced shrinkage of protoplasm. As compared to uninoculated wheat plants, microbial inoculated plants showed less shrinkage of their protoplasm and membrane damage. Furthermore, the accumulation of osmolytes (glucose, trehalose, proline, glycine betaine, and polyamines) maintains cell turgidity and stabilizes membranes and enzymes (Sharma et al. 2019). In our study, wheat plants inoculated with microbes displayed lower levels of electrolyte leakage, lipid peroxidation, and LOX activity, which are critical for membrane integrity. Moreover, wheat plants inoculated with microbes possess more antioxidant molecules (proline, α -tocopherols, and polyamines), enzymatic activity (SOD, CAT, and APX), and nutrients (Ca^{2+} and K^{+}) which play a vital role in membrane protection. In a stressful environment, proline is an effective scavenger of ROS, preventing oxidative stress from damaging cells. Furthermore, α -tocopherol scavenges free radicals in the host tissue, protecting it from lipid peroxidation (Niki 2021). Polyamines are important for membrane stability (Wang et al. 2022). Antioxidant enzymes (CAT, SOD, and APX) can scavenge ROS and protect membranes from oxidative stress in adverse environmental conditions (Ru et al. 2023). Calcium forms intermolecular links that maintain membrane integrity, stabilize cell walls, and regulate ion transport (de Bang et al. 2021). In plants, potassium plays a critical role in osmoregulation and membrane potential (Sardans and Peuelas, 2021). Microbial inoculation leads to the accumulation of osmolytes, nutrients, and antioxidants, which reduce membrane damage (Tyagi et al. 2023). In our study, more vesicles were formed in microbial-inoculated wheat plants than in the control. Under stress conditions, vesicle formation increases (Karim et al. 2014). By increasing the membrane surface area, vesicles facilitate ion transport and storage, protecting the cells from ionic imbalance. Therefore, the higher rate of vesicle formation observed in this study suggests that microbial-inoculated wheat plants are able to tolerate salinity better. However, PGPB has a greater ability to alleviate salinity stress than *P. indica* and AMF in wheat plants at all levels of salinity in terms of maintaining the ultrastructure of the plasma membrane.

4.3 PART III

To study the role of soil microbes on growth, changes in nutritional, biochemical and physiological parameters, ultrastructural changes, and antioxidant accumulation in maize under drought stress.

To accomplish the objective, the percentage of AMF and *P. indica* colonization, plant growth and biomass, macronutrients and micronutrients content, rate of physiological processes, photosynthetic pigments content, osmolytes content, lipid peroxidation, relative permeability, LOX activity, protein content, and α -tocopherol content was estimated in microbial inoculated and uninoculated maize plants under drought stress.

4.3.1 Results

4.3.1.1 Microbial colonization

AMF and *P. indica* successfully colonized the roots of maize plants at all levels of water stress. However, the percentage of colonization was higher in AMF-inoculated maize plants than in *P. indica*-inoculated plants during drought stress. Plant roots not inoculated with AMF and *P. indica* did not show microbial colonization (**Figure 4.23**) Different levels of water regime significantly affected the percent root colonization. A gradual decline in microbial colonization was observed with increasing water stress levels (**Table 4.7**).

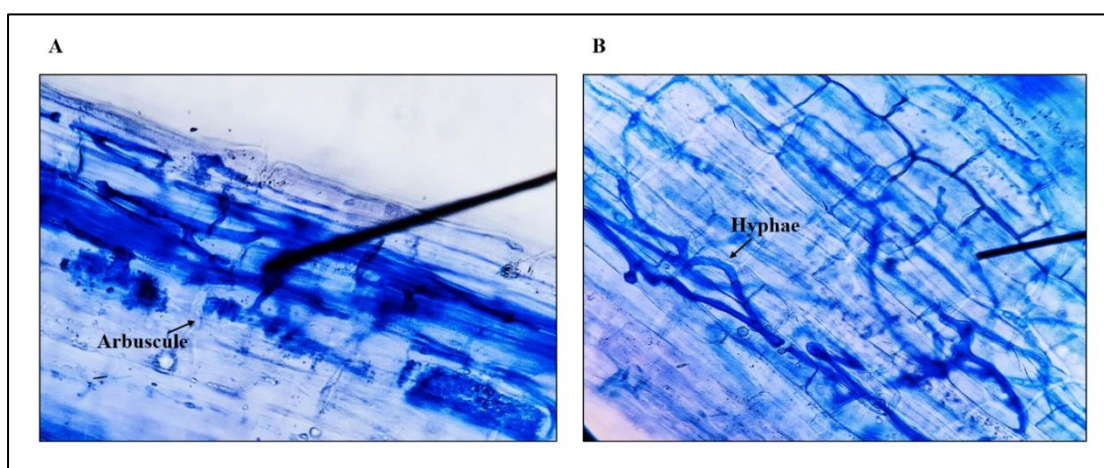


Figure 4.23: Microscopic observation of root colonization of (A) AMF-inoculated maize plants and (B) *P. indica*-inoculated maize plants under drought stress.

Table 4.7: Influence of drought stress and microbe inoculations on percentage microbe colonization, length, and biomass of shoot and root in maize plants.

FC (%)	Microbe	Shoot length (cm)	Root length (cm)	Shoot biomass (g)	Root biomass (g)	Leaf area (cm ²)	Root colonization (%)
75	Control	67.60±2.07def	24.97±0.42j	6.41±0.48gh	2.79±0.07ghi	55.58±0.87hi	-
	<i>P. indica</i>	69.10±0.17cde	29.00±1.24f	7.58±0.28de	3.83±0.57cd	63.31±2.81ef	67
	PGPB	75.33±2.01a	34.64±1.07a	8.81±0.63a	4.91±0.54a	71.66±2.52 a	-
	AMF	73.22±1.12ab	32.77±2.11c	8.11±0.08bcd	4.48±0.13ab	69.16±1.55abc	72
55	Control	66.83±1.04efg	25.69±0.68i	6.12±0.43h	2.73±0.03ghi	55.33±0.31hi	-
	<i>P. indica</i>	69.04±1.4cde	27.89±0.39gh	7.46±0.14ef	3.31±0.20ef	66.28±1.34efg	62
	PGPB	75.37±1.64a	33.77±2.13b	8.45±0.39ab	4.72±0.20ab	70.22±0.26ab	-
	AMF	73.33±0.15ab	30.89±2.59e	7.72±0.11cde	4.27±0.22bc	68.04±1.48bcd	65
45	Control	64.30±1.01g	23.44±0.74k	5.87±0.50h	2.60±0.21hi	52.82±2.01ij	-
	<i>P. indica</i>	67.33±0.55efg	27.44±1.08h	7.25±0.22ef	3.07±0.03fg	60.04±0.79g	55
	PGPB	72.00±0.38bc	32.00±2.17c	8.36±0.54ab	4.46±0.22 b	68.51±0.57bcd	-
	AMF	69.63±0.76cde	31.67±1.25d	7.30±0.06ef	3.99±0.16 cd	66.82±1.97cd	57
35	Control	55.40±1.13h	21.50±1.22l	4.4±0.26 i	2.40±0.29i	50.96±2.88j	-
	<i>P. indica</i>	65.10±0.43fg	23.56±0.78k	6.85±0.52fg	2.89±0.14fgh	56.22±2.47h	32
	PGPB	70.78±2.73bcd	30.50±1.89e	8.26±0.07abc	4.01±0.04cd	65.59±0.55de	-
	AMF	68.00±2.01def	28.23±0.15g	7.16±0.03ef	3.66±0.14de	60.75±1.08fg	37
Significance							
Drought		***	***	***	**	***	***
<i>P. indica</i>		***	***	***	***	***	***
<i>P. indica</i> ×Drought		**	***	*	ns	ns	***
PGPB		***	***	***	***	***	-
PGPB×Drought		***	***	*	ns	ns	-
AMF		***	***	***	***	***	***
AMF×Drought		**	***	**	ns	ns	***

Values represent averages across replicates. Letters within a column represent significant differences at $P < 0.05$. ns: not significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

4.3.1.2 Growth parameters

Growth parameters of microbial inoculated and uninoculated maize plants at four different water regimes are shown in **Table 4.7**. At all levels of water regimes, the microbial-inoculated maize plants performed significantly better in terms of shoot length, root length, shoot biomass, root biomass, and leaf area compared to uninoculated plants. Under severe drought conditions (35% FC), PGPB inoculation increased the shoot and root length by 27.76% and 41.86%, respectively, followed by AMF inoculation which improved it by 22.74% and 31.30% respectively, while *P. indica* inoculation increased it by 17.51% and 9.58%, respectively, compared to uninoculated plants. A 35% FC PGPB-inoculated plant showed an increase in the biomass of its shoots and roots of 84.86% and 66.25%, respectively. This was preceded by AMF inoculation which raised it by 60.25% and 51.87% each, and *P. indica* inoculation enhanced it by 53.32% and 20.06%, respectively, as opposed to uninoculated plants. The leaf area of maize plants was significantly improved with inoculation with PGPB (28.69%) followed by AMF (19.18%) and *P. indica* (10.31%) in comparison to non-inoculated maize plants. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, and AMF on plant growth, biomass, and leaf area (**Table 4.7**). On comparing the potential of microbes to increase growth parameters, PGPB-inoculated plants showed higher drought resistance as compared to AMF and *P. indica*-inoculated plants.

4.3.1.3 Mineral nutrients content

Table 4.8 provides comprehensive data on the macronutrient (Ca^{2+} , Mg^{2+} , K^+ , Na^+) and micronutrient (Zn^{2+} , Fe, Mn^{2+} , Cu) composition of maize plants subjected to drought stress and microbial colonization. Both stress and microbial inoculation significantly influenced nutrient levels. Increasing drought stress resulted in notable reductions in Ca^{2+} , Mg^{2+} , K^+ , Zn^{2+} , Fe, Mn^{2+} , and Cu levels, accompanied by an increase in Na^+ levels in both inoculated and uninoculated plants. At 35% field capacity (FC), PGPB-inoculated plants displayed the highest increase in Ca^{2+} (177.30%), followed by *P. indica*-inoculated plants (132.94%) and AMF-inoculated plants (121.94%). Similarly, at 35% FC, PGPB-inoculated plants exhibited the greatest increases in Mg^{2+} (469.95%) and K^+ (161.74%), followed by AMF-inoculated plants (231.75% and 122.25%, respectively) and *P. indica*-inoculated plants (280.93% and 30.44%, respectively).

Table 4.8: Influence of water stress and microbial inoculation on the content of macro-nutrients and micro-nutrients in leaves of the maize plants.

FC (%)	Microbe	Macronutrients (mg kg ⁻¹)				Micronutrients (mg kg ⁻¹)			
		Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	Zn ²⁺	Fe	Mn ²⁺	Cu
75	Control	541.06±32.37g	2734.83±101.62e	2493.63±38.49h	487.30±13.06j	66.74±4.55cd	543.80±24.62ef	54.81±3.52e	93.80±4.96de
	<i>P. indica</i>	1055.16±26.47b	5183.37±45.32 a	4945.35±28.39a	483.62±29.27j	107.68±8.43ab	835.40±24.62b	69.42±3.53d	227.80±6.67b
	PGPB	949.67±44.37c	3798.56±68.59b	4495.43±74.64b	374.08±29.22k	97.21±5.40b	847.11±10.76b	88.37±2.16a	334.27±4.96a
	AMF	837.95±29.77d	3154.26±30.58d	3400.94±39.46d	451.37±26.89j	113.04±8.07a	741.52±12.44c	74.89±5.10cd	95.14±7.11de
55	Control	545.89±45.54g	2309.10±127.71f	2480.20±2.59h	659.84±26.89fg	41.95±8.63f	448.84±17.35g	53.69±5.39e	66.91±4.06ef
	<i>P. indica</i>	1136.63±33.55a	2851.90±118.47e	4072.05±65.40c	609.88±38.43ghi	76.40±1.94c	825.11±39.20b	69.05±3.04d	127.18±4.96c
	PGPB	731.74±27.94e	3760.80±19.89b	3327.45±82.73d	556.29±26.89i	72.13±11.69c	779.39±15.12c	73.48±4.84cd	127.58±6.89c
	AMF	629.98±23.24f	2266.02±117.35h	2657.13±39.08g	596.15±38.43i	74.12±12.85b	816.30±10.58f	72.08±4.25cd	90.87±6.89de
45	Control	414.92±21.41h	673.37±107.88 k	1453.35±29.79k	937.69±25.51b	27.68±7.54g	327.40±24.62h	51.23±4.90ef	55.25±8.16fg
	<i>P. indica</i>	821.83±28.24d	1102.03±56.26i	1760.18±17.63j	671.50±29.22ef	65.48±3.51cd	747.26±32.20c	54.42±4.67e	93.49±9.95de
	PGPB	708.65±15.85e	3499.70±38.28c	3028.75±26.15f	600.04±41.27hi	56.32±5.67de	756.25±21.00c	67.34±3.19de	103.34±0.48cd
	AMF	558.33±23.81g	2162.46±107.78g	2335.51±27.48i	652.947±46.52fgh	66.03±8.47cd	914.88±16.77a	68.03±5.32d	99.08±4.52cd
35	Control	230.84±26.45i	287.56±81.83l	643.91±2919m	1030.80±23.41a	23.17±2.66g	261.46±29.58i	37.78±4.48g	20.65±9.79h
	<i>P. indica</i>	537.72±33.10g	1095.39±86.83i	839.89±36.46l	861.54±25.51c	50.38±9.17ef	584.10±10.58d	43.76±6.67fg	37.17±4.04gh
	PGPB	640.11±17.90f	1638.93±47.44h	1685.34±23.24j	717.81±27.27e	56.13±2.97de	579.32±17.26de	49.30±4.85ef	96.47±4.52de
	AMF	512.31±11.76g	953.97±32.75j	1431.10±14.38k	801.28±28.93d	54.43±9.54def	772.63±12.44c	45.82±5.13fg	89.94±4.52de
Significance									
Drought		***	***	***	***	***	***	***	**
<i>P. indica</i>		***	***	***	***	***	***	Ns	***
<i>P. indica</i> × Drought		***	***	***	***	ns	***	***	***
PGPB		***	***	***	***	***	***	***	***
PGPB × Drought		***	***	***	***	ns	***	***	***
AMF		***	***	***	***	***	***	Ns	*
AMF × Drought		***	***	***	***	ns	***	**	***

Values represent the mean of replicates. Different letters within a column indicate significant differences at P<0.05. ns: not significant. ***P<0.001, **P<0.01, *P<0.05.

Furthermore, at 35% FC, PGPB-inoculated plants showed the highest decrease in Na^+ content (30.36%), followed by *P. indica*-inoculated plants (22.27%), and AMF-inoculated plants (16.42%). Regarding micronutrients, at 35% FC, PGPB, AMF, and *P. indica*-inoculated plants exhibited elevated levels of Zn^{2+} (2.42, 2.35, and 2.17-fold increase, respectively), Fe (1.30, 2.96, and 2.23-fold increase, respectively), Mn^{2+} (2.22, 1.21, and 1.16-fold increase, respectively), and Cu (4.67, 4.36, and 1.80-fold increase, respectively) compared to non-inoculated plants. Notably, AMF showed superior efficacy in mitigating drought stress in terms of Fe content when compared to *P. indica* and PGPB. Overall, PGPB-inoculated plants consistently exhibited enhanced levels of Ca^{2+} , Mg^{2+} , K^+ , Zn^{2+} , Mn^{2+} , and Cu content, along with reduced Na^+ content in leaves, relative to plants inoculated with AMF and *P. indica*.

4.3.1.4 Physiological parameters

Physiological parameters of microbial-inoculated and uninoculated maize plants at four different water regimes are depicted in **Figure 4.24**. At all levels of water regimes, microbial-inoculated maize plants showed substantially higher photosynthetic rate, transpiration rate, stomatal conductance, and internal CO_2 levels than uninoculated maize plants. PGPB (357.03%) and AMF (282.44%) significantly increased photosynthetic rates in comparison to uninoculated plants at 35% FC stress, led by *P. indica* (137.37%). The stomatal conductance of maize plants was significantly enhanced with inoculation with PGPB (197.08%) followed by AMF (184.58%) and *P. indica* (155%) in comparison to uninoculated maize plants. The transpiration rate of maize plants inoculated with PGPB, AMF and *P. indica* increased to 243.98%, 174%, and 169.11% respectively at 35% FC. Plants inoculated with PGPB had an internal CO_2 increase of 369.03% under severe drought conditions, while plants inoculated with AMF and *P. indica* had an increase of 328.73% and 203.71%, respectively, in contrast to uninoculated plants. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, and AMF physiological parameters (**Table 4.9**). Therefore, this indicates that PGPB is the most efficient microbe in alleviating drought stress at all levels of the water regime as compared to AMF and *P. indica*.

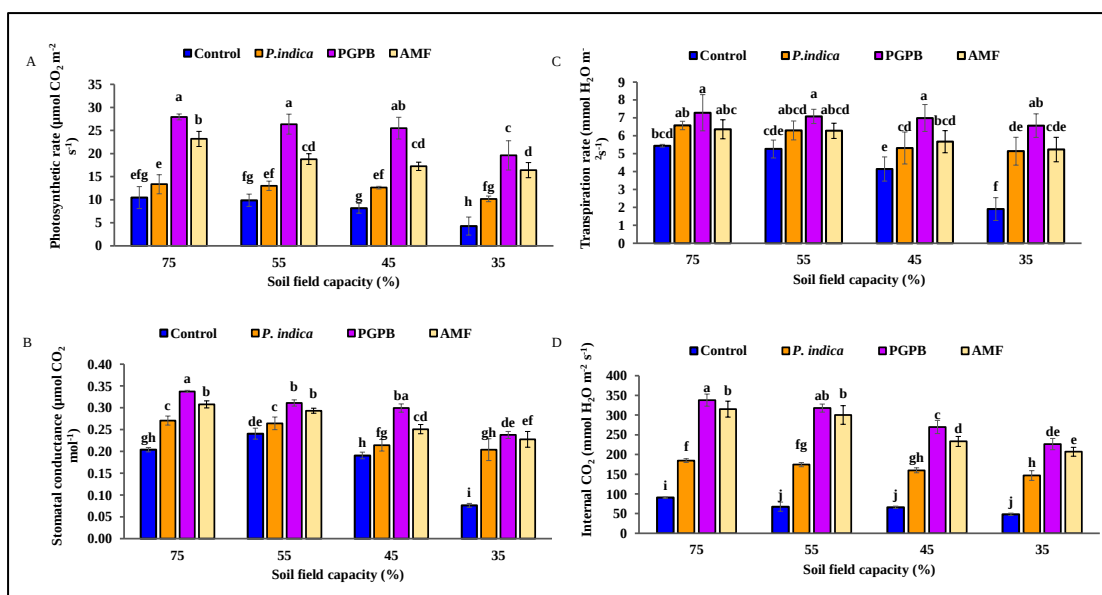


Figure 4.24: Effects of drought and microbial inoculation in maize on (A) photosynthetic rate; (B) stomatal conductance; (C) transpiration rate; and (D) internal CO_2 . Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.3.1.5 Biochemical parameters

4.3.1.5.1 Photosynthetic pigment content

Photosynthetic pigments viz. chlorophyll a, chlorophyll b, total chlorophylls, and carotenoid content in microbial-inoculated maize plants showed substantially higher levels than uninoculated maize plants at all levels of water regimes (**Figure 4.25**). With increasing water stress levels plants' photosynthetic pigment content significantly decreases. At the highest level of drought (35% FC), the chlorophyll a content of maize plants inoculated with PGPB, AMF and *P. indica* increased to 190.18%, 172.73%, and 139.71% respectively as compared to uninoculated plants. Plants inoculated with PGPB had an increased chlorophyll b content of 60.52% under severe drought conditions, while plants inoculated with AMF and *P. indica* had an increase of 46.39% and 41.18%, respectively. The total chlorophyll content was higher in PGPB-inoculated (111.19%) compared to AMF (95.77%) and *P. indica* (79.68%) as compared to the control at the highest level of stress. At 35% FC carotenoid content was higher in PGPB (58.76%) inoculated plants, followed by AMF (37.15%) and *P. indica* (28.42%) inoculated plants in comparison to uninoculated plants. Two-way ANOVA

demonstrated a significant independent effect of drought, *P. indica*, PGPB, AMF, and interaction of drought $\times$ *P. indica*, drought $\times$ PGPB, and drought $\times$ AMF on chlorophyll a and total chlorophyll content (**Table 4.9**). Therefore, this indicates that PGPB is the most efficient microbe in alleviating drought stress at all levels of the water regime as compared to AMF and *P. indica* in maize plants.

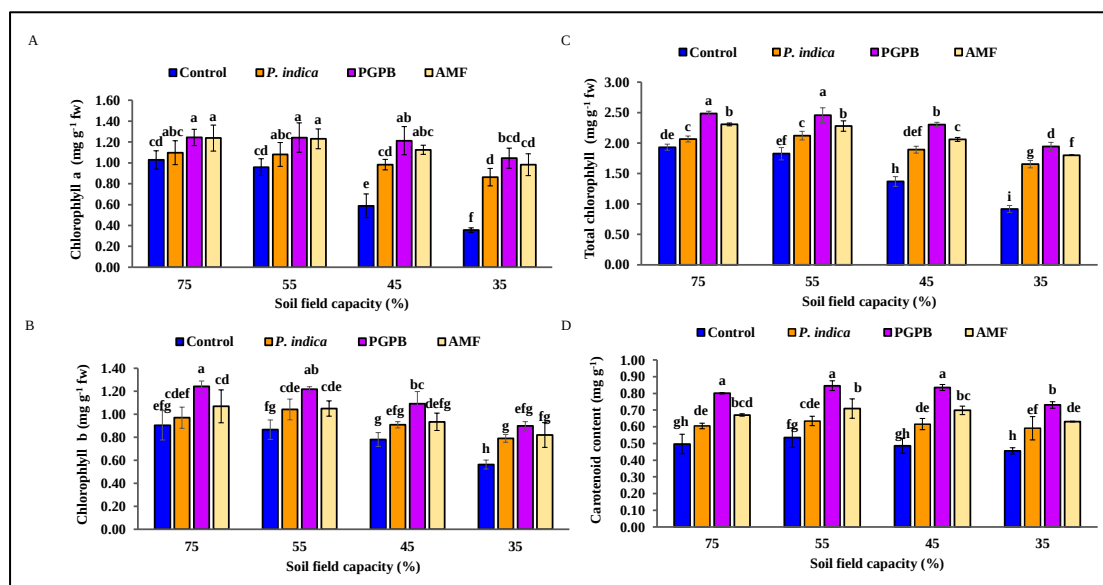


Figure 4.25: Effects of drought and microbial inoculation in maize on the content of (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; and (D) carotenoid. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.3.1.5.2 Osmolyte content

With increasing water stress levels, the content of glycine betaine exhibited a linear and significant increase in leaves of microbial-inoculated and uninoculated maize plants (**Figure 4.26A**). Comparing 35% FC and 75% FC glycine betaine content increased by about 1.9-fold in AMF-inoculated plants, 1.6-fold in *P. indica* plants, and 1.5-fold in uninoculated plants. As compared to uninoculated plants, *P. indica* and AMF-inoculated plants had significantly higher glycine betaine content at all water regimes. At 35% FC, AMF-inoculated plants had a 1.2-fold higher leaf glycine betaine content than the control, which was led by *P. indica*-inoculated plants (1.1-fold). PGPB inoculation did not affect glycine betaine content, which decreased with increasing

drought stress levels. Thus, AMF outperforms PGPB and *P. indica* at all levels of water regimes in alleviating drought stress.

In both microbial inoculated and uninoculated maize plants, total sugar content increases with increased levels of drought stress in maize plants (**Figure 4.26B**). At all levels of drought stress, however, microbes facilitated higher total sugar accumulation in leaves than in corresponding uninoculated. Plants inoculated with PGPB displayed 2.5, 1.6, 1.7, and 1.8-fold higher total sugar content than plants uninoculated at 75%, 55%, 45%, and 35% FC, respectively. Total sugar content in AMF-inoculated plants was 2.4, 1.5, 1.5, and 1.4-fold greater than that of uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. As a result of inoculation with *P. indica*, total sugar content increased 1.3, 1.3, 1.3, and 1.2-fold when compared with uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, AMF, and interaction of drought $\times$ *P. indica*, drought $\times$ PGPB, and drought $\times$ AMF on trehalose content (**Table 4.9**). Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF-inoculated plants. Water stress and microbial inoculation significantly enhanced total sugar content.

In microbial-inoculated and uninoculated maize plants, the amount of trehalose increased linearly with increasing levels of water stress (**Figure 4.26C**). A significantly higher trehalose content was detected in microbial-inoculated plants compared to uninoculated plants at all water regimes. Trehalose content in PGPB-inoculated plants was 3.0, 1.8, 2.5, and 2.1-fold higher than in uninoculated plants at 75%, 55%, 45%, and 35% FC respectively. Plants inoculated with AMF had trehalose contents of 2.5, 1.6, 2.1, and 1.9-fold higher than uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. In *P. indica* inoculated plants, trehalose content was 2.4, 1.3, 1.6, and 1.3-fold higher than in uninoculated plants at 75%, 55%, 45%, and 35% FC. Therefore, PGPB is more efficient than AMF and *P. indica* inoculated plants at all water regimes in alleviating drought stress. Trehalose content was significantly enhanced by water stress and microbial inoculation interaction.

Proline content in maize leaves was significantly influenced by water stress and microbial inoculation (**Figure 4.26D**). Both microbial inoculated and uninoculated maize leaves exhibited an increase in proline content under water stress. However, microbial-inoculated maize plants showed considerably better performance in terms of proline content compared to uninoculated plants at all levels of water regimes. At 35% FC, PGPB-inoculated plants had a 49.80% higher leaf proline content than control, which was led by AMF-inoculated plants (36.73%) and *P. indica*-inoculated plants (32.34%).

Water stress and microbial inoculation significantly influenced polyamine content (putrescine and spermidine). However, spermine was not detected in both microbial-inoculated or uninoculated plants at all water regimes. Increasing the level of water stress to plants showed a contrasting effect on the content of putrescine and spermidine in microbial inoculated and uninoculated plants (**Figure 4.26E-F**). PGPB, AMF, *P. indica*, and uninoculated plants showed an increase in putrescine content by 3.1, 2.9, 5.0, and 2.7-fold respectively, at 35% FC, as compared to well-watered plants. Spermidine content recorded 1.3, 1.3, and 1.1-fold increases in AMF, PGPB, and *P. indica* inoculated plants at 35% FC respectively, over uninoculated plants. Spermidine content in PGPB-inoculated plants was 2.7, 2.0, 1.4, and 1.3-fold higher than in uninoculated plants at 75%, 55%, 45%, and 35% FC respectively. Plants inoculated with AMF had spermidine contents of 2.8, 2.1, 1.5, and 1.3-fold higher than uninoculated plants at 75%, 55%, 45%, and 35% FC, respectively. In *P. indica* inoculated plants, spermidine content was 2.2, 1.4, 1.0, and 1.1-fold higher than in uninoculated plants at 75%, 55%, 45%, and 35% FC respectively. However, microbial-inoculated plants recorded higher putrescine and spermidine content over uninoculated plants at all water regimes. Thus, AMF outperforms PGPB and *P. indica* at all levels of water regimes in alleviating drought stress in terms of polyamine content. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, and AMF on glycine betaine, trehalose, proline, and polyamine content (**Table 4.9**).

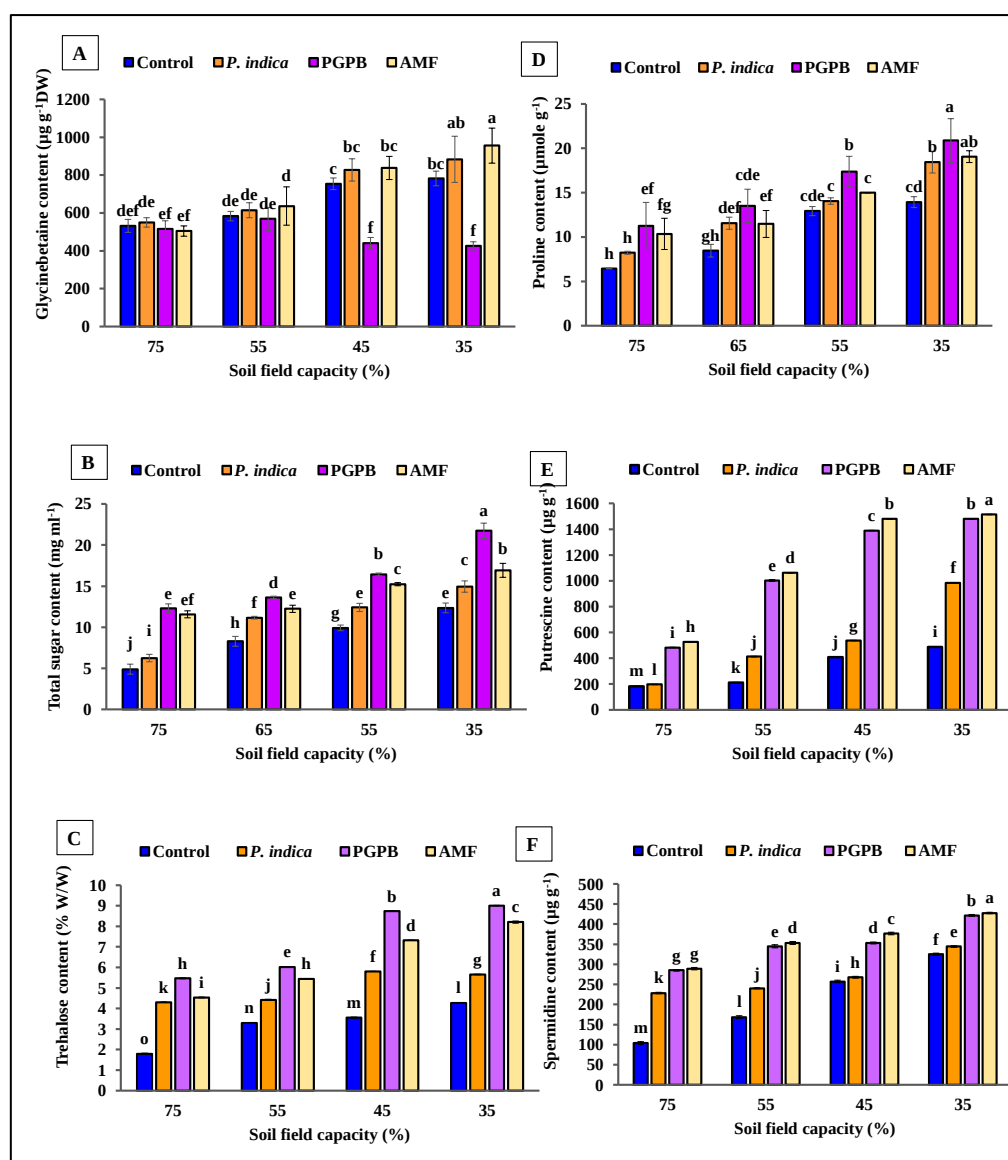


Figure 4.26: Effects of drought and microbial inoculation in maize on the content of (A) glycine betaine; (B) total sugar; (C) trehalose; (D) proline; (E) putrescine; and (F) spermidine. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.3.1.5.3 Protein

Water stress significantly increased protein content in both microbial inoculated and uninoculated maize plants till 55% FC followed by a decrease at 45% and 35% FC. However, under water stress, microbial-inoculated plants possessed significantly higher levels of protein than uninoculated plants (**Figure 4.27A**). As compared with uninoculated maize, PGPB, AMF, and *P. indica* inoculated plants produced 139.80%, 119.13%, and

86.79% more protein respectively at 35% FC. Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF-inoculated plants. Water stress and microbial inoculation significantly enhanced protein content.

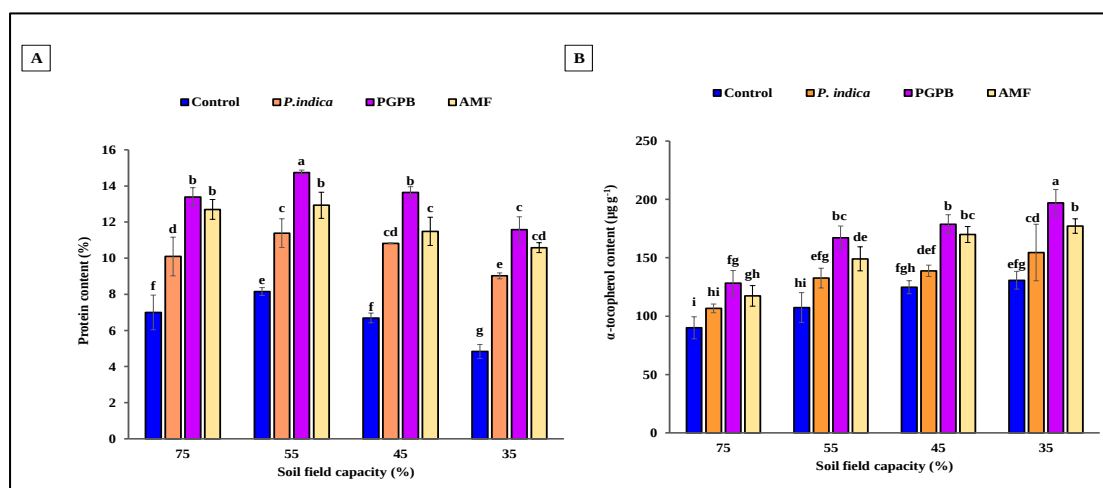


Figure 4.27: Effects of drought and microbial inoculation in maize on the content of (A) protein; and (B) α -tocopherol. Values represent averages across replicates. Different letters represent significant differences at $P < 0.055$.

4.3.1.5.4 α -tocopherol content

Water stress significantly increased α -tocopherol content in both microbial inoculated and uninoculated maize plants. However, under water stress, microbial-inoculated plants possessed significantly higher levels of α -tocopherol than uninoculated plants (**Figure 4.27B**). As compared with uninoculated maize, PGPB, AMF, and *P. indica* inoculated plants produced 50.73%, 35.48%, and 18.16% more α -tocopherol respectively at 35% FC. Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF-inoculated plants. Water stress and microbial inoculation significantly enhanced α -tocopherol content.

4.3.1.5.5 Membrane damage

In **Figure 4.28A**, the concentration of MDA is used to measure the levels of lipid peroxidation in leaves of maize plants exposed to different levels of water stress. In both microbial inoculated and uninoculated maize plants, exposure to water stress significantly increased MDA levels. However, uninoculated plants have a higher MDA

content. At all water regimes, microbial-inoculated plants had significantly lower MDA levels. The MDA content of *P. indica*, AMF, and PGPB-inoculated plants decreased by 23.01%, 35.10%, and 41.55% over uninoculated plants at 35% FC, respectively. The relative membrane permeability of maize plants grown under water stress was significantly affected by microbial inoculation and water stress. Electrolyte leakage increased in maize plants in response to water stress. PGPB, AMF, and *P. indica* inoculated plants maintained lower electrolyte leakage at all water stress levels than uninoculated maize (Figure 4.28B).

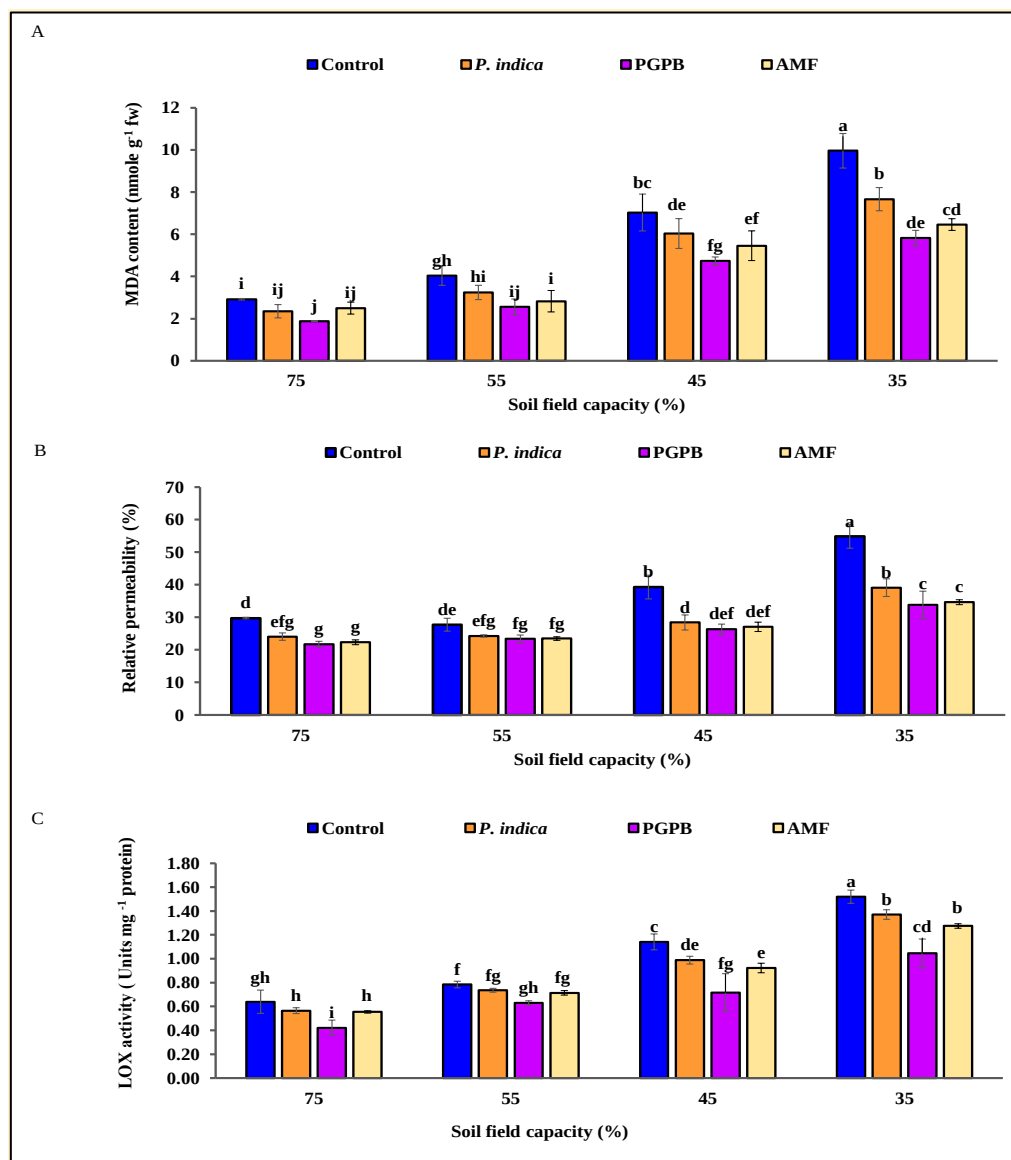


Figure 4.23: Effects of drought and microbial inoculation in maize on (A) MDA content; and (B) relative permeability (C) LOX activity. Values represent averages across replicates. Letters within a column represent significant differences at $P < 0.055$.

At 35% FC, PGPB, AMF, and *P. indica* inoculated plants showed 38.36%, 36.86%, and 28.69% lower relative permeability, respectively than uninoculated plants. Water stress increased LOX activity in both microbial-inoculated and uninoculated plants; however, a significant difference in LOX activity was observed between these plants (**Figure 4.28C**). Plants inoculated with microbes displayed significantly lower LOX activity. At 35% FC, PGPB, AMF, and *P. indica* inoculated plants showed 31.23%, 16.14%, and 9.82% lower LOX activity, respectively than uninoculated plants. All levels of water stress, however, showed lower activity of LOX in PGPB-inoculated plants than in AMF and *P. indica*. Two-way ANOVA demonstrated a significant independent effect of drought, *P. indica*, PGPB, AMF, and interaction of drought $\times$ *P. indica*, and drought $\times$ PGPB on membrane damage (**Table 4.9**). Thus, at all water regimes, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF in maize plants.

4.3.1.6 Antioxidant enzyme activity

Under water stress and well-watered conditions, microbial colonization enhanced SOD activity in maize plants (**Figure 4.29A**). Water stress increased SOD activity in microbial and uninoculated maize plants. Under severe drought conditions (35% FC), PGPB inoculation raised SOD activity by 22.30% followed by AMF inoculation which improved it by 19.36% while *P. indica* inoculation increased it by 12.47% compared to uninoculated maize plants. With increasing levels of water stress, PGPB and *P. indica*-inoculated maize plants displayed increased CAT activity. Conversely, AMF-inoculated plants exhibited decreased CAT activity in response to increased water stress in comparison to the control. Plants inoculated with PGPB increased CAT activity. Conversely, AMF-inoculated plants exhibited decreased CAT activity in response to increased water stress in comparison to the control. Plants inoculated with PGPB and *P. indica* displayed CAT activity increases of 54.13% and 24.21% as opposed to uninoculated maize plants respectively at 35% FC (**Figure 4.29B**). Microbial inoculated plants displayed higher APX activity than uninoculated plants under all water regimes. At each level of water stress, PGPB-inoculated plants exhibited significantly higher APX activity than *P. indica* and AMF-inoculated maize plants. Plants inoculated with PGPB had APX activities 1.34, 1.23, 1.43, and 1.43-fold higher than those uninoculated maize plants at 75%, 55%, 45%, and 35% FC, respectively.

Maize plants inoculated with AMF had APX activities 1.24, 1.13, 1.37, and 1.28-fold higher than those uninoculated maize plants at 75%, 55%, 45%, and 35% FC, respectively. At all levels of water stress, *P. indica* inoculated maize plants showed 1.1-fold higher APX activity than uninoculated plants (Figure 4.29C).

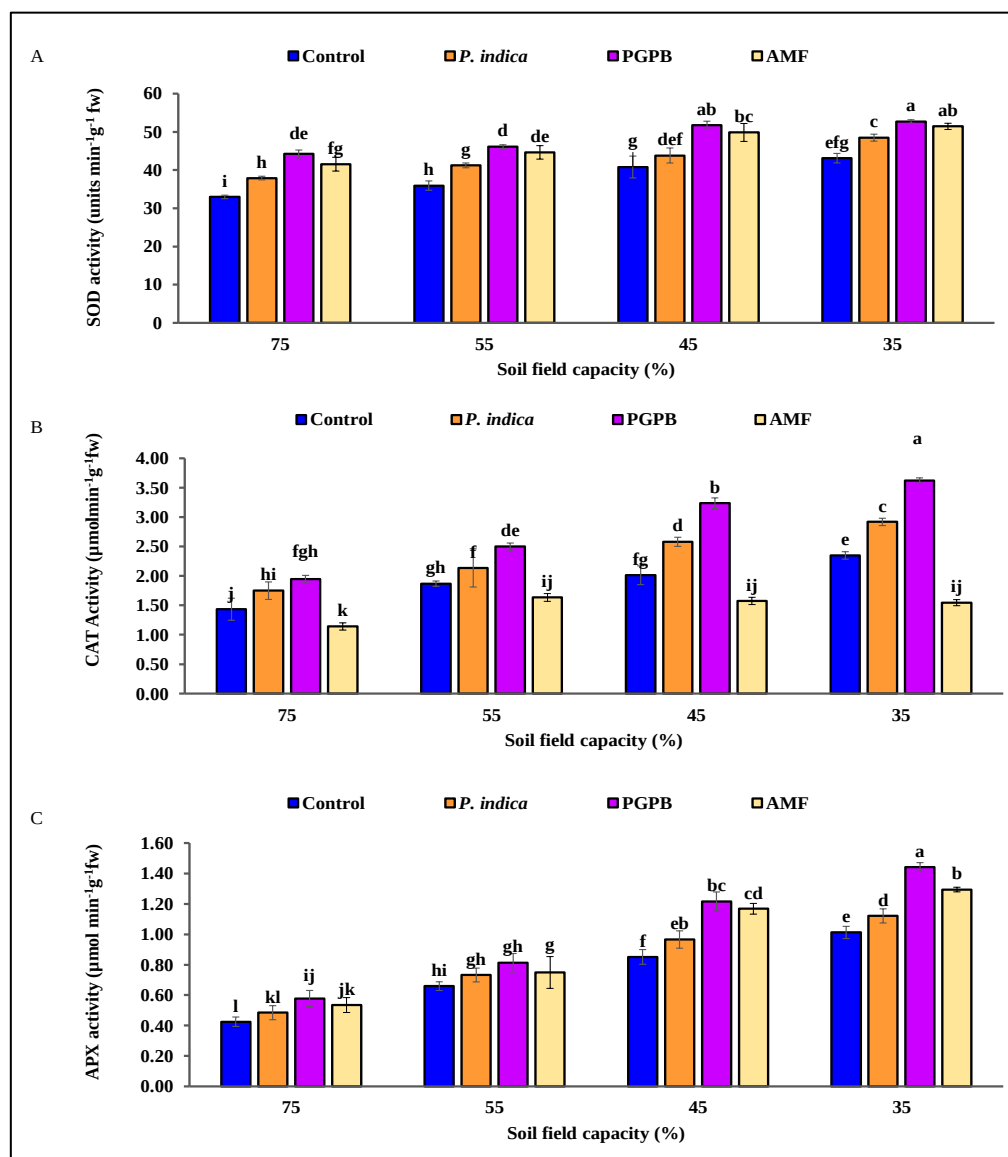


Figure 4.29: Effects of drought and microbial inoculation in maize on the activity of (A) SOD; (B) CAT; and (C) APX. Values represent averages across replicates. Different letters represent significant differences at P<0.05.

4.3.1.7 Cell ultrastructural studies

At all water regimes, the electron micrographs show qualitative differences between microbial inoculated and uninoculated maize plants.

4.3.1.7.1 Chloroplast

Figures 4.30 and **4.31** show changes in chloroplast ultrastructure in microbial-inoculated and uninoculated maize leaves under water stress. Microbial inoculated and uninoculated maize plants displayed chloroplasts with distinct thylakoids and grana surrounded by double-layered membranes under well-watered conditions (75% FC). However, PGPB-inoculated plants exhibited a greater number of thylakoids and granum than AMF and *P. indica*-inoculated plants. Under 55% FC water stress, uninoculated plants showed decreased numbers of thylakoids and grana, and increased numbers of plastoglobules. Under low drought stress, the thylakoid membrane contracted thereby reducing the thylakoid lumen (**Figures 4.30** and **4.31**). The chloroplast structures were almost the same in microbial-inoculated plants at 55% FC and 75% FC. A 45% water stress led to swelling in the thylakoid lumen resulting in destacking of grana. Moreover, the accumulation of smaller-size plastoglobules was more in uninoculated plants. Microbial-inoculated plants showed chloroplasts with intact thylakoids and grana and fewer but large size plastoglobules accumulation, though the size of the plastoglobules was large. In contrast, plants inoculated with PGPB displayed more intact thylakoids, grana, and a lesser accumulation of plastoglobules than AMF or *P. indica*. Consequently, at 35% FC, the chloroplast in uninoculated plants was characterized by disorganized thylakoids and grana and the accumulation of a large number of smaller-sized plastoglobules (**Figure 4.30D**). Contrary to this, microbial-inoculated plants contained intact thylakoids, disorganized grana, and fewer but larger-sized plastoglobules. As compared to AMF and *P. indica*, plants inoculated with PGPB showed more intact thylakoids and fewer but large-size plastoglobules at severe drought conditions.

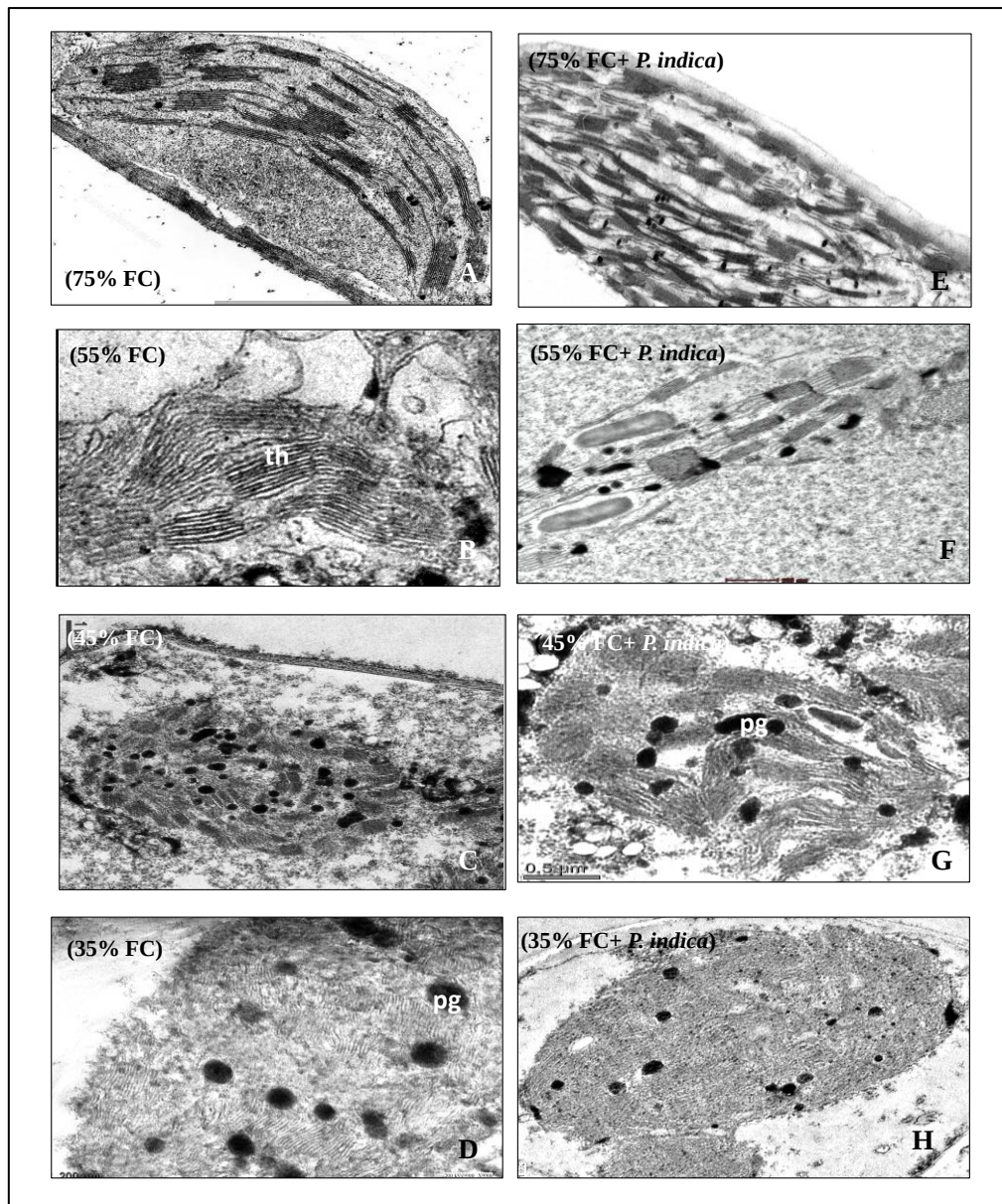


Figure 4.30: Transmission electron micrographs of chloroplast in mesophyll cells of (A) Control maize plant not exposed to drought stress (75% FC); distinct thylakoids and grana surrounded by a well-defined double-layered membrane. (B) Control maize plant subjected to 55% FC; with a lesser number of thylakoids and grana. Some plastoglobules are seen. (C) Control maize plant exposed to 45% FC; disorganized thylakoids and grana. Increased accumulation of plastoglobules. (D) The control maize plant was subjected to 35% FC; disorganized thylakoids and grana. Accumulation of a large number of plastoglobules. (E) *P. indica* inoculated maize plant not exposed to drought stress (75% FC); well-developed thylakoids and properly stacked grana surrounded by a double membrane. (F) *P. indica* maize inoculated plant subjected to 55% FC; more grana and fewer plastoglobules. (G) *P. indica* inoculated maize plant subjected to 45% FC; structure of thylakoids and grana is still maintained. Fewer plastoglobules are seen. (H) *P. indica* inoculated maize plant subjected to 35% FC; intact thylakoids and fewer large plastoglobuli visible. Slight disintegration of grana. The values in the bracket indicate the level of drought stress imposed on plants.

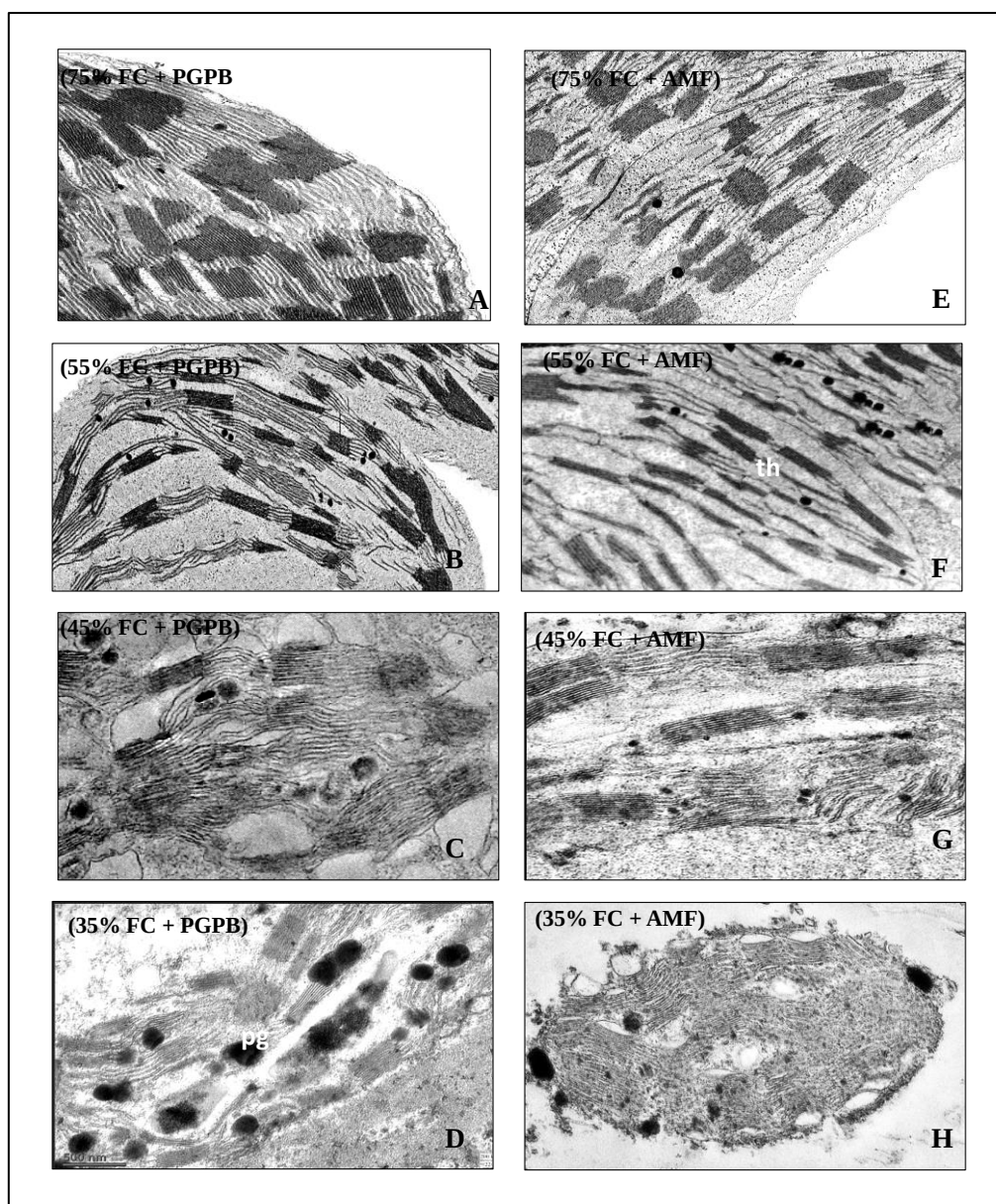


Figure 4.31: Transmission electron micrographs of chloroplast in mesophyll cells of (A) PGPB-inoculated maize plant not exposed to drought stress (75% FC); distinct thylakoids and well-developed grana surrounded double-layered membrane. (B) PGPB-inoculated maize plant subjected to 55% FC; intact thylakoids and grana with fewer plastoglobules. (C) PGPB-inoculated maize plant exposed to 45% FC; grana organization still maintained and fewer plastoglobules. (D) PGPB-inoculated maize plant subjected to 35% FC; large-sized plastoglobules have accumulated; structure of grana and thylakoid is still maintained. (E) AMF-inoculated maize plant not exposed to drought stress (75% FC); properly stacked grana in the stroma (F) AMF-inoculated maize plant subjected to 55% FC; fewer thylakoids and plastoglobules. (G) AMF-inoculated maize plant subjected to 45% FC; the chloroplast shows intact thylakoids and increased accumulation of plastoglobules. (H) AMF-inoculated maize plant subjected to 35% FC; fewer large plastoglobules have accumulated. Intact thylakoids and disorganized grana were seen. The values in the bracket denote the level of drought stress imposed on plants.

4.3.1.7.2 Plasma membrane

Under well-watered (75% FC) conditions the plasma membrane of both microbial inoculated and uninoculated maize plants closely adhered to the cell wall (**Figures 4.32 and 4.33**). As a result of water stress, the plasma membrane detached from the cell wall, forming an apoplastic space. In response to increased water stress, the space between the plasma membrane and the cell wall became larger. Plasma membrane detachment, however, was less pronounced in microbial-inoculated plants, resulting in a smaller cell wall-plasma membrane gap. At all levels of water stress, PGPB-inoculated plants showed comparatively less plasma membrane separation from the cell wall than AMF and *P. indica*. Furthermore, at higher water stress levels, invaginations of the plasma membrane led to the formation of vesicles in the cytoplasm. Compared with their microbial-inoculated counterparts, uninoculated plants displayed less vesicle formation.

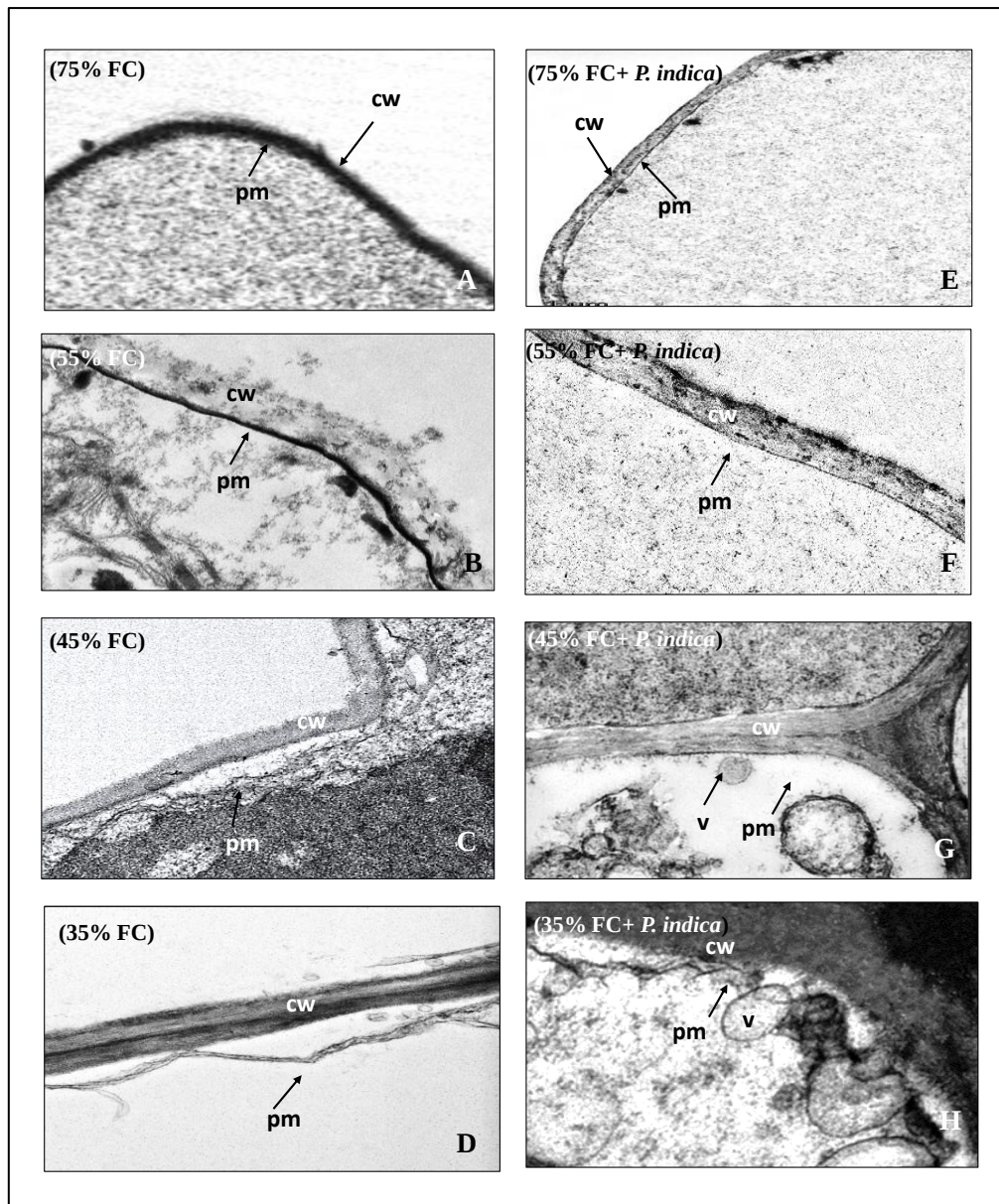


Figure 4.32: Transmission electron micrographs of the cell wall and plasma membrane in mesophyll cells of (A) Control maize plants not exposed to drought stress (75% FC); plasma membrane closely appressed to the cell wall. (B) Control plant subjected to 55% FC stress; receding plasma membrane. (C) Control maize plants exposed to 45% FC stress; increased space between the plasma membrane and cell wall; vesicles initiated. (D) Control maize plants subjected to 35% FC stress; tremendous increase in the apoplastic space between the plasma membrane and cell wall. (E) *P. indica* inoculated maize plants not exposed to drought stress (75% FC); well-preserved plasma membrane in close association with the cell wall. (F) *P. indica* inoculated maize plants subjected to 55% FC stress; plasma membrane in close association with the cell wall. (G) *P. indica* inoculated maize plants subjected to 45% FC stress; incipient plasmolysis. (H) *P. indica* inoculated maize plants subjected to 35% FC stress; a slight increase in the space between the cell wall and plasma membrane. The values in the bracket denote the level of water regimes imposed on plants. cw = cell wall; pm = plasma membrane; v = vesicle.

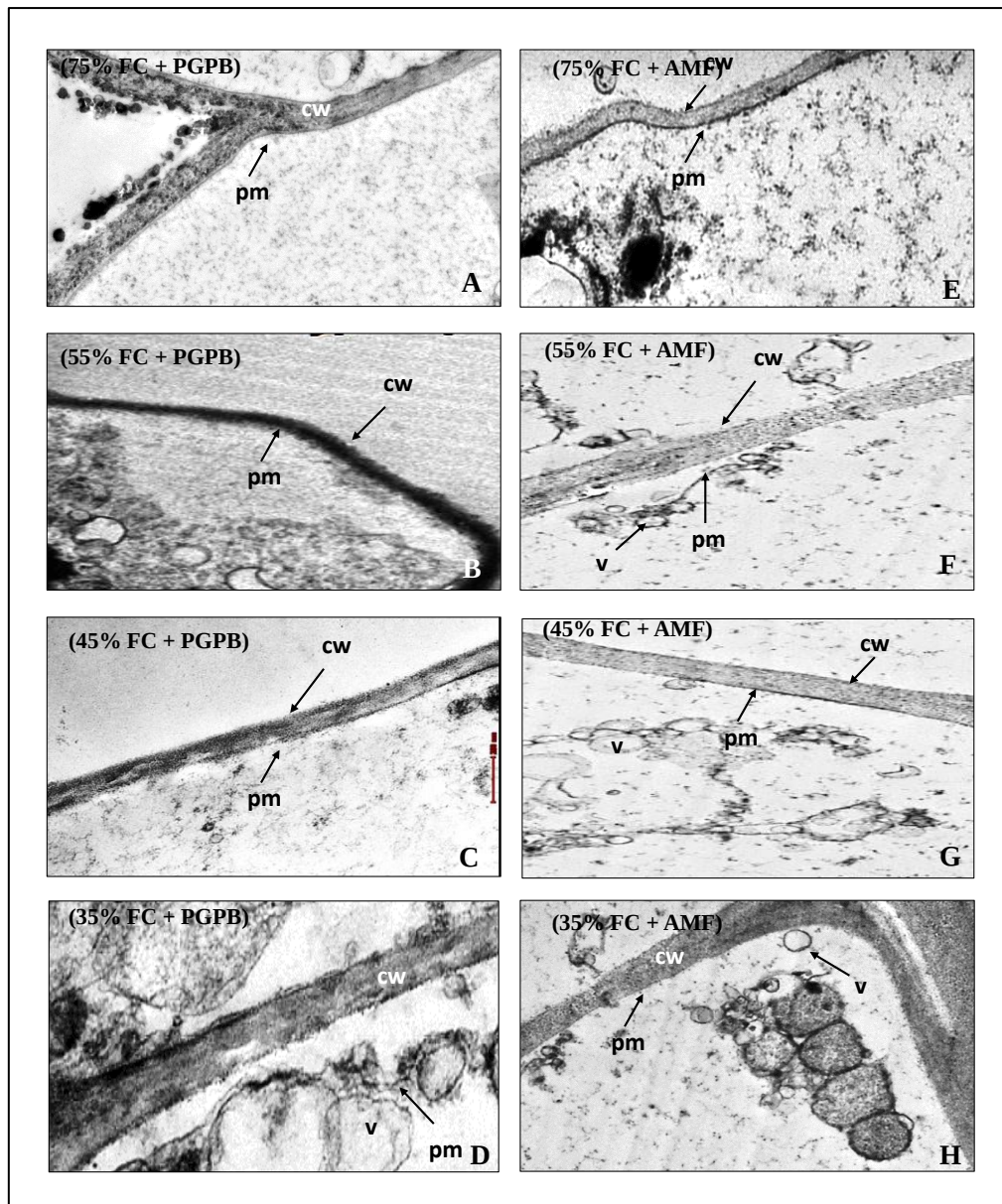


Figure 4.33: Transmission electron micrographs of the cell wall and plasma membrane in mesophyll cells of (A) PGPB-inoculated maize plants not exposed to drought stress (75% FC); plasma membrane closely appressed to the cell wall. (B) PGPB-inoculated maize plants subjected to 55% FC stress; plasma membrane closely appressed to the cell wall. (C) PGPB-inoculated maize plants exposed to 45% FC; plasma membrane well preserved. (D) PGPB-inoculated maize plants subjected to 35% FC stress; plasma membrane structure still intact. (E) AMF-inoculated maize plants not exposed to drought stress (75% FC); well-preserved plasma membrane in close association with the cell wall. (F) AMF-inoculated maize plants subjected to 55% FC; well-preserved plasma membrane in close association with the cell wall. (G) AMF-inoculated maize plants subjected to 45% FC stress; plasma membrane slightly shrank from the cell wall; vesicle formation is seen. (H) AMF-inoculated maize plants subjected to 35% FC stress; plasma membrane slightly detached from the cell wall. The values in the bracket denote the level of water regimes imposed on plants. cw = cell wall; pm = plasma membrane; v = vesicle.

Table 4.9: Effect of drought, *P. indica*, drought × *P. indica*, PGPB, drought × PGPB, AMF, drought × AMF on physiological processes, photosynthetic pigments, protein, α-tocopherol, membrane damage, osmolytes contents, and antioxidant enzyme activity in maize plants.

Parameters	Drought	<i>P. indica</i>	Drought × <i>P. indica</i>	PGPB	Drought × PGPB	AMF	Drought × AMF
Photosynthetic rate	***	***	ns	***	ns	***	ns
Transpiration	***	***	**	***	**	***	**
Stomatal conductance	***	***	***	***	***	***	***
Internal CO ₂	***	***	ns	***	***	***	***
Chlorophyll a	***	***	***	***	***	***	**
Chlorophyll b	***	***	ns	***	ns	***	ns
Total chlorophyll	***	***	***	***	***	***	***
Carotenoid	ns	***	ns	***	ns	***	ns
Protein	ns	***	ns	***	ns	***	ns
α-tocopherol	***	***	ns	***	ns	***	ns
Lipid peroxidation	***	***	ns	***	***	***	**
Relative permeability	***	***	**	***	***	***	***
LOX	***	***	ns	***	**	***	**
Trehalose	***	***	***	***	***	***	***
Total sugars	***	***	ns	ns	***	***	**
Proline	***	***	**	***	ns	***	ns
Glycine betaine	***	*	ns	***	***	**	ns
Putrescine	***	***	***	***	***	***	***
Spermidine	***	***	***	***	***	***	***
SOD	***	***	ns	***	ns	***	ns
CAT	***	***	ns	***	***	***	***
APX	***	***	ns	***	***	***	**

Significant interactions at $P < 0.05$. ns: not significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. AMF, arbuscular mycorrhizal fungi; PGPB, plant growth-promoting bacteria; LOX, lipoxygenase; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase.

4.3.2 Discussion

In the present study, we observed that the ability of AMF and *P. indica* to colonize maize roots decreased with increasing drought stress levels. The results are consistent with previous studies in which drought stress reduces hyphal growth and decreases mycorrhizal colonization (Ostadi et al. 2022). The colonization of AMF and *P. indica*

decreased under severe drought-stress conditions. This resulted in a decrease in shoot and root mass, suggesting that the beneficial effects of AMF and *P. indica* depend on root colonization.

Both microbial-inoculated plants and uninoculated maize plants under drought stress showed reduced plant growth. This finding may be explained by reduced nutrient uptake and photosynthesis in the plant due to drought stress (Ullah and Farooq 2022). However, drought-stressed maize plants inoculated with PGPB, AMF, and *P. indica* exhibited larger shoots and roots, and greater biomass levels than uninoculated plants. Our study's results agree with earlier reports on AMF colonization in *Dalbergia sissoo* (Bhardwaj et al. 2023), and *Commiphora myrrha* (Birhane et al. 2023). Based on the results of a study by Ghabooli and Kaboosi 2022, inoculating *Solanum lycopersicum* with *P. indica* under water stress conditions enhanced root and shoot biomass and root volume. We found that microbial-inoculated maize plants had higher leaf area than uninoculated maize plants under drought stress. Oliveira et al. 2022 reported that AMF-inoculated soybean plants had increased leaf area as compared to uninoculated plants. The improved nutrient uptake (Rajput et al. 2022), increased osmolyte accumulation and antioxidant activity, and decreased relative permeability led to improved drought tolerance and increased plant growth in microbial-inoculated plants (Tyagi et al. 2023). On comparing the potential of microbes to increase growth parameters, PGPB-inoculated maize plants showed higher drought resistance as compared to AMF and *P. indica* inoculated maize plants.

Our study showed that the nutrient contents of both microbial inoculated and uninoculated maize plants decreased with the increased level of drought. This is because of decreased root development and less water availability during drought, maize plants cannot absorb essential nutrients. However, at all levels of drought stress, microbial inoculation provided better nutrient acquisition than uninoculated maize plants. It is believed that the increased absorption surface of extraradical hyphae is responsible for enhancing nutrient status in AMF and *P. indica* inoculated plants (Ortiz et al. 2015). In maize plant growth and development, nutrients play a vital role. Calcium is essential for membrane integrity, cell wall stability, and ion transport (de Bang et al. 2021). Drought induces Ca^{2+} deficiency in plants. During drought, Na^+ replaces Ca^{2+} in the membranes and cell walls, reducing cell turgidity and hydraulic conductivity. Our

study showed that microbial-inoculated maize plants were better at absorbing Ca^{2+} ions which is in line with Devarajan et al. (2021) work.

K^+ maintains osmotic and membrane potential (Sardans and Peñuelas 2021) and regulates the synthesis of proteins, sugars, and enzyme activity (Hasanuzzaman et al. 2018). We found that drought treatment reduced K^+ levels both in inoculated and uninoculated maize plants. As Na^+ and K^+ have similar physiological properties, they are inevitable competitors. Consequently, cytoplasmic Na^+ competes with K^+ for binding sites, inhibiting metabolic processes that rely on K^+ . However, microbial-inoculated maize plants showed higher uptake of K^+ ions than uninoculated plants. According to our findings, higher osmolyte accumulation, protein synthesis, and antioxidant enzyme activity can be attributed to high K^+ accumulation (Hasanuzzaman et al. 2018) in microbial-inoculated plants under drought stress.

Magnesium is an essential component of chlorophyll, and it is required for photosynthesis and protein synthesis (Kleczkowski and Igamberdiev 2021). It was reported that drought stress reduced chlorophyll content and photosynthesis in plants, but PGPB and *P. indica* inoculation increased these parameters (Inbaraj 2021). Similarly, drought reduced Mg^{2+} uptake in wheat, but *Azospirillum* alleviated this effect. We found that drought and microbial inoculation significantly affected Mg^{2+} concentration in maize plant tissues. This may be attributed to the low availability of Ca^{2+} under drought stress. Ca^{2+} is a strong competitor of Mg^{2+} (Pandey and Sanyal 2021) and has more affinity for plasma membrane sites than Mg^{2+} . In our study, higher chlorophyll content and photosynthesis can also be correlated with increased Mg^{2+} content in microbial-inoculated maize plants under drought stress.

There was a rectilinear increase in Na^+ content in maize plants in both microbial-inoculated and uninoculated plants. However, uninoculated maize plants had higher Na^+ contents compared to PGPB, AMF, and *P. indica* which implies that symbioses control Na^+ uptake. AMF excludes Na^+ by discriminating its uptake from soil or plant transfer. The findings indicate that microbes influence Na^+ translocation to the aerial parts. Through exopolysaccharide binding to sodium ions in the soil, PGPB can confer drought tolerance to plants by decreasing sodium absorption from the soil and increasing nutrient uptake by roots (Bhagat et al. 2021). Low Na^+ content found in

AMF-treated roots can be attributed to the ability of the fungus to retain Na^+ in intraradical hyphae or compartmentalize it in root cell vacuoles. The mycorrhizal symbiosis enhanced plant growth and physiological process, thereby diluting these ions in tissues.

In this study, drought stress and microbial inoculation were investigated for their impact on micronutrient uptake in maize plants. Unfortunately, most plant nutrient studies under drought stress have paid little attention to micronutrients. Micronutrients are essential for maize plant growth and development. Zn^{2+} plays a crucial role in the synthesis of proteins, chlorophyll, and enzymes (Kumar et al. 2021). Chlorophyll synthesis and chloroplast structure and function require Fe. (Kumar et al. 2021). In plant cells, copper plays a vital role in photosynthesis, respiration, redox reactions, and the maintenance of cell wall structure (Kumar 2015a). In photosystem II, Mn^{2+} contributes to the structure of the water-splitting system which provides electrons for photosynthesis. In maize plants, drought reduces micronutrient uptake. We observed that with an increase in drought levels, the concentrations of Cu, Fe, Mn^{2+} , and Zn^{2+} in microbial-inoculated and uninoculated maize plants decreased. This is because Cu, Fe, and Zn^{2+} are less soluble and mobile in soil solutions during drought conditions. With the increasing drought, copper, zinc, and iron levels decrease, and a depletion zone forms around the roots of maize plants. Because of this, uninoculated maize plants have limited access to these nutrients. In addition, decreases in Cu, Zn^{2+} , Mn^{2+} , and Fe in microbial-inoculated maize plants may be due to decrease hyphal growth during drought. However, at each level of drought stress, microbial inoculation exhibited better micronutrient acquisition than uninoculated maize plants. It is consistent with earlier studies showing an increase in Zn^{2+} , Fe, and Cu content following mycorrhizal inoculation. In our study, higher chlorophyll content and photosynthesis can also be correlated with increased micronutrient content in microbial-inoculated maize plants under drought stress.

Photosynthesis is integral to plant growth and is therefore an important indicator of a plant's potential for growth and drought tolerance (Liang et al. 2019). In response to drought stress, stomatal closure decreases gas exchange and internal CO_2 levels, resulting in a decrease in photosynthetic pigment production, thereby reducing the photosynthetic rate (Liang et al. 2019), which was further confirmed in the present

study. Moreover, the transpiration rate decreases during drought leading to reduced nutrient and water uptake in maize plants. In contrast, drought-stressed maize plants inoculated with PGPB, AMF, and *P. indica* had an increase in photosynthetic rate, stomatal conductance, internal CO₂, and transpiration rate. Our results are in agreement with previous reports on PGPB inoculation improved plant photosynthetic efficiency by increasing the CO₂ assimilation rate, stomatal conductance, and transpiration rate (Vishnupradeep et al. 2022), and reducing structural damages to the photosynthetic apparatus by enhancing osmolyte accumulation in plants. Additionally, the results indicated that microbial inoculation significantly increased the carotenoid content in maize plants. The antioxidant property of carotenoids protects the photosynthetic apparatus against free radical damage, inhibits lipid peroxidation, stabilizes cell membranes, and plays a critical role in light-harvesting complex assembly (Farooq et al. 2009). Increased photosynthesis rates are a result of higher chlorophyll content and leaf area in maize plants.

As light-absorbing pigments, chlorophylls and carotenoids are crucial photosynthesis in maize plants. Drought decreases chlorophyll and carotenoid content (Khazaei et al. 2020), which is consistent with our findings. It was observed that PGPB, AMF, and *P. indica* maize inoculated plants had higher chlorophyll a and chlorophyll b levels, total chlorophyll and carotenoid levels than uninoculated maize plants. This suggests that microbial inoculation reduces the impact of drought on maize. In a similar study, an AMF substantially increased the chlorophyll and carotenoid content of *Cupressus arizonica* (Aalipour et al. 2023). A study found that maize plants colonized with PGPB had more chlorophyll and carotenoid content (Vishnupradeep et al. 2022). Our studies are in line with earlier studies suggesting higher photosynthetic pigments are probably a consequence of antioxidant accumulation, protecting the chloroplast structure, and increasing the solubilization and bioavailability of organic minerals, such as Mg (Khan et al. 2019). Our results coincide with the findings that *Bacillus* strains increase chlorophyll levels in maize due to increases in nutrient balance (Moreno-Galván et al. 2020).

We observed significantly higher osmolyte accumulation in both microbial inoculated (PGPB, AMF, and *P. indica*) and uninoculated maize plants during drought stress. However, microbial inoculated maize plants had a greater amplitude of osmolyte increases than uninoculated maize plants. The results of this study are consistent with a

previous study that demonstrated increased proline and sugar content in maize inoculated with the microbial consortium under drought stress (Hashem et al. 2016). *Acinetobacter*, *calcoaceticus*, and *Penicillium* sp. effectively ameliorated drought-induced stress in foxtail millet by increasing glycine betaine, proline, and sugar accumulation (Kour et al. 2020) by maintaining cell turgidity and membrane integrity. According to Liu et al. 2022, rhizobium symbiosis enhances drought tolerance in *Astragalus sinicus* through the production of putrescine, spermidine, and spermine. Based on these results, microbial inoculated maize plants are more efficient at osmotic adjustment than their uninoculated counterparts. However, PGPB outpaces AMF and *P. indica* in osmotic adjustment in maize plants under drought stress.

There was a remarkable difference in protein content in microbial inoculated and uninoculated maize plants under all levels of drought stress. Plants inoculated with PGPB, *P. indica*, and AMF had higher protein content. In microbial inoculated maize plants, higher protein content is associated with higher photosynthetic rates, chlorophyll contents, membrane integrity, and osmolyte accumulation. Similarly, Boutasknit et al. (2020) found that drought stress could be mitigated by AMF-colonized carob plants with higher levels of protein. According to this study, inoculating maize plants with PGPB, *P. indica*, or AMF enhanced drought tolerance through protein regulation.

In our study, maize plants inoculated with PGPB, *P. indica*, and AMF have a high level of α -tocopherol, which indicates that α -tocopherol is a free radical scavenger as well as a modulator of lipid peroxidation during abiotic stress (Kim et al. 2019). This study substantiates the findings of Fresno et al. (2023) that higher levels of α -tocopherol in AMF colonized *Trifolium repens* plants mitigate drought stress. Based on the current study, inoculating maize plants with PGPB, *P. indica*, and AMF enhanced drought tolerance by regulating α -tocopherol content, thus maintaining a high level of photosynthetic efficiency and chlorophyll content and reducing ion leakage.

A key physiological indicator of drought in plants is malondialdehyde, a by-product of membrane lipid peroxidation (Yasmeen et al. 2022). Lipid peroxidation alters cell membrane ultrastructure and permeability in maize plants during drought stress. Additionally, the LOX enzyme converts lipids into hydroperoxyl fatty acids and releases free radicals that damage cells. Prevention of lipid peroxidation and relative

permeability are key factors in drought tolerance (Kour et al. 2020). We observed a rectilinear increase in lipid peroxidation, relative membrane permeability, and LOX activity in maize plants inoculated with microbes (PGPB, AMF, and *P. indica*) and uninoculated maize plants with increasing stress levels. However, the amplitude of the increase in lipid peroxidation and electrolyte leakage in microbial inoculated maize plants was lower than that of uninoculated plants, which is in accordance with earlier reports (Begum et al. 2022). The reduced LOX activity in microbial inoculated maize plants is inconsistent with a previous report in which the co-inoculation of AMF and PGPB resulted in an increase in LOX activity in tobacco plants (Begum et al. 2022). In our study, the microbial inoculated maize plant maintained a lower MDA concentration and relative membrane permeability by regulating adaptive responses such as the synthesis of non-enzymatic molecules (carotenoids, proline, and α -tocopherol) and enzymatic antioxidants (CAT, APX, and SOD). These findings are similar to previous findings that PGPB-inoculated plants showed improved drought stress tolerance by maintaining a high level of carotenoids, proline, and tocopherol (Timmusk et al. 2014), as well as CAT, APX, and SOD activity (Chiappero et al. 2019), thereby reducing ROS accumulation.

In our study, the antioxidant enzyme activity of uninoculated and inoculated maize plants differed significantly. This result is consistent with an earlier study demonstrating that microbial inoculation improved plant growth under drought stress because of the enhanced activity of the ROS-scavenging antioxidant enzyme (Tyagi et al. 2023). Thus, microbial inoculation reduces lipid peroxidation and membrane permeability in plants by increasing antioxidant enzyme activity (Tyagi et al. 2023). Earlier studies have found that microbial consortium-treated maize exhibited increased antioxidant enzyme activity in stress conditions (Abbas et al., 2020), which lends weight to our study. Tyagi et al. 2023 reported that deploying a microbial consortium of *Serendipita indica*, *Rhizophagus intraradices*, and *Azotobacter chroococcum* boosts drought tolerance in maize by enhancing the activity of antioxidant enzymes. CAT activity in AMF-inoculated maize plants decreased with increasing levels of drought, which is consistent with previous findings that PGPB reduced CAT activity during drought stress in maize (Siddique et al. 2022). The current study proposes the use of PGPB, AMF, and *P. indica* to enhance antioxidant defense mechanisms in maize and

reduce drought stress. Future agriculture practices may benefit from the use of microbes as a bio-inoculant for promoting plant growth.

The chloroplast is the most susceptible cell organelle to drought stress (Gunasekera and Ratnasekera 2023). Our present study illustrated that the ultrastructure of both microbial-inoculated and uninoculated maize plants was affected by drought stress. Under low drought stress, the thylakoid membrane contracted thereby reducing the thylakoid lumen. Thylakoid lumen contraction may be a consequence of osmotic collapse (Semenova 2002). As the drought level increased, the lumen swelled, resulting in the destacking of grana. A number of studies have shown that osmotic stress causes thylakoid swelling and membrane damage (Aldesuquy et al. 2018). Microbially inoculated maize plants, however, seem to have less chloroplast damage than uninoculated plants. This may be attributed to more accumulation of osmolytes (glycine betaine, polyamines, and sugars) in microbial-inoculated plants. More accumulation of osmolytes prevents osmotic collapse, while polyamines inhibit membrane degradation by interacting with negatively charged membrane sites in grana and stroma thylakoids (Ali et al. 2020). In our study, microbial-inoculated maize plants showed reduced chloroplast membrane damage, possibly due to larger plastoglobule sizes that prevented oxidative damage. It has been reported that plastoglobules increase in size during drought (Zhu et al. 2021). Plastoglobules are lipoprotein bodies present in the chloroplast and function as sites for α -tocopherol synthesis under oxidative stress (Brehelin et al. 2007). In our study, microbial-inoculated maize plants had higher levels of α -tocopherols than uninoculated plants. α -tocopherols have been reported to protect membrane lipids and photosystem II against photooxidation. In microbial-inoculated plants, plastoglobule accumulation may be attributed to higher polyamine levels (Shu et al. 2015). Thus, in this study, microbial-inoculated maize plants had reduced damage to the ultrastructure of chloroplast due to high levels of osmolytes and large plastoglobules that prevented oxidative damage. However, PGPB has a greater ability to alleviate drought stress than *P. indica* and AMF-inoculated plants at all levels of drought stress in terms of maintaining the ultrastructure of the chloroplast.

Cell membrane stability in plants is considered a reliable indicator of drought tolerance (Abobatta 2019). Electron micrographs showed that plasma membranes of maize plant cells disintegrated under drought stress. With increasing drought levels, the distance

between the plasma membrane and cell wall increases. A similar finding was reported for *Ormosia hosiei* seedlings (Liu and Wei 2021). The disintegration of membranes occurs due to the peroxidation of polyunsaturated fatty acids by free radicals (Zhang et al. 2021) and the shrinkage of protoplasm during drought conditions. Compared to uninoculated maize plants, however, microbial-inoculated plants showed less membrane damage and protoplasm shrinkage. This statement is further strengthened by the accumulation of osmolytes (glucose, trehalose, proline, glycine betaine, and polyamines), which maintain cell turgor pressure and stabilize membranes, enzymes, and proteins (Sharma et al. 2019). We found lower levels of electrolyte leakage, lipid peroxidation, and LOX activity in microbial-inoculated plants, which play a significant role in membrane protection. This is because microbial-inoculated maize plants acquire more non-enzymatic antioxidant molecules (proline, α -tocopherols, and polyamines), enzymatic antioxidants (SOD, CAT, APX), and nutrients (calcium and potassium) compared with uninoculated plants. Proline is an effective ROS scavenger, protecting cells from damage by oxidative stress under a stressful environment (Ashraf and Foolad 2007). Moreover, α -tocopherol protects host tissue from lipid peroxidation and maintains the integrity of their membrane by scavenging free radicals (Niki 2021). Polyamines provide stability to membranes (Wang et al. 2022). In adverse environmental conditions, antioxidant enzymes (CAT, SOD, and APX) scavenge ROS and protect membranes from oxidative stress (Ru et al. 2023). By forming intermolecular links, calcium maintains the structural integrity of plant membranes, stabilizes cell walls, and regulates ion transport and selectivity (de Bang et al. 2021). Potassium plays a vital role in plants, including osmoregulation and membrane potential (Sardans and Peñuelas, 2021). So, microbial inoculation reduces membrane damage through osmolyte, nutrient, and antioxidant-mediated reductions of lipid peroxidation in plants (Tyagi et al. 2023). Vesicle formation was more in microbial-inoculated maize plants as compared to uninoculated plants. Vesicle formation increases under stress conditions (Karim et al. 2014). They increase the membrane surface area, which facilitates ion transport and storage, thus protecting the cells from ionic imbalance. Thus, more vesicle formation observed in this study indicates a better ability of microbial-inoculated plants to tolerate droughts.

4.4 PART IV

To study the role of soil microbes on growth, changes in nutritional, biochemical and physiological parameters, ultrastructural changes, and antioxidant accumulation in maize under salinity stress.

To accomplish the objective, the percentage of AMF and *P. indica* colonization, plant growth and biomass, macronutrients and micronutrients content, rate of physiological processes, photosynthetic pigments content, osmolytes content, lipid peroxidation, relative permeability, LOX activity, protein content, and α -tocopherol content was estimated in microbial inoculated and uninoculated maize plants under salinity stress.

4.4.1 Results

4.4.1.1 Microbial colonization

AMF and *P. indica* successfully colonized maize plants' roots at all salinity stress levels. However, the percentage of colonization was higher in AMF-inoculated maize plants than in *P. indica*-inoculated plants during salinity stress. No microbial colonization was observed in roots not inoculated with AMF and *P. indica* (**Figure 4.34**). Root colonization rates were significantly affected by different levels of salinity stress. Increased salinity levels caused a gradual decline in microbial colonization (**Table 4.10**).

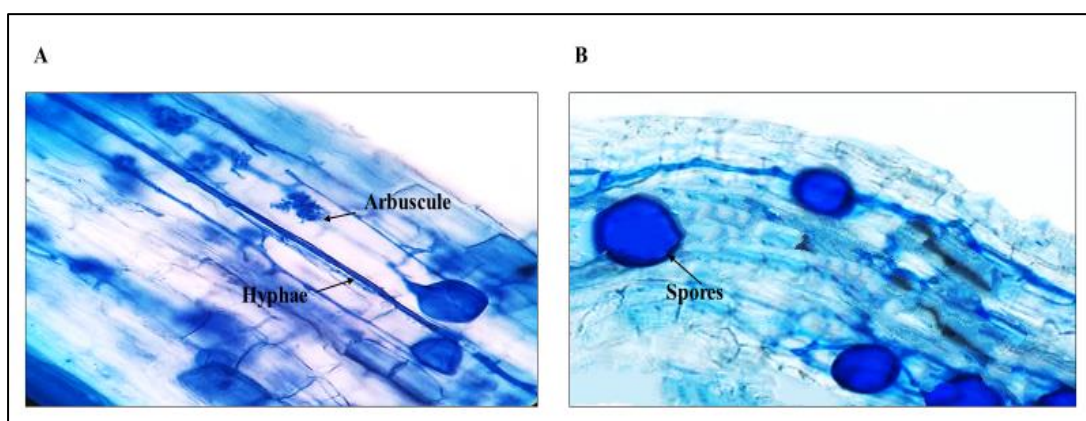


Figure 4.34: Microscopic observation of root colonization of (A) AMF-inoculated maize plants; (B) *P. indica*-inoculated maize plants under salinity stress.

Table 4.10: Influence of salinity stress and microbe inoculations on percentage microbe colonization, length, and biomass of shoot and root in maize plants.

NaCl (mM)	Microbe	Shoot length (cm)	Root length (cm)	Shoot biomass (g)	Root biomass (g)	Leaf area (cm ²)	Root colonization (%)
0	Control	70.20±1.01g	27.30±1.74e	6.41±0.48gh	3.29±0.20ef	59.12±1.07ij	-
	<i>P. indica</i>	75.00±0.35de	31.33±1.47d	7.58±0.28def	4.33±0.58cd	66.85±2.98efg	67
	PGPB	81.20±1.93b	36.10±0.85bc	8.81±0.63ab	5.28±0.33ab	74.61±0.56ab	-
	AMF	78.23±0.80c	35.10±1.73bc	7.54±0.56def	4.85±0.12bc	72.11±1.90bcd	72
50	Control	74.70±1.35de	30.80±1.48d	6.95±0.69fg	3.49±0.25ef	61.53±2.15hi	-
	<i>P. indica</i>	77.33±0.80cd	34.33±1.88c	7.89±0.60cde	4.67±0.38bc	67.75±2.17ef	63
	PGPB	86.40±0.61a	39.10±1.65a	9.45±0.39a	5.62±0.45a	77.84±2.15a	-
	AMF	82.10±1.10b	37.33±1.10ab	8.66±0.37abc	5.21±0.43ab	74.66±2.17ab	64
100	Control	66.40±1.66h	24.10±0.85ef	5.87±0.50h	3.10±0.08f	56.37±2.22jk	-
	<i>P. indica</i>	71.10±1.25fg	27.10±1.00e	7.02±0.01fg	4.18±0.16cd	63.58±1.25gh	54
	PGPB	76.33±1.89cde	37.30±0.30ab	8.36±0.54bcd	5.16±0.31ab	73.46±1.57bc	-
	AMF	73.67±1.65ef	34.33±0.55c	7.30±0.06ef	4.76±0.67bc	69.77±2.37de	56
200	Control	58.70±1.67j	20.60±1.22f	4.80±0.60i	2.90±0.17f	54.50±2.59k	-
	<i>P. indica</i>	62.67±2.41i	25.67±1.14ef	6.85±0.52fg	3.77±0.21def	59.76±2.28ij	32
	PGPB	70.20±2.59g	30.60±0.35d	8.26±0.07bcd	4.78±0.52bc	70.21±1.57cde	-
	AMF	67.17±1.76h	27.33±0.55e	7.13±0.15efg	4.30±0.13cd	65.36±0.36fg	36
Significance							
NaCl		***	***	***	**	***	***
<i>P. indica</i>		***	***	***	***	***	***
NaCl × <i>P. indica</i>		ns	ns	ns	ns	ns	***
PGPB		***	***	***	***	***	-
NaCl × PGPB		ns	***	ns	ns	ns	-
AMF		***	***	***	***	***	***
NaCl × AMF		ns	***	ns	ns	ns	***

Values represent averages across replicates. Letters within a column represent significant differences at $P < 0.05$. ns: not significant. *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$.

4.4.1.2 Growth parameters

Table 4.10 shows the growth parameters of microbial inoculated and uninoculated maize plants under four different levels of salinity stress. Plants inoculated with microbes performed significantly better than uninoculated plants at all salinity levels in terms of shoot and root length, shoot and root biomass, and leaf area. PGPB inoculation improved shoot and root length by 1.2 and 1.5-fold respectively under severe salinity conditions (200 mM NaCl) while AMF inoculation improved them by 1.14 and 1.3-fold, respectively. In contrast to uninoculated plants, plants inoculated with *P. indica* exhibited 1.07 and 1.2-fold increases, respectively. PGPB-inoculated plants displayed increases of 1.7-fold in shoot and root biomass at 200 mM NaCl stress. In comparison to uninoculated plants, AMF inoculation raised it by 1.5-fold, and *P. indica* inoculation raised it by 1.4 and 1.3-fold respectively. PGPB significantly increased the leaf area of maize plants by 1.3-fold followed by AMF (1.2-fold) and *P. indica* (1.1-fold) in comparison to uninoculated maize plants. There was a significant independent effect of salinity, *P. indica*, PGPB, and AMF on plant growth, biomass, and leaf area in the two-way ANOVA (**Table 4.10**). When comparing the ability of microbes to increase growth parameters, PGPB-inoculated plants showed higher salinity resistance as compared to AMF and *P. indica*-inoculated plants.

4.4.1.3 Mineral nutrients content

Table 4.11 shows the macro- and micronutrient content of maize plants with respect to salinity stress and microbial inoculation. Microbial inoculation and salinity stress had significant effects on leaf macronutrient (Ca^{2+} , Mg^{2+} , K^+ , Na^+) and micronutrient (Zn^{2+} , Fe, Mn^{2+} , and Cu) contents.

Ca^{2+} levels decreased linearly as salinity stress levels increased both in microbial-inoculated and uninoculated plants (**Table 4.11**). The presence of microbes, however, led to higher Ca^{2+} levels than those in plants without microbes. The Ca^{2+} content of plants treated with PGPB, *P. indica*, and AMF was 2.0, 2.4, and 2.3-fold higher than that of uninoculated plants at 200 mM NaCl. In comparison with PGPB and AMF-

inoculated plants, *P. indica* is more effective at alleviating salinity stress based on Ca^{2+} content at maximum salinity levels.

Mg^{2+} uptake in maize leaves was affected by microbe inoculation and salinity stress. Mg^{2+} levels decreased in maize plants as salinity levels increased. However, the Mg^{2+} levels of plants inoculated with microbes were higher than those of uninoculated plants at the same salinity level (**Table 4.11**). Mg^{2+} uptake in uninoculated plants and *P. indica* was inconsistent under salinity stress. Initially, leaf Mg^{2+} uptake increased at 50 mM NaCl, but then it decreased at 100 mM NaCl and 200 mM NaCl in both *P. indica* inoculated and control plants. In contrast, Mg^{2+} content decreased gradually with increasing salinity levels in PGPB and AMF-inoculated plants. When plants were inoculated with PGPB, *P. indica*, and AMF, their Mg^{2+} contents were 10.1, 8.1, and 4.4-fold greater than those of uninoculated plants. In all treatments, plants inoculated with PGPB had higher Mg^{2+} content in leaves compared to those inoculated with *P. indica* or AMF.

The K^{+} content of maize plants declined as salinity stress increased. Plants inoculated with microbes, however, maintained higher K^{+} levels at the same level of salinity stress (**Table 4.11**). As a result of salinity stress, K^{+} uptake in the PGPB, the *P. indica*, the AMF, and the control plants exhibited inconsistent responses. In PGPB, *P. indica*, AMF, and control plants, K^{+} uptake in leaves increased up to 50 mM NaCl, then decreased to 100 mM NaCl and 200 mM NaCl. In plants inoculated with PGPB, *P. indica*, and AMF, K^{+} content was 2.5, 1.6, and 1.7-fold higher than those without inoculation. All treatments showed that microbial-inoculated plants had higher leaf K^{+} content than uninoculated plants.

A linear increase in sodium content was observed in the maize leaves of both microbial inoculated and uninoculated plants with increasing salinity stress levels (**Table 4.11**). Microbial inoculated plants, however, possess substantially less Na^{+} than uninoculated plants at all levels of salinity stress. In addition, the Na^{+} content of PGPB-inoculated plants was 30.2% lower than that of control plants, followed by AMF (20.5%) and *P. indica* (16.2%) colonized plants. As a result, PGPB has a greater ability to alleviate salinity stress than *P. indica* and AMF-inoculated plants at all levels of salinity.

Salinity negatively affected Zn^{2+} accumulation in maize plants (**Table 4.11**). A significant decrease in Zn^{2+} levels was observed in microbial-inoculated plants as well as control plants under salinity stress. Conversely, plants inoculated with microbes maintained higher levels of Zn^{2+} under similar salinity conditions. At 200 mM NaCl, PGPB, *P. indica*, and AMF-inoculated plants had 2.5, 1.0, and 2.1-fold higher Zn^{2+} content than controls. PGPB is more effective in alleviating salinity stress at all levels of salinity stress than *P. indica* and AMF-inoculated plants. Salinity stress and microbial inoculation significantly enhanced Zn^{2+} content.

There was a substantial decrease in Fe levels in the leaves of maize plants under salinity stress. In contrast, microbial-inoculated plants maintained higher levels of Fe in response to the same level of salinity stress (**Table 4.11**). At 200 mM NaCl, *P. indica*, PGPB, and AMF-inoculated plants increased their Fe content by 1.1, 1.9, and 1.0-fold, respectively. Salinity stress is alleviated more effectively by PGPB than by *P. indica* and AMF-inoculated plants.

In both microbial inoculated and uninoculated plants, Mn^{2+} content decreased significantly with increased salinity stress. PGPB and AMF-inoculated plants, however, maintained higher levels of Mn^{2+} at the same salinity level (**Table 4.11**). Mn^{2+} content decreased by 60.1% in *P. indica* inoculated plants compared to controls at the highest salinity treatment. Plants inoculated with PGPB and AMF showed an increase in Mn^{2+} by 152.4% and 133.3%, respectively, at 200 mM NaCl. In terms of Mn^{2+} content, PGPB plants can alleviate salinity stress better than *P. indica* and AMF plants. In maize plants, salinity stress negatively affected Cu accumulation (**Table 4.11**). There was a significant decrease in Cu levels in leaves of both inoculated and control plants when exposed to salinity stress. Microbe-inoculated plants, however, showed higher levels of Cu at the same salinity stress level. Both *P. indica* and control plants responded inconsistently to salinity stress. In *P. indica* and control plants, Cu uptake increased until 50 mM NaCl, then decreased to 100 mM NaCl and 200 mM NaCl. In contrast to controls, PGPB, *P. indica*, and AMF-inoculated plants increased their Cu concentrations 6.7, 4.6, and 2.8-fold at 200 mM NaCl, respectively.

Table 4.11: Influence of salinity stress and microbial inoculation on the content of macro- and micro-nutrients in leaves of the maize plants.

NaCl (mM)	Microbe	Macronutrients (mg kg ⁻¹)				Micronutrients (mg kg ⁻¹)			
		Ca ²⁺	Mg ²⁺	K ⁺	Na ⁺	Zn ²⁺	Fe	Mn ²⁺	Cu
0	Control	762.17±13.58e	2266.03±14.08f	2750.70±9.37h	556.56±15.84f	54.13±3.47b	570.32±5.36f	97.36±4.79b	57.58±6.59j
	<i>P. indica</i>	956.26±13.46a	2761.26±3.58e	3756.61±6.69b	490.47±7.18g	60.32±0.51f	747.06±5.72bc	105.51±0.40b	312.20±12.37a
	PGPB	854.06±9.63bc	4667.94±15.85a	3274.89±10.19e	369.93±9.22h	75.05±3.99b	867.99±4.97a	120.56±6.17a	258.59±1.07b
	AMF	877.45±8.83b	3551.16±4.08b	3161.54±8.88f	460.05±3.66g	65.15±3.97c	786.32±10.29b	99.14±8.07b	105.35±4.00fg
50	Control	759.38±18.29e	1873.99±15.66g	3425.45±16.34cd	747.63±14.29d	46.49±4.52e	524.43±6.20g	56.68±3.19e	84.43±7.04gh
	<i>P. indica</i>	836.13±13.93d	3044.26±12.57d	3842.28±20.78b	617.35±18.43e	56.04±0.51d	733.53±12.44c	98.09±0.94b	323.24±6.23a
	PGPB	805.01±5.76d	3348.07±5.39c	4490.04±13.37a	494.69±14.65g	63.92±3.15c	783.11±15.04b	86.15±10.45c	226.14±4.38c
	AMF	698.94±16.32f	1753.81±9.89g	3338.45±12.73de	502.53±12.26fg	49.144.50e	618.93±10.10e	97.61±3.25b	105.31±9.51fg
100	Control	362.75±13.10j	1173.68±6.21h	1453.36±15.43k	935.72±15.51b	30.99±1.40cd	451.94±13.40h	56.86±4.28e	61.61±8.85ij
	<i>P. indica</i>	626.62±22.91g	2181.01±16.34f	3450.30±16.33c	727.11±8.43d	48.34±1.54e	665.57±12.41d	80.23±6.99cd	314.17±14.82a
	PGPB	594.96±20.26gh	2257.77±13.87f	3299.94±16.33e	634.54±17.28e	62.41±5.22cd	773.54±18.64b	82.63±9.10c	203.31±6.57d
	AMF	601.33±12.72gh	1128.69±3.32h	2869.50±15.64g	662.42±16.89e	38.05±1.50f	556.74±14.26fg	83.83±1.93f	82.08±7.70hi
200	Control	248.30±7.24k	121.15±4.56k	1406.51±13.42k	1033.79±15.94a	17.48±1.09g	340.43±9.13j	30.78±5.12f	26.44±6.57k
	<i>P. indica</i>	595.53±8.55gh	975.26±6.66i	2260.39±3.09j	865.95±3.66b	17.64±5.54g	383.86±3.47i	12.27±0.53g	121.49±8.04f
	PGPB	501.49±12.65i	1228.71±12.29h	3463.90±16.33c	721.55±8.93d	43.04±1.54ef	658.53±13.63d	77.70±0.30cd	176.87±2.73e
	AMF	560.34±12.01h	537.23±5.72j	2429.58±7.59i	822.35±1.74c	36.84±8.23a	356.19±5.40ij	71.80±0.79d	74.14±3.43hij
Significance									
Salinity		***	***	***	***	***	***	***	***
<i>P. indica</i>		***	***	***	***	***	***	***	***
<i>P. indica</i> × Salinity		***	***	***	*	***	***	***	***
PGPB		***	***	***	***	***	***	***	***
PGPB × Salinity		***	***	***	*	***	ns	**	***
AMF		***	***	**	***	***	***	***	***
AMF × Salinity		***	***	***	**	***	***	***	ns

Values represent averages across replicates. Letters within a column represent significant differences at P<0.05. ns: not significant. ***P<0.001, **P<0.01, *P<0.05.

4.4.1.4 Physiological parameters

Figure 4.35 depicts the physiological parameters of microbial-inoculated and uninoculated maize plants under four different levels of salinity stress. The microbial-inoculated maize plants displayed a significantly higher photosynthetic rate, transpiration rate, stomatal conductance, and internal CO₂ level than the uninoculated maize plants at all salinity levels. Photosynthetic rates were significantly increased by PGPB, AMF, and *P. indica* by 2.9, 2.2, and 1.7-fold as compared to uninoculated plants under 200 mM NaCl stress. PGPB-inoculated maize plants had 3.9-fold increased stomatal conductance, followed by AMF (3.5-fold), and *P. indica* (3.1-fold) as compared to uninoculated plants. In maize plants inoculated with PGPB, AMF, and *P. indica*, transpiration rates increased to 6.9, 5.4, and 4.7-fold, respectively at 200 mM NaCl salinity stress. Under severe salinity stress conditions, PGPB-inoculated plants had an internal CO₂ increase of 4.1-fold, while AMF and *P. indica*-inoculated plants had an increase of 3.7 and 2.6-fold respectively. The results of the two-way ANOVA showed a significant independent effect of salinity on PGPB, *P. indica*, and AMF physiological parameters (**Table 4.12**). Therefore, PGPB has been found to alleviate salinity stress better than both AMF and *P. indica*.

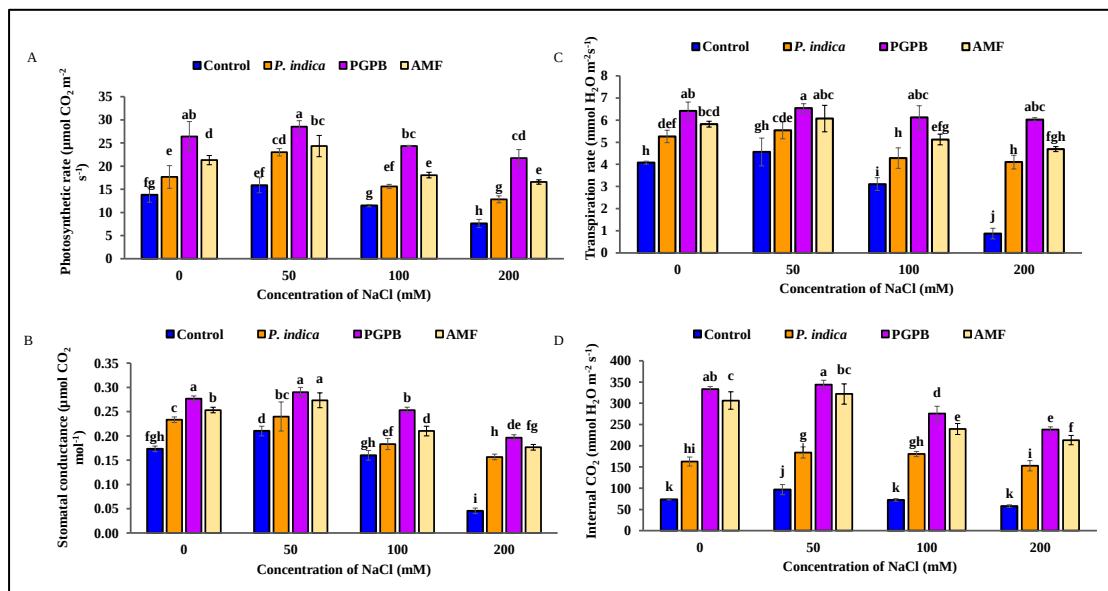


Figure 4.35: Effects of salinity and microbial inoculation in maize on (A) photosynthetic rate; (B) stomatal conductance; (C) transpiration rate; and (D) internal CO₂. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.4.1.5 Biochemical parameters

4.4.1.5.1 Photosynthetic pigment content

Photosynthetic pigments viz. chlorophyll a, chlorophyll b, total chlorophylls, and carotenoid content in microbial-inoculated maize plants showed substantially higher levels than uninoculated maize plants at all levels of salinity stress (**Figure 4.36**).

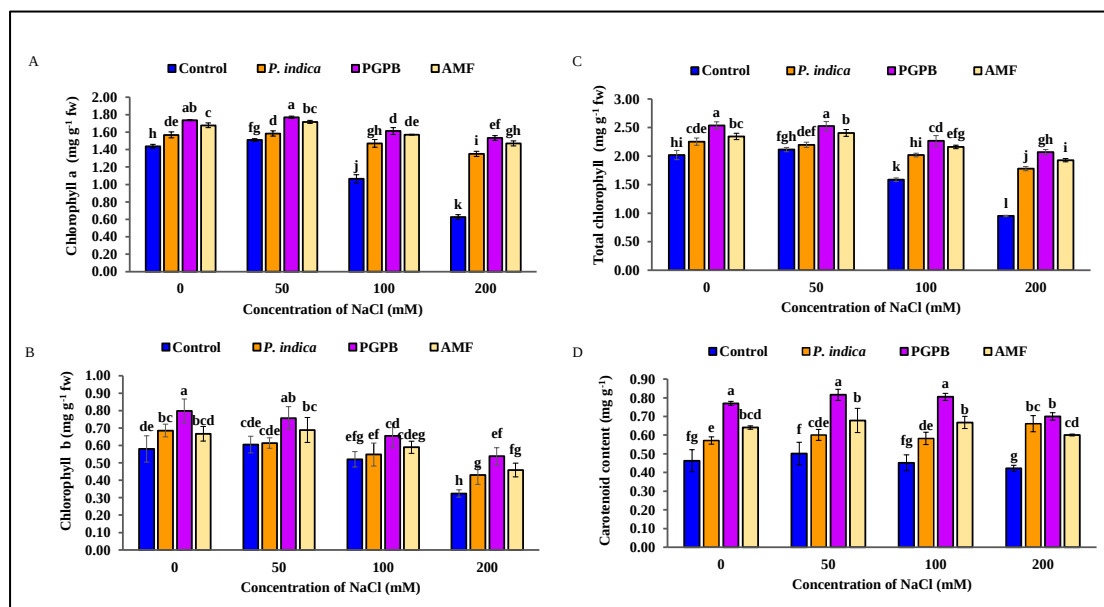


Figure 4.36: Effects of salinity and microbial inoculation in maize on the content of (A) chlorophyll a; (B) chlorophyll b; (C) total chlorophyll; and (D) carotenoid. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

A substantial increase in chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid contents was observed in microbial-inoculated maize plants under all levels of salinity stress (**Figure 4.36**). Up to 50 mM NaCl, maize plant photosynthetic pigment content increased, while at 100 and 200 mM NaCl, it decreased significantly. Plants inoculated with PGPB, AMF, and *P. indica* increased their chlorophyll a content to 2.4, 2.3, and 2.1-fold, respectively compared to uninoculated plants, when exposed to the highest level of salinity stress (200 mM NaCl). As a result of PGPB inoculation, chlorophyll b content was increased by 1.7-fold under severe salinity conditions, whereas AMF and *P. indica* inoculation increased it by 1.4-fold and 1.3-fold, respectively. At the highest stress level, the total chlorophyll content in PGPB-inoculated plants was 2.2-fold higher than in the control plants, followed by AMF (2-fold) and *P. indica* (1.9-fold). In comparison with uninoculated plants, PGPB (1.7-fold) inoculated plants had higher

carotenoid contents than *P. indica* (1.6-fold) and AMF (1.4-fold) inoculated plants. A two-way ANOVA demonstrated that salinity, *P. indica*, PGPB, AMF, and their interactions significantly impacted chlorophyll a and total chlorophyll content (**Table 4.12**). Therefore, PGPB is more effective at alleviating salinity stress than AMF and *P. indica* maize plants.

4.4.1.5.2 Osmolyte content

Glycine betaine content increased linearly with increasing salinity stress levels in microbial inoculated and uninoculated maize plants (**Figure 4.37A**). Comparing between 0 and 200 mM NaCl stress treatment glycine betaine content increased by about 1.6-fold in AMF-inoculated plants, led by *P. indica* (1.4-fold), PGPB (1.4-fold), and uninoculated (1.5-fold) plants. The glycine betaine content of microbial-inoculated plants was significantly higher at all salinity levels compared to uninoculated plants. The leaf glycine betaine content of AMF and PGPB-inoculated plants was 1.3-fold higher than that of control plants at 200 mM NaCl, followed by *P. indica* inoculated plants (1.2-fold). Glycine betaine content in maize plants was significantly affected by salinity stress and microbial inoculation.

In response to salinity stress, both microbial inoculated and uninoculated maize plants showed rectilinear increases in total sugar content (**Figure 4.37B**). The presence of microbes, however, facilitated higher sugar accumulation in leaves at all levels of salt stress. The total sugar content of plants inoculated with PGPB at 0, 50, 100, and 200 mM NaCl stress was 2.3, 1.7, 1.6, and 1.6-fold higher than that of plants uninoculated. AMF-inoculated plants had total sugar contents 2.2, 1.4, 1.5, and 1.3-fold higher than uninoculated plants at 0, 50, 100, and 200 mM NaCl stress. Inoculating plants with *P. indica* increased total sugar content 1.2, 1.3, 1.2, and 1.2-fold when compared with uninoculated plants under 0, 50, 100, and 200 mM NaCl stress. PGPB alleviates salinity stress more effectively than *P. indica* and AMF-inoculated plants at all salinity stress levels. In maize plants, salinity stress and microbial inoculation increased total sugar content significantly.

A linear increase in trehalose content was observed in both microbial-inoculated and uninoculated maize plants under salinity stress (**Figure 4.37C**). However, microbial-

inoculated plants displayed significantly higher trehalose contents than uninoculated plants at all levels of salinity stress. As a result of PGPB inoculation, trehalose content was 3.3, 1.7, and 1.7-fold higher than in uninoculated plants, respectively, at 0, 50, 100, and 200 mM NaCl stress. Upon AMF inoculation, the trehalose contents of the plants were 2.7, 2.6, 1.4, and 1.3-fold higher than those of uninoculated plants at 0, 50, 100, and 200 mM NaCl stress. *P. indica* inoculated plants produced 2, 2, 1.2, and 1.2-fold more trehalose than uninoculated plants at 0, 50, 100, and 200 mM NaCl stress, respectively. Based on two-way ANOVA, salinity, *P. indica*, PGPB, and AMF have significant independent effects and interaction of salinity $\times$ *P. indica*, salinity $\times$ PGPB, and salinity $\times$ AMF on trehalose content (**Table 4.12**). Thus, PGPB alleviates salinity stress more efficiently than AMF or *P. indica*-inoculated plants at all salinity levels.

Microbial inoculation and salinity stress significantly influenced maize leaf proline content (**Figure 4.37D**). Both microbial inoculated and uninoculated maize leaves exhibited an increase in proline content under salinity stress. Microbial-inoculated maize plants performed significantly better under all salinity stress levels compared to uninoculated plants. Leaf proline content was 1.6-fold higher in PGPB-inoculated plants compared to the control, followed by AMF-inoculated plants (1.5-fold) and *P. indica*-inoculated plants (1.4-fold). Polyamine content (putrescine and spermidine) was significantly influenced by salinity stress and microbial inoculation. Spermine was, however, not detected in both microbial inoculated and uninoculated plants under all salinity conditions. Microbial inoculated and uninoculated plants demonstrated contrasting effects of salinity stress on putrescine and spermidine (**Figure 4.37E-F**). As compared to plants not exposed to salinity stress, PGPB, AMF, *P. indica*, and uninoculated plants displayed increases in putrescine levels by 2.6, 2.8, 1.9, and 6.6-fold, respectively at 200 mM NaCl. Plants inoculated with PGPB had spermidine levels 2.6, 1.8, 1.4, and 1.3-fold higher than uninoculated plants under 0, 50, 100, and 200 mM NaCl stress, respectively. As a result of AMF inoculation, spermidine levels of plants were 3.1, 1.9, 1.5, and 1.5-fold higher than those of uninoculated plants under 0, 50, 100, and 200 mM NaCl stress. A 0, 50, 100, and 200 mM NaCl stress increased spermidine levels in *P. indica* inoculated plants by 2.1, 1.3, 1.1, and 1.2-fold that of uninoculated plants. Despite salinity stress, microbial-inoculated plants recorded higher

putrescine and spermidine contents than uninoculated plants. At all levels of salinity stress, AMF outperforms PGPB and *P. indica* in terms of polyamine content. Two-way ANOVA demonstrated a significant independent effect of salinity, *P. indica*, PGPB, and AMF on glycine betaine, trehalose, proline, and polyamine content (Table 4.12).

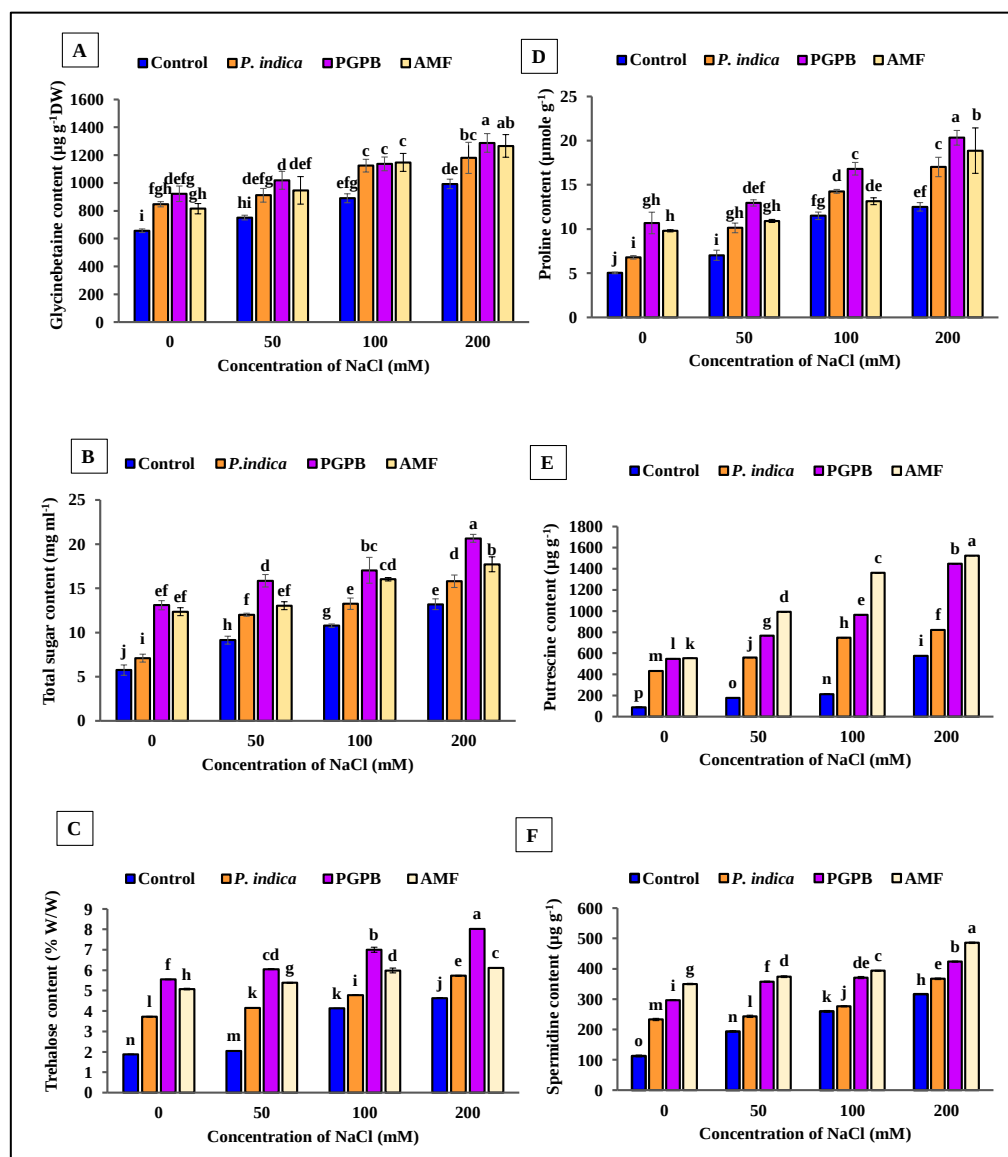


Figure 4.37: Effects of salinity and microbial inoculation in maize on the content of (A) glycine betaine; (B) total sugar; (C) trehalose; (D) proline; (E) putrescine; and (F) spermidine. Values represent averages across replicates. Different letters represent significant differences at $P < 0.05$.

4.4.1.5.3 Protein

Both microbial inoculated and uninoculated maize plants exhibited significant increases in protein content under salt stress till 50 mM NaCl followed by decrease at 100 and

200 mM NaCl stress. Microbial inoculated plants, however, produced significantly more protein than uninoculated plants when exposed to salinity stress (**Figure 4.38A**). Plants inoculated with PGPB, AMF, and *P. indica* produced 2.1, 2.0, and 1.7-fold more protein than uninoculated maize at 200 mM NaCl, respectively. As a result, PGPB has a greater ability to alleviate salinity stress than *P. indica* and AMF-inoculated plants at all salinity levels. Protein content was significantly increased by salinity stress and microbial inoculation.

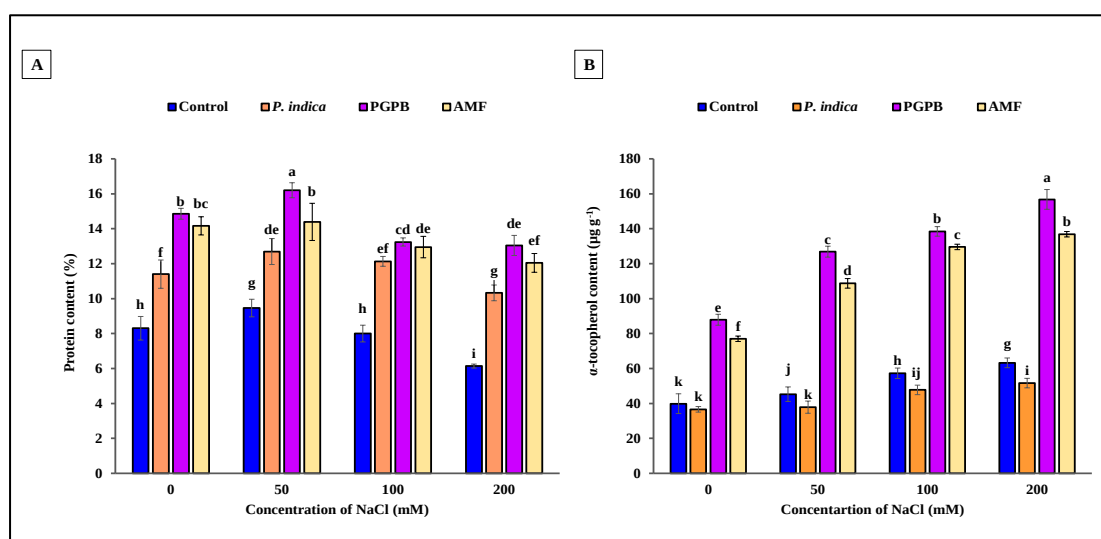


Figure 4.38: Effects of salinity and microbial inoculation in maize on the content of (A) protein; and (B) α -tocopherol. Values represent averages across replicates. Different letters represent significant differences at $P < 0.055$.

4.4.1.5.4 α -tocopherol content

Both microbial inoculated and uninoculated maize plants showed an increase in α -tocopherol in response to salinity stress. However, microbial-inoculated plants exhibited significantly higher levels of α -tocopherol under salinity stress (**Figure 4.38B**). Plants inoculated with PGPB and AMF produced 2.5 and 2.2 more α -tocopherol than uninoculated plants at 200 mM NaCl, respectively. There was no impact of *P. indica* colonization on α -tocopherol content in maize at all the levels of salinity stress. The PGPB-inoculated plants are, therefore, more efficient at alleviating salinity stress than the *P. indica* and AMF-inoculated plants at all salinity levels. The α -tocopherol content of plants was significantly enhanced by salinity stress and microbial inoculation.

4.4.1.5.5 Membrane damage

The content of MDA in **Figure 4.39A** is used to measure lipid peroxidation in maize plants exposed to different levels of salinity stress. Both microbial inoculated and uninoculated maize plants displayed increased MDA content under salinity stress. Uninoculated plants, however, have higher levels of MDA. There was a significant reduction in MDA contents in plants that were microbial inoculated regardless of salinity level.

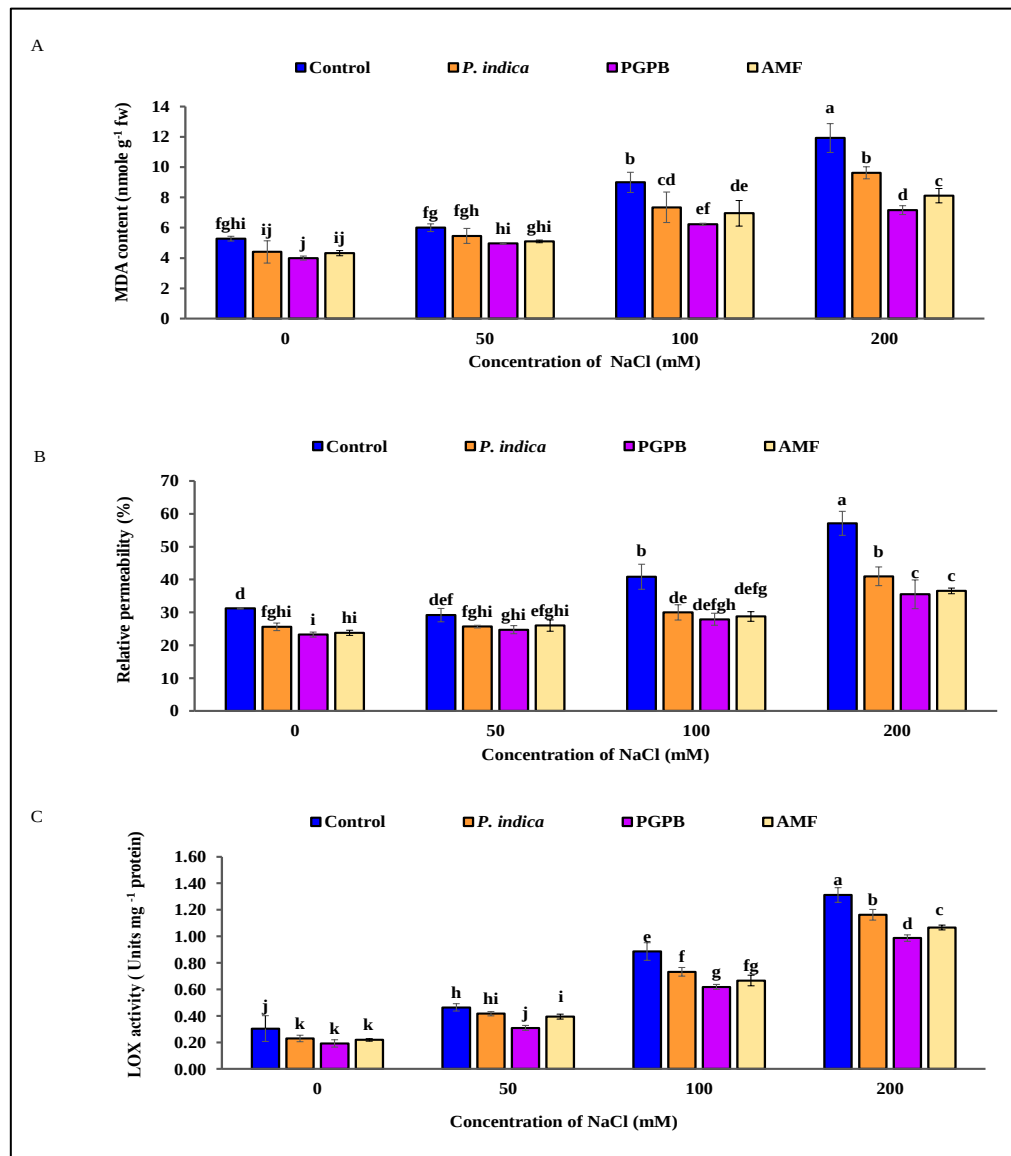


Figure 4.39: Effects of salinity and microbial inoculation in maize on (A) MDA content; and (B) relative permeability (C) LOX activity. Values represent averages across replicates. Letters within a column represent significant differences at $P < 0.055$.

P. indica, AMF, and PGPB-inoculated plants showed decreases in MDA content of 19.2%, 31.9%, and 39.9%, respectively over uninoculated plants, when exposed to 200 mM NaCl stress. Microbial inoculation and salinity stress significantly affected the relative membrane permeability of maize plants. The relative permeability in maize plants increased as a result of salinity stress. At all salinity levels, PGPB, AMF, and *P. indica* inoculated plants exhibited lower electrolyte leakage than the control (**Figure 4.39B**). Plants inoculated with PGPB, AMF, and *P. indica* exhibited 37.8%, 36.0%, and 28.2% lower relative permeability, respectively than the control at 200 mM NaCl. In both microbial inoculated and uninoculated plants, salinity stress increased LOX activity, but a significant difference was observed between them (**Figure 4.39C**). Microbe-inoculated plants had significantly lower LOX levels. Plants inoculated with PGPB (24.8%) showed lower LOX activity than those inoculated with AMF (18.7%) and *P. indica* (11.38%) as compared to control under all levels of salinity stress. Two-way ANOVA revealed a significant independent effect of salinity, *P. indica*, PGPB, AMF, and interaction of salinity $\times$ AMF, and salinity $\times$ PGPB on membrane damage (**Table 4.12**). PGPB is therefore more effective at alleviating salinity stress in terms of membrane damage at all salinity levels than *P. indica* and AMF in maize plants.

4.4.1.6 Antioxidant enzyme activity

Both microbial and uninoculated maize plants displayed increased SOD activity with increasing salinity stress levels. However, microbial colonization increased SOD activity in maize plants under salinity stress conditions more than in control plants (**Figure 4.40A**). As compared to uninoculated plants at 200 mM NaCl, PGPB inoculation raised SOD activity by 1.3-fold, AMF inoculation increased it by 1.2-fold, and *P. indica* inoculation increased it by 1.1-fold. As salinity stress increased, PGPB and *P. indica* inoculated plants displayed increased CAT activity. The CAT activity of plants inoculated with AMF decreased with an increase in salinity stress as opposed to control plants. A 2.1-fold and 1.5-fold increase in CAT activity was observed in plants inoculated with PGPB and *P. indica* as compared to uninoculated plants at 200 mM

NaCl, respectively (**Figure 4.40B**). The APX activity of PGPB, *P. indica*, and AMF-inoculated plants was higher than that of uninoculated plants at all salinity levels. At 200 mM NaCl stress, PGPB-inoculated plants had 1.4-fold higher APX activities than their uninoculated counterparts, followed by AMF-inoculated plants (1.3-fold) and *P. indica* (1.1-fold) (**Figure 4.40C**).

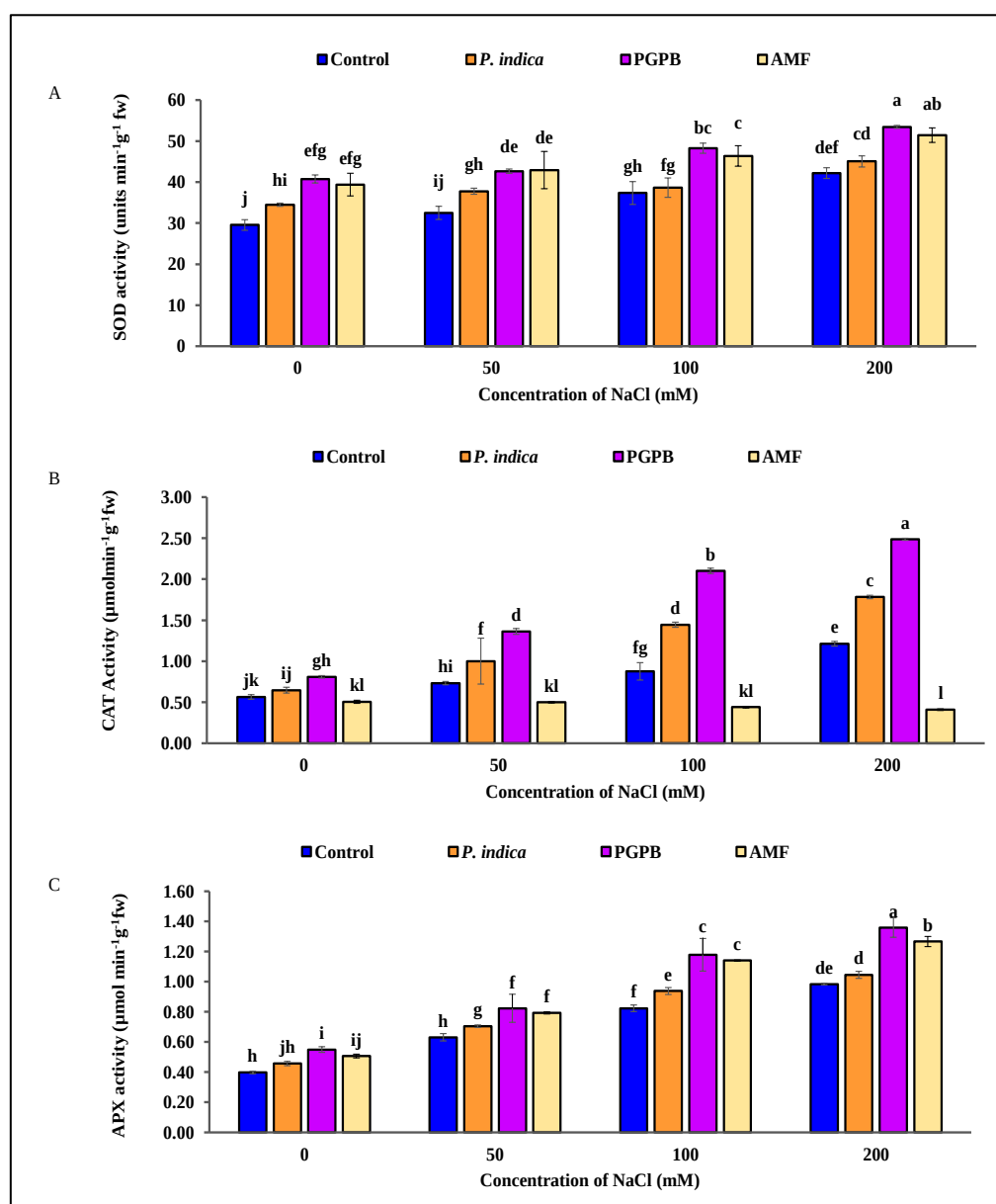


Figure 4.40: Effects of salinity and microbial inoculation in maize on the activity of (A) SOD; (B) CAT; and (C) APX. Values represent averages across replicates. Different letters represent significant differences at $P < 0.055$.

4.4.1.7 Cell ultrastructural studies

4.4.1.7.1 Chloroplast

At all levels of salinity, the electron micrographs show qualitative differences between microbial inoculated and uninoculated maize plants. **Figures 4.41** and **4.42** show changes in chloroplast ultrastructure in microbial-inoculated and uninoculated maize leaves under salinity stress. Microbial inoculated and uninoculated maize plants displayed chloroplasts with distinct thylakoids and grana surrounded by double-layered membranes under 0 mM NaCl stress. However, PGPB-inoculated plants exhibited a greater number of thylakoids and granum than AMF and *P. indica*-inoculated plants. Under 50 mM NaCl salinity stress, uninoculated plants showed decreased numbers of thylakoids and grana, and increased numbers of plastoglobules. Under low salinity stress, the thylakoid membrane contracted thereby reducing the thylakoid lumen (**Figures 4.41** and **4.42**). The chloroplast structures were almost the same in microbial-inoculated plants at 0 and 50 mM NaCl. A 100 mM NaCl salinity stress led to swelling in the thylakoid lumen resulting in destacking of grana. Moreover, the accumulation of smaller-size plastoglobules was more common in uninoculated plants. Microbial-inoculated plants showed chloroplasts with intact thylakoids and grana and fewer but large size plastoglobules accumulation, though the size of the plastoglobules was large. In contrast, plants inoculated with PGPB displayed more intact thylakoids, grana, and a lesser accumulation of plastoglobules than AMF or *P. indica*. Consequently, at 200 mM NaCl, the chloroplast in uninoculated plants was characterized by disorganized thylakoids and grana and the accumulation of a large number of smaller-sized plastoglobules (**Figure 4.41D**). Contrary to this, microbial-inoculated plants contained intact thylakoids, disorganized grana, and fewer but larger-sized plastoglobules. As compared to AMF and *P. indica*, plants inoculated with PGPB showed more intact thylakoids and fewer but fewer large-size plastoglobules at severe salinity conditions.

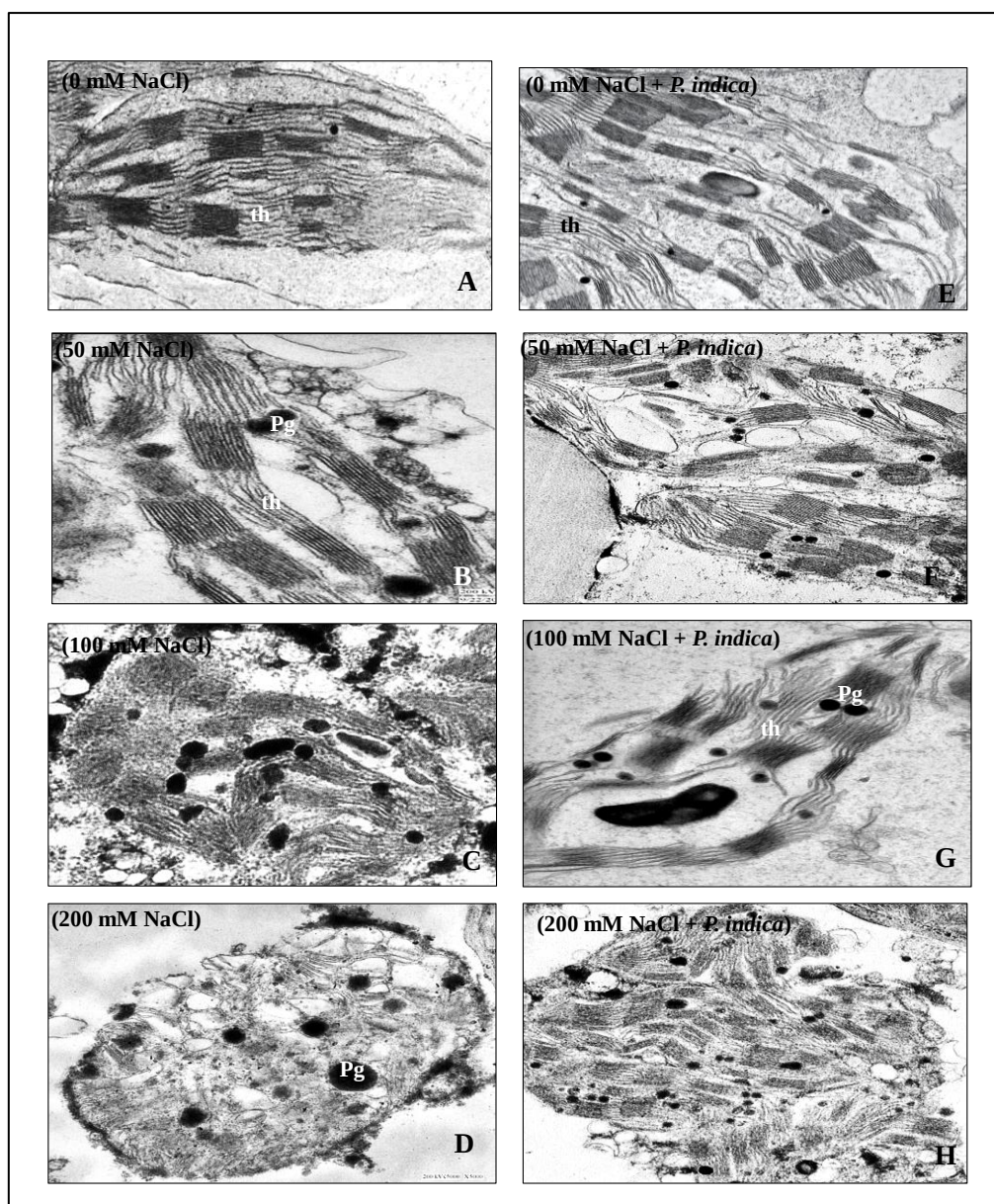


Figure 4.41: Transmission electron micrographs of chloroplast in mesophyll cells of (A) Control maize plants not exposed to NaCl; chloroplast with intact thylakoids and grana surrounded. (B) Control maize plant subjected to 50 mM NaCl; with a lesser number of thylakoids and some plastoglobules. (C) Control maize plant not exposed to 100 mM NaCl; disorganized grana, and increased accumulation of plastoglobules. (D) Control maize plant subjected to 200 mM NaCl; thylakoids and grana disorganized; a large number of plastoglobules seen (E) *P. indica* inoculated maize plant not exposed to NaCl; Grana stacked neatly with well-developed thylakoids (F) *P. indica* inoculated maize plant subjected to 50 mM NaCl; more thylakoids and fewer plastoglobules. (G) *P. indica* inoculated maize plant subjected to 100 mM NaCl; structure of thylakoid and grana still maintained. (H) *P. indica* inoculated maize plant subjected to 200 mM NaCl; fewer plastoglobules have accumulated; slight disintegration of grana and thylakoid was seen. Pg= plastoglobuli; th = thylakoids. The values in the bracket indicate the concentration of NaCl added to the soil.

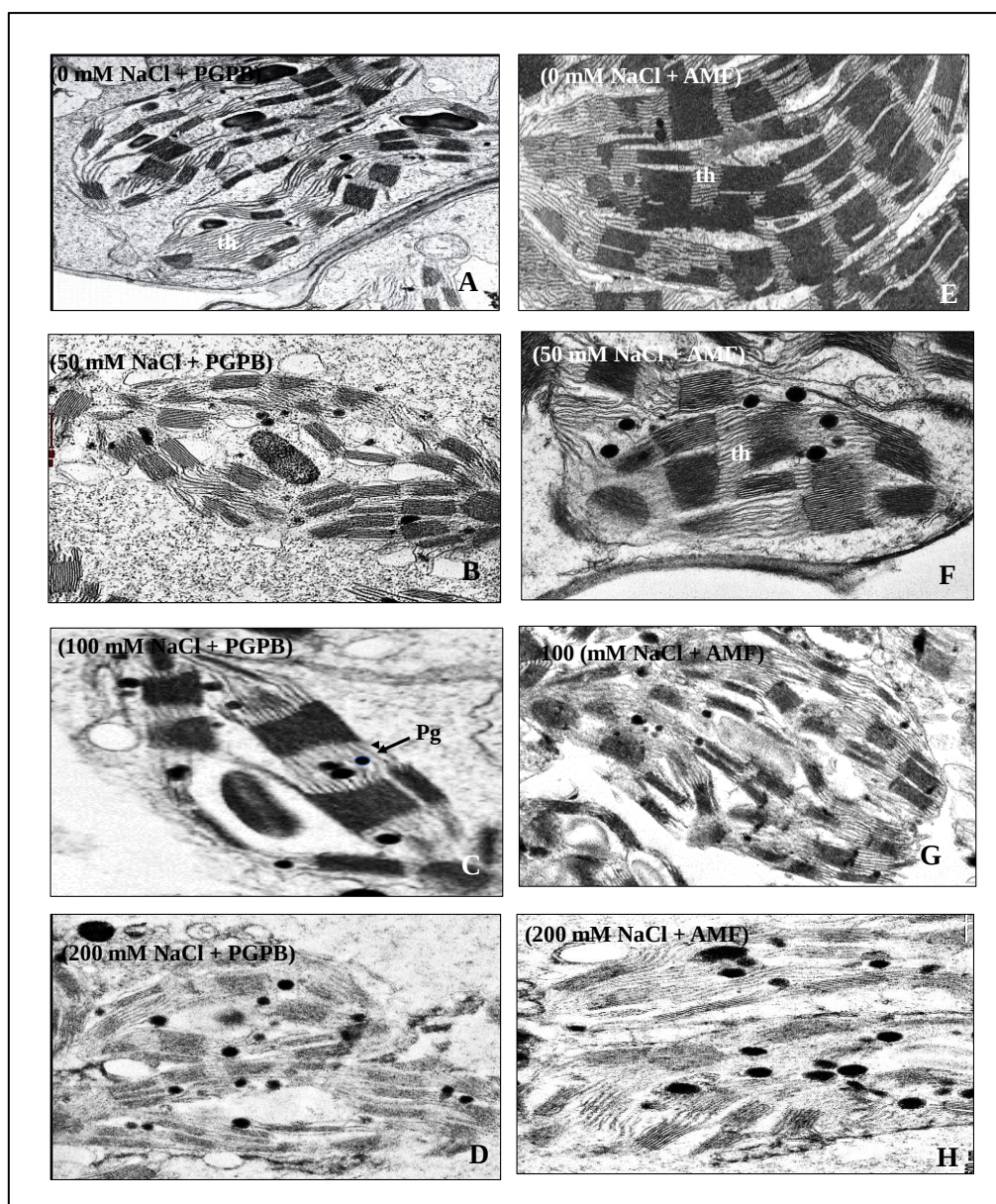


Figure 4.42: Transmission electron micrographs of the chloroplast of mesophyll cells of (A) PGPB-inoculated maize plant not exposed to NaCl; well-developed with distinct thylakoids and grana seen (B) PGPB-inoculated maize plant subjected to 50 mM NaCl; more thylakoids and fewer plastoglobules. (C) PGPB-inoculated maize plant exposed to 100 mM NaCl; structure of grana is still maintained and fewer plastoglobules. (D) PGPB-inoculated maize plant subjected to 200 mM NaCl; fewer plastoglobules have accumulated; structure of grana and thylakoid is still maintained. (E) AMF-inoculated maize plant not exposed to NaCl; well-developed thylakoids neatly stacked into grana (F) AMF-inoculated maize plant subjected to 50 mM NaCl; more thylakoids and fewer plastoglobules. (G) AMF-inoculated maize plant subjected to 100 mM NaCl; the chloroplast shows a lesser number of thylakoids and fewer accumulation of plastoglobules. (H) AMF-inoculated maize plant subjected to 200 mM NaCl; fewer plastoglobules have accumulated, and slight disintegration of the thylakoid is seen. Pg= plastoglobuli; th = thylakoids. The values in the bracket indicate the concentration of NaCl added to the soil.

4.4.1.7.2 Plasma membrane

Under 0 mM NaCl conditions the plasma membrane of both microbial inoculated and uninoculated maize plants closely adhered to the cell wall (**Figures 4.43** and **4.44**). As a result of salinity stress, the plasma membrane detached from the cell wall, forming an apoplastic space. In response to increased salinity stress, the space between the plasma membrane and the cell wall became larger. Plasma membrane detachment, however, was less pronounced in microbial-inoculated plants, resulting in a smaller cell wall-plasma membrane gap. At all levels of salinity stress, PGPB-inoculated plants showed comparatively less plasma membrane separation from the cell wall than AMF and *P. indica*. Furthermore, at higher salinity stress levels, invaginations of the plasma membrane led to the formation of vesicles in the cytoplasm. Compared with their microbial-inoculated counterparts, uninoculated plants displayed less vesicle formation.

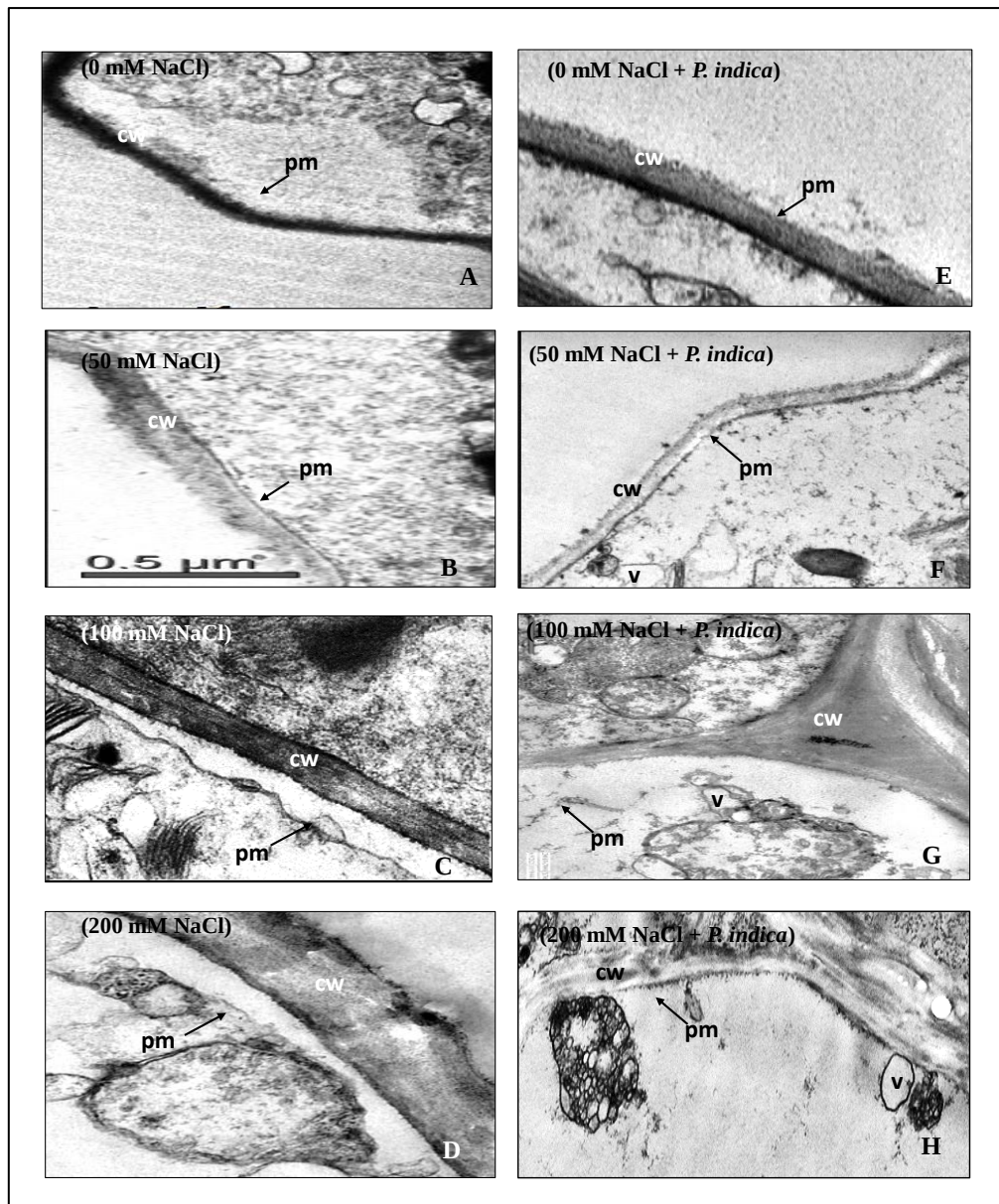


Figure 4.43: Transmission electron micrographs of the mesophyll cell wall and plasma membrane (A) Control maize plants not exposed to NaCl; plasma membrane firmly attached to the cell wall. (B) Control maize plants subjected to 50 mM NaCl; receding plasma membrane. (C) Control maize plants exposed to 100 mM NaCl; the gap between the plasma membrane and cell wall increased. (D) Control maize plants subjected to 200 mM NaCl; a marked increase in space between the plasma membrane and cell wall. (E) *P. indica* inoculated maize plants not exposed to NaCl; plasma membrane tightly attached to the cell wall. (F) *P. indica* inoculated maize plants subjected to 50 mM NaCl; the plasma membrane is closely bound to the cell membrane. (G) *P. indica* inoculated maize plants subjected to 100 mM NaCl; incipient plasmolysis and vesicle formation initiated. (H) *P. indica* inoculated maize plants subjected to 200 mM NaCl; numerous vesicles near the plasma membrane.

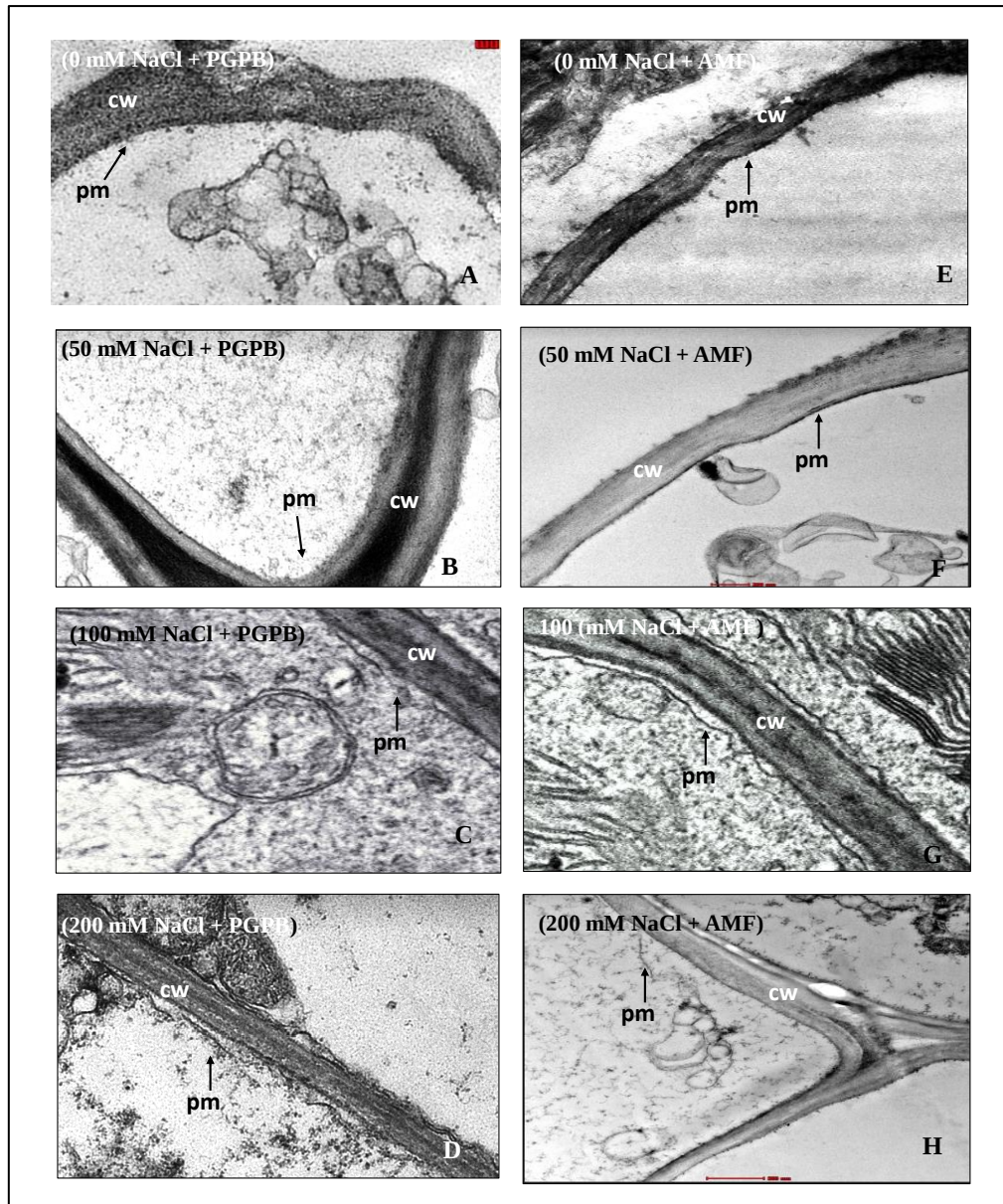


Figure 4.44: Transmission electron micrographs of the mesophyll cell wall and plasma membrane: (A) PGPB-inoculated maize plants without NaCl treatment; plasma membrane closely associated with the cell wall. (B) PGPB-inoculated maize plants subjected to 50 mM NaCl; plasma membrane closely attached to the cell wall. (C) PGPB-inoculated maize plants subjected to 100 mM NaCl; slight separation between the plasma membrane and cell wall; vesicles are seen near the membrane. (D) PGPB-inoculated maize plants subjected to 200 mM NaCl; distance between the plasma membrane and cell wall slightly increased (E) AMF-inoculated maize plants not exposed to NaCl; well-preserved plasma membrane firmly adhered to the cell wall. (F) AMF-inoculated maize plants subjected to 50 mM NaCl; well-preserved plasma membrane firmly attached to the cell wall. (G) AMF-inoculated maize plants subjected to 100 mM NaCl; plasma membrane slightly detached from the cell wall; vesicles are seen near the membrane. (H) AMF-inoculated maize plants subjected to 200 mM NaCl; numerous vesicles near the plasma membrane. The values in brackets indicate the concentration of NaCl added to the soil. cw = cell wall; pm = plasma membrane; v = vesicle.

Table 4.12: Effect of NaCl, *P. indica*, NaCl × *P. indica*, PGPB, NaCl × PGPB, AMF, NaCl × AMF on physiological processes, photosynthetic pigments, protein, α-tocopherol, membrane damage, osmolytes contents, and antioxidant enzyme activity in maize plants.

Parameters	NaCl	<i>P. indica</i>	NaCl × <i>P. indica</i>	PGPB	NaCl × PGPB	AMF	NaCl × AMF
Photosynthetic rate	***	***	ns	***	ns	***	ns
Transpiration	***	***	***	***	***	***	***
Stomatal conductance	***	***	***	***	***	***	***
Internal CO ₂	***	***	ns	***	***	***	***
Chlorophyll a	***	***	***	***	***	***	***
Chlorophyll b	***	**	**	***	ns	***	ns
Total chlorophyll	***	***	***	***	***	***	***
Carotenoid	ns	***	*	***	ns	***	ns
Protein	***	***	ns	***	*	***	ns
α-tocopherol	***	***	ns	***	***	***	***
Lipid peroxidation	***	***	ns	***	***	***	***
Relative permeability	***	***	**	***	***	***	***
LOX	***	***	ns	***	*	***	**
Trehalose	***	***	***	***	***	***	***
Total sugars	***	***	ns	***	ns	***	**
Proline	***	***	**	***	*	***	**
Glycine betaine	***	***	ns	***	ns	***	ns
Putrescine	***	***	***	***	***	***	***
Spermidine	***	***	***	***	***	***	***
SOD	***	***	ns	***	ns	***	ns
CAT	***	***	**	***	***	***	***
APX	***	***	ns	***	***	***	***

Significant interactions at P<0.05. ns: not significant. ***P<0.001, **P<0.01, *P<0.05. AMF, arbuscular mycorrhizal fungi; PGPB, plant growth-promoting bacteria; LOX, lipoxygenase; SOD, superoxide dismutase; CAT, catalase; APX, ascorbate peroxidase.

4.4.2 Discussion

Our study showed that increasing salinity stress levels reduced the ability of AMF and *P. indica* to colonize maize roots. According to previous studies, salinity stress reduces hyphal growth and mycorrhizal colonization (Saravi et al. 2022). In response, shoot and root biomass decreased, suggesting that AMF and *P. indica* benefits are dependent on root colonization.

A reduction in maize plant growth was seen for both microbial inoculated and uninoculated plants under salinity stress. The reduction in plant growth may be attributed to decreased nutrient uptake and photosynthesis as a result of salinity stress (Azeem et al. 2023). However, AMF, PGPB, and *P. indica* inoculated maize plants displayed longer shoots and roots, as well as higher biomass under salinity stress conditions. Our results are consistent with earlier reports on PGPB colonization in mustard (Khan et al. 2023). Studies by Ghabooli and Kaboosi 2022 found that inoculating legume plants with AMF increased shoot biomass under salinity stress conditions. The results of our study showed that microbial-inoculated maize plants exhibited higher leaf area under salinity stress compared to uninoculated plants. Khan et al. (2023) reported that mustard plants inoculated with PGPB had increased leaf area. Plant growth was increased in microbial-inoculated plants as a result of improved nutrient uptake (Masrahi et al. 2023) and osmolyte accumulation, in addition to increased antioxidant activity and a decreased lipid peroxidation process (Khan et al. 2023). As compared to AMF and *P. indica* inoculated plants, PGPB-inoculated maize plants showed higher salinity resistance.

Our study showed that inoculated and uninoculated maize plants had nutrient depletion due to salinity stress. Maize plants are unable to absorb essential nutrients due to poor root development and a lack of water availability during salinity. However, microbial inoculation led to better nutrient acquisition than an uninoculated maize plant at every level of salinity stress. Enhanced nutrient status is attributed to the increased absorption surface of extraradical hyphae in AMF and *P. indica* inoculated plants (Ortiz et al. 2015). Nutrients play a crucial role in maize plant growth and development. The mineral calcium is critical for the integrity of membranes, the stability of cell walls, and ion transport (de Bang et al. 2021). Plants become deficient in Ca^{2+} when they are exposed to salinity. During salinity stress, the Ca^{2+} present in membranes and cell walls is replaced by Na^{+} resulting in the reduction of cell turgidity. According to our study, maize plants inoculated with microbes were better at absorbing Ca^{2+} ions, consistent with the findings of Ait-El-Mokhtar et al. (2020).

K^{+} maintains membrane potential and osmotic pressure (Sardans and Peuelas, 2021). Moreover, it modulates protein and sugar synthesis, and enzyme activity

(Hasanuzzaman et al. 2018). We found that both inoculated and uninoculated maize plants showed a reduction in K^+ levels following salinity treatment. Because K^+ and Na^+ are inherently competitors due to their similar physiological properties. Because of this, cytoplasmic Na^+ competes with K^+ for binding sites, inhibiting K^+ -dependent metabolic processes. In contrast, inoculated maize plants substantially elevated K^+ content more than uninoculated maize plants, consistent with previous findings that PGPB inoculation enhanced K^+ uptake in *Salvia Officinalis* (Aslani et al. 2021). Higher accumulation of osmolytes, protein synthesis, and antioxidant enzyme activity was associated with higher K^+ accumulation (Hasanuzzaman et al. 2018) in plants colonized with microbes under salinity stress.

Mg^{2+} is a vital component of chlorophyll and is essential for the photosynthesis and synthesis of proteins (Kleczkowski and Igamberdiev 2021). Salinity stress reduced chlorophyll content and photosynthesis in rice plants; however, AMF inoculation increased these parameters (Tisarum et al. 2020). Similarly, Mg^{2+} uptake was also inhibited by salinity in *Pisum sativum*, but AMF mitigated this effect (Parihar et al. 2020). We found that Mg^{2+} content in maize plant tissues was significantly affected by salinity and microbial inoculation. It may be due to a lack of Ca^{2+} availability under salinity stress. The Ca^{2+} ion competes with the Mg^{2+} ion (Pandey and Sanyal 2021) for plasma membrane sites. We found that increased levels of Mg^{2+} correlate with higher photosynthetic pigments and photosynthetic rates in microbial-inoculated maize plants under salinity stress.

During salinity stress, Na^+ content increased rectilinearly in both microbial inoculated and control maize plants. However, uninoculated maize was higher in Na^+ than PGPB, AMF, and *P. indica*, indicating that microbes play a crucial role in Na^+ uptake. Na^+ is excluded from plants by AMF because it inhibits its uptake from soil or its translocation in plants. Apparently, microbes influence Na^+ translocation to aerial parts. PGPB secrete exopolysaccharides that bind to Na^+ present in soil and restrict its absorption by plants and increase nutrient uptake by roots (Bhagat et al. 2021). AMF-colonized maize plants have low Na^+ content, possibly due to the ability of the fungus to confine Na^+ in intraradical hyphae or root cell vacuoles. Maize plant growth and physiological processes are enhanced by mycorrhizal symbiosis, therefore diluting these ions in tissues.

Micronutrients play a crucial role in maize plant growth and development. Synthesis of proteins and photosynthetic pigments requires Zn^{2+} (Kumar et al. 2021). A vital component of chloroplast structure and function is Fe, which is necessary for chlorophyll synthesis (Kumar et al. 2021). The role of copper in plant cells includes photosynthesis, respiration, and the structure of the cell wall (Kumar 2015a). In photosystem II, Mn^{2+} contributes to the structure of the water-splitting system which provides electrons for photosynthesis. Micronutrient uptake in maize plants is reduced by salinity. We observed that increasing salinity levels reduced the content of Cu, Fe, Mn^{2+} , and Zn^{2+} in both microbial-inoculated and control maize plants. This is because salinity reduces the solubility and mobility of Cu, Fe, and Zn^{2+} in soil solutions, and a depletion zone develops around them. Thus, control maize plants are unable to access these nutrients. Furthermore, decreasing Cu, Zn^{2+} , Mn^{2+} , and Fe levels in microbial-inoculated maize plants may be the result of decreased hyphal growth. Microbial inoculation, however, improved micronutrient acquisition at all levels of salinity stress. According to earlier studies, Zn^{2+} , Fe, and Mn^{2+} content in *Vitis vinifera* was increased after AMF and PGPB (Karimi and Noori 2022) which is in line with our results. Bacteria chelate and acidify soil to make Cu, Fe, Mn^{2+} , and Zn^{2+} more accessible to plants (Etesami et al. 2014). In our study, higher photosynthetic content and photosynthesis are also associated with higher micronutrient content in maize plants colonized with microbes.

The ability of a plant to sustain photosynthesis determines its potential for growth and salinity tolerance (Tisarum et al. 2020). Stomatal closure reduces gas exchange and internal CO_2 levels during salinity stress, thereby reducing photosynthetic pigment production and photosynthesis (Meng et al. 2023 Mar), which was further confirmed in the present study. Additionally, during salinity, the transpiration rate decreased, resulting in reduced nutrient and water uptake in maize plants. However, maize plants inoculated with PGPB, AMF, and *P. indica* exhibited an increase in photosynthetic rate, stomatal conductance, internal CO_2 , and transpiration rate at all levels of salinity. The results of our study are consistent with previous studies that have found that AMF inoculation improves plant photosynthetic efficiency by improving CO_2 assimilation rate, stomatal conductance, and transpiration rate. Moreover, osmolyte accumulation in

AMF-colonized plants reduces structural damage to the photosynthetic apparatus (Puccio et al. 2023). Furthermore, carotenoid content was significantly increased by microbial inoculation in maize plants in our study. As an antioxidant, carotenoids protect the chloroplast structure, inhibit MDA formation, stabilize membranes, and play a critical role in the assembly of light-harvesting complexes (Gómez-Sagasti et al. 2023). In our study, higher chlorophyll and carotenoid levels, combined with a larger leaf area, resulted in higher photosynthesis rates in maize plants.

A critical component of photosynthesis is chlorophyll and carotenoids, pigments that absorb light in maize plants. Chlorophyll and carotenoid contents are decreased by salinity stress (Khan et al. 2023), which is in line with our results. We found that maize plants inoculated with PGPB, AMF, and *P. indica* had higher chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid levels than the control maize plants. Hence, microbial inoculation reduces the detrimental effects of salinity on maize plants. A similar study found that PGPB colonization substantially increased the contents of chlorophyll and carotenoid in maize (Solórzano-Acosta et al. 2023). According to a study, *Colocasia esculenta* colonized with AMF contained a higher level of chlorophyll and carotenoids (Baltazar-Bernal 2022). The present study is consistent with previous studies suggesting higher photosynthetic pigments may result from antioxidant accumulation, chloroplast protection, and greater solubilization and bioavailability of nutrients (Parihar et al. 2020). Our results are in agreement with the finding that AMF increases chlorophyll levels in maize through improved uptake of nutrients (Parihar et al. 2020).

We observed significantly higher osmolyte accumulation in both microbial inoculated (PGPB, AMF, and *P. indica*) and uninoculated maize plants during salinity stress. However, microbial inoculated plants had a greater amplitude of osmolyte increases than uninoculated plants. The results of this study are consistent with a previous study that demonstrated increased proline, glycine betaine, and sugar content in mustard inoculated with the PGPB under salinity stress (khan et al. 2023). AMF effectively ameliorated salinity-induced stress in *Colocasia esculenta* by increasing glycine betaine, proline, and sugar accumulation (Baltazar-Bernal et al. 2022) by maintaining cell turgidity and membrane integrity. According to Yaghoubian et al. 2022, *P. indica*

and azotobacter symbiosis enhances salinity tolerance in *Silybum marianum* through the production of glycine betaine, proline, and sugar accumulation. Based on these results, microbial inoculated maize plants are more efficient at osmotic adjustment than their uninoculated counterparts. However, PGPB outpaces AMF and *P. indica* in osmotic adjustment in maize plants under salinity stress.

In our study, there was a remarkable difference in protein content in microbial inoculated and uninoculated maize plants under all levels of salinity stress. Maize plants inoculated with PGPB, *P. indica*, and AMF had higher protein content. In microbial-inoculated plants, higher protein content is associated with higher photosynthetic rates, chlorophyll contents, membrane integrity, and osmolyte accumulation. Similarly, Solórzano-Acosta et al. (2023) found that salinity stress could be mitigated by PGPB and AMF-colonized *Persea americana* plants with higher levels of protein. According to this study, inoculating maize plants with PGPB, *P. indica*, or AMF enhanced salinity tolerance through protein regulation.

In our study, maize plants inoculated with PGPB, *P. indica*, and AMF have a high level of α -tocopherol, which indicates that α -tocopherol is a free radical scavenger as well as a modulator of lipid peroxidation during abiotic stress (Kim et al. 2019). This study substantiates the findings of Afshar and Abbaspour (2023) that higher levels of α -tocopherol in AMF colonized pistachio plants mitigate salinity stress. Based on the current study, inoculating maize plants with PGPB, *P. indica*, and AMF enhanced salinity tolerance by regulating α -tocopherol content, thus maintaining a high level of photosynthetic efficiency and chlorophyll content and reducing ion leakage.

In plants, malondialdehyde, a by-product of membrane lipid peroxidation, is considered an important physiological indicator of salinity (Xu et al. 2022). Peroxidation of lipids alters the ultrastructure and permeability of cell membranes. As a result of LOX activity, lipids are converted into hydroperoxyl fatty acids, which release free radicals that cause damage to the cells in maize plants during salinity stress. Salinity tolerance depends on reduced lipid peroxidation and relative membrane permeability (Afshar et al. 2023). In our study, MDA content, relative membrane permeability, and LOX activity of both the inoculated plants and the control maize plants increased rectilinearly

with increasing salinity levels. In microbial- inoculated maize plants, however, MDA content, LOX activity, and electrolyte leakage were less pronounced than that in control plants, which is consistent with previous reports (Afshar et al. 2023). Microbial-inoculated maize plants have reduced LOX activity, in contrast to a previous report in which the co-inoculation of PGPB and AMF resulted in a higher LOX activity (Begum et al. 2022). In our study, inoculated maize plants exhibited decreased MDA concentrations and relative membrane permeability by synthesizing non-enzymatic molecules like carotenoids, proline, and α -tocopherol, as well as enzymatic antioxidants (CAT, APX, and SOD). A similar finding was reported by Khan et al. (2023) that PGPB-inoculated plants were able to reduce ROS formation by maintaining their carotenoids and proline content, and CAT, APX, and SOD activities.

Uninoculated plants and inoculated maize plants displayed significantly different antioxidant enzyme activities in our study. The antioxidant activity of microbial-inoculated plants was higher than that of control plants. Our result is consistent with a previous study that demonstrated that microbial inoculation is effective in improving plant growth under salinity stress by enhancing the activity of ROS-scavenging antioxidant enzymes (Solórzano-Acosta et al. 2023). Therefore, microbial inoculation reduces membrane permeability and lipid peroxidation in plants by increasing antioxidant enzyme activity (Tyagi et al. 2023). It has previously been reported that *P. indica* colonized tomato exhibited increased antioxidant enzyme activity under salinity stress conditions (Xu et al., 2022) which supports our findings. According to Zhang et al. 2023, the use of *P. indica* increases salinity tolerance in soybean through the enhancement of antioxidant enzyme activity. Our study found that as salinity levels increased, CAT activity in AMF-inoculated maize plants decreased. The findings are consistent with previous studies showing that PGPB reduces CAT activity during abiotic stress in maize (Siddique et al. 2022). A study conducted by Samaddar et al. (2019) reported a significant decrease in APX activity during salinity stress in plants inoculated with PGPB which is in line with our study. In the current study, PGPB, AMF, and *P. indica* are proposed to enhance antioxidant defense mechanisms and salinity tolerance in maize.

Chloroplasts are highly susceptible to salinity stress (Liu et al. 2022). The present study showed that salinity stress affected the ultrastructure of chloroplasts of both microbial inoculated and control maize plants. Salinity reduces the thylakoid lumen by contracting the membrane which may be a consequence of osmotic collapse (Semenova 2002). When salinity levels increased, thylakoid swelling caused destacking of grana (Kotula et al. 2019). In several studies, it has been demonstrated that osmotic stress damages thylakoid membranes and causes swelling (Goussi et al. 2018). The chloroplasts of microbial-inoculated maize plants, however, seem to be less damaged than their uninoculated counterparts in our study. This may be attributed to a better accumulation of osmolytes viz. glycine betaine, polyamines, and sugars in plants that have been inoculated with microbes. As osmolytes accumulate in plants, they prevent osmotic collapse during salinity stress. Ali et al. (2020) reported that polyamines inhibit membrane degradation in grana and stroma thylakoids through their interaction with negatively charged membrane sites. In our study, the plastoglobule sizes of microbial-inoculated maize plants were larger, which prevented oxidative damage to the chloroplast membranes. According to Sheteiwy et al. (2022), plastoglobules grow bigger in size during salinity. Plastoglobules are lipoprotein structures that are attached to thylakoids, and they play an important role in chloroplast metabolism and stress response (Woodson 2022). In the presence of oxidative stress, they serve as sites for the synthesis of α -tocopherol. In our study, plants inoculated with microbes had higher α -tocopherol levels than the control. The antioxidant activity of α -tocopherol has been linked to the protection of membrane lipids and photosystem II from photooxidation. Enhanced polyamine levels may be responsible for plastoglobule accumulation in microbially inoculated plants (Shu et al. 2015). This study showed that maize plants inoculated with microbes had reduced oxidative damage to chloroplasts due to their high osmolyte levels and large plastoglobule sizes. However, PGPB has a greater ability to alleviate salinity stress than *P. indica* and AMF-inoculated maize plants at all levels of salinity in terms of maintaining the ultrastructure of the chloroplast.

Salt tolerance in plants can be predicted based on cell membrane stability (Pathiana et al. 2023). Electron micrographs showed that plasma membranes of maize plants disintegrated when exposed to salinity. In response to increasing salinity levels, the

space between the plasma membrane and cell wall increases. Similar findings also were found for *Halodule wrightii* (Ferreira et al. 2017). The membrane disintegrates due to the peroxidation of polyunsaturated fatty acids by free radicals (Zhang et al. 2021) as well as salt-induced shrinkage of protoplasm. As compared to uninoculated maize plants, microbial inoculated plants showed less shrinkage of their protoplasm and membrane damage. Furthermore, the accumulation of osmolytes (glucose, trehalose, proline, glycine betaine, and polyamines) maintains cell turgidity and stabilizes membranes and enzymes (Sharma et al. 2019). In our study, maize plants inoculated with microbes displayed lower levels of electrolyte leakage, lipid peroxidation, and LOX activity, which are critical for membrane integrity. Moreover, plants inoculated with microbes possess more antioxidant molecules (proline, α -tocopherols, and polyamines), enzymatic activity (SOD, CAT, and APX), and nutrients (Ca^{2+} and K^{+}) which play a vital role in membrane protection. In a stressful environment, proline is an effective scavenger of ROS, preventing oxidative stress from damaging cells. Furthermore, α -tocopherol scavenges free radicals in the host tissue, protecting it from lipid peroxidation (Niki 2021). Polyamines are important for membrane stability (Wang et al. 2022). Antioxidant enzymes (CAT, SOD, and APX) can scavenge ROS and protect membranes from oxidative stress in adverse environmental conditions (Ru et al. 2023). Calcium forms intermolecular links that maintain membrane integrity, stabilize cell walls, and regulate ion transport (de Bang et al. 2021). In plants, potassium plays a critical role in osmoregulation and membrane potential (Sardans and Peuelas, 2021). Microbial inoculation leads to the accumulation of osmolytes, nutrients, and antioxidants, which reduce membrane damage (Tyagi et al. 2023). In our study, more vesicles were formed in microbial-inoculated maize plants than in the control. Under stress conditions, vesicle formation increases (Karim et al. 2014). By increasing the membrane surface area, vesicles facilitate ion transport and storage, protecting the cells from ionic imbalance. Therefore, the higher rate of vesicle formation observed in this study suggests that microbial-inoculated plants are able to tolerate salinity better. However, PGPB has a greater ability to alleviate salinity stress than *P. indica* and AMF in maize plants at all levels of salinity in terms of maintaining the ultrastructure of the plasma membrane.

5. SUMMARY AND CONCLUSION

Worldwide, drought and salinity are major abiotic stress that adversely affects crop growth and productivity. In plants exposed to drought and salinity, changes occur in morphology, physiology, biochemistry, and ultrastructure that inhibit growth and development. Additionally, drought and salinity affect enzyme activity, stomatal conductance, photosynthesis, and reactive oxygen species (ROS) production. ROS produced by cells damage cell membranes, lipids, proteins, DNA, and RNA, as well as induce programmed cell death. Abiotic stress adversely affects nutrient uptake, osmotic balance, membrane integrity, and the overall growth of plants. Drought and salinity stress strongly modify plant ultrastructure. Drought and salinity damage most structural components of plants, leading to their loss of function and eventual death. Among a vast variety of soil microbes living in the rhizosphere, arbuscular mycorrhizal fungi (AMF), plant growth-promoting bacteria (PGPB), and *Piriformospora indica* are ubiquitous. These fungi and bacteria are important components of natural ecosystems and can survive droughts and saline conditions. In an array of host plants, *P. indica* symbiosis alleviates drought stress in plants such as *Salvia officinalis*, *Solanum lycopersicum*, and *Poncirus trifoliata*. *P. indica* symbiosis also mitigates salinity stress in plants such as *Brassica napus*, *Ocimum basilicum*, and rice. AMF symbiosis has been shown to alleviate drought stress in *Dalbergia sissoo*, *Camellia sinensis*, and *Trifolium repens*. AMF symbiosis also mitigates salinity stress in legumes, pistachio, and barley. PGPB has been shown to alleviate drought stress in an array of host plants such as *Oryza sativa*, *Juglans regia*, and *Salvia officinalis*. PGPB also mitigates salinity stress in various host plants such as *Glycyrrhiza glabra*, *Arabidopsis*, and *Vigna radiata*. AMF, PGPB, and *P. indica* alleviate drought and salinity-induced growth inhibition, but the mechanisms involved remain unclear. Based on these considerations, the following objectives were set to investigate the mechanisms underlying drought and salinity tolerance in wheat and maize colonized by PGPB (*Azotobacter chroococcum*, *Enterobacter asburiae*, and *Lactococcus lactis*), AMF (*Glomus intraradices*, *Glomus mosseae*, and *Scutellospora*) and *P. indica*.

1. **To study the role of soil microbes on growth, changes in nutritional, biochemical, and physiological parameters of wheat and maize plants under salt and drought stresses.**
2. **To study the role of soil microbes during salt and drought stresses on the antioxidant accumulation in wheat and maize.**
3. **To study the role of soil microbes on cell ultrastructural changes in wheat and maize plants under salt and drought stresses.**

To accomplish these objectives, a pot experiment was conducted in the botanical garden of Swami Shraddhanand College, University of Delhi, India. The experiment was laid out for wheat and maize in a completely randomized block design with two factors: microbial inoculation and drought levels. There were four levels of water stress treatment (75%, 55%, 45%, and 35% of soil field capacity) and four microbial conditions (PGPB-inoculated, AMF-inoculated, *P. indica*-inoculated, and uninoculated). Therefore, 16 treatments (4 x 4) with three replicates each were used. Another pot experiment was laid out for wheat and maize in a completely randomized block design with two factors: microbial inoculation and salinity levels. There were four levels of salinity treatment (0, 50, 100, and 200 mM NaCl) and four microbial conditions (PGPB-inoculated, AMF-inoculated, *P. indica*-inoculated, and uninoculated). Therefore, 16 treatments (4 x 4) with three replicates each were used.

The major findings of the investigation are summarized as follows:

1. AMF and *P. indica* successfully colonized the roots of maize plants at all levels of drought salinity stress. However, the ability of AMF and *P. indica* to colonize wheat and maize roots decreased with increasing drought and salinity stress levels. However, despite of decline in AMF and *P. indica* colonization, microbial-inoculated plants showed better growth and biomass compared to uninoculated plants. The percentage of colonization was higher in AMF-inoculated wheat and maize plants as compared to *P. indica*-inoculated plants during drought and salinity stress. These observations suggest that the ability of AMF and *P. indica* in the alleviation of drought and salinity stress is not affected.

2. As the level of drought and salinity increased, the concentrations of growth and biomass declined gradually in maize and wheat plants. However, at all levels of drought and salinity, the microbial-inoculated wheat and maize plants performed significantly better in terms of shoot length, root length, shoot biomass, root biomass, and leaf area compared to uninoculated plants. On comparing the potential of microbes to increase growth parameters, PGPB-inoculated plants showed higher drought and salinity resistance as compared to AMF and *P. indica*-inoculated plants. This indicates that drought and salinity affected both the growth and biomass of plants. On comparing the potential of microbes to increase growth parameters, PGPB-inoculated wheat and maize plants showed higher drought and salinity resistance as compared to AMF and *P. indica*-inoculated plants.
3. With the increase in the level of drought and salinity, macronutrients (Ca^{2+} , Mg^{2+} , and K^{+}), micronutrients (Zn^{2+} , Fe, Mn^{2+} , and Cu) content of wheat and maize plant tissue showed a significant decline in both microbial-inoculated and uninoculated plants. Since macronutrients and micronutrients are involved in many physiological and biochemical processes in the plant, the low nutrient content is detrimental to plant growth. However, at all levels of drought and salinity, microbial-inoculated plants displayed more macronutrients and micronutrients than uninoculated wheat and maize plants. Due to the ability of microbes to absorb nutrients via extra radical hyphae or solubilization, immobile metal nutrients are more readily absorbed. This suggests that microbial colonization could overcome the negative effects and microbial-inoculated wheat and maize plants were more tolerant to drought and salt stress.
4. Microbial inoculation, drought, and salinity stress had significant effects on leaf Na^{+} contents in wheat and maize plants. With increasing drought and salinity stress levels, Na^{+} increased in PGPB, AMF, and *P. indica* inoculated, and uninoculated plants. At all levels of drought and salinity stress, however, the presence of microbes led to lower Na^{+} levels than uninoculated plants. This indicated that microbe colonization influenced the translocation of Na^{+} ions to plant aerial parts, thus preventing their toxicity.

5. Photosynthesis is integral to plant growth and is, therefore, an important indicator of a plant's potential for growth and drought and salinity tolerance. In response to drought and salinity stress, stomatal closure decreases gas exchange and internal CO₂ levels, resulting in a decrease in photosynthetic pigment production, thereby reducing the photosynthetic rate in wheat and maize plants. Moreover, the transpiration rate decreased during drought and salinity stress leading to reduced nutrient and water uptake in wheat and maize plants. In contrast, drought-stressed wheat and maize plants inoculated with PGPB, AMF, and *P. indica* had an increase in photosynthetic rate, stomatal conductance, internal CO₂, and transpiration rate. Additionally, the results indicated that microbial inoculation significantly increased the carotenoid content in wheat and maize plants. The antioxidant property of carotenoids protects the photosynthetic apparatus against free radical damage, inhibits lipid peroxidation, stabilizes cell membranes, and plays a critical role in light-harvesting complex assembly. Increased photosynthesis rates are a result of- (i) higher chlorophyll content ; (ii) higher leaf area; (iii) increased CO₂ assimilation rate; (iv) higher stomatal conductance; (v) more transpiration rate; (vi) increased carotenoid content responsible for protecting the photosynthetic apparatus against free radical damage, inhibits lipid peroxidation, stabilizes cell membranes, and plays a critical role in light-harvesting complex assembly; and (vii) reduced structural damage to the photosynthetic apparatus by enhanced osmolyte accumulation in plants.
6. As light-absorbing pigments, chlorophylls, and carotenoids are crucial for photosynthesis in wheat and maize plants. Drought and salinity decrease chlorophyll and carotenoid content in maize and wheat plants. PGPB, AMF, and *P. indica* inoculation increased the chlorophyll a, chlorophyll b levels, total chlorophyll, and carotenoid levels in wheat and maize plants as compared to their uninoculated counterparts. This suggests that microbial inoculation reduces the impact of drought and salinity on wheat and maize. Higher photosynthetic pigments are probably a consequence of higher antioxidant accumulation, prevention of ultrastructure of chloroplast, and increasing the solubilization and bioavailability of nutrients. PGPB is the most efficient microbe in alleviating

drought and salinity stress as compared to AMF and *P. indica* inoculated plants in terms of photosynthetic pigments.

7. A major consequence of drought and salinity stress is the accumulation of osmolytes (glycine betaine, trehalose, sugars, proline, and polyamines). With an increase in stress level, osmolyte content in wheat and maize increased proportionately. At each level of drought and salinity, microbial-inoculated wheat and maize plants accumulated more osmolytes compared to uninoculated plants. However, the proline content decreased in AMF-inoculated wheat plants with increasing levels of salinity. High osmolytes content in microbial-inoculated wheat and maize plants indicates more tolerance to drought and salinity stress and a better capacity to maintain osmotic balance and integrity of cell ultrastructure.
8. There was a remarkable difference in protein content in microbial inoculated and uninoculated wheat and maize plants under all levels of drought and salinity stress. Wheat and maize plants inoculated with PGPB, *P. indica*, and AMF had higher protein content. Proteins are organic molecules that form the structural and functional units of a cell. These compounds improve chlorophyll content, protect chloroplast structure, activate ROS scavenging, and enhance osmolyte synthesis. Higher protein content in microbial-inoculated wheat and maize plants under drought and salinity stress is associated with higher photosynthetic rates, chlorophyll contents, membrane integrity, and osmolyte accumulation. PGPB is the most efficient microbe in alleviating drought and salinity stress as compared to AMF and *P. indica* inoculated plants in terms of protein content.
9. Since α -tocopherol is an antioxidant, it inhibits ROS generation during photosynthesis (mainly $^1\text{O}_2$ and $\text{OH}\cdot$) and reduces lipid peroxidation. In our study, wheat and maize plants inoculated with PGPB, *P. indica*, and AMF have a high level of α -tocopherol, which indicates that α -tocopherol is a free radical scavenger as well as a modulator of lipid peroxidation during abiotic stress. Based on the current study, inoculating wheat and maize plants with PGPB, *P. indica*, and AMF enhanced drought and salinity tolerance by regulating α -tocopherol content, thus maintaining a high level of photosynthetic efficiency and chlorophyll content and reducing ion leakage. PGPB is the most efficient microbe in alleviating

- drought and salinity stress at all levels of the drought and salinity as compared to AMF and *P. indica* inoculated plants in terms of α -tocopherol content.
10. In wheat and maize plants, the extent of membrane damage is determined by the level of lipid peroxidation, electrolyte leakage, and LOX activity under drought and salinity stress. However, at all the levels of drought and salinity stress, the membrane damage was lower in microbial-inoculated plants as compared to uninoculated plants. This may be explained by better non-enzymatic (carotenoids, proline, and α -tocopherol), and enzymatic antioxidants (CAT, APX, and SOD) accumulation in microbial inoculated wheat and maize plants as compared to their uninoculated counterparts prevented peroxidation of membrane lipids. PGPB is the most efficient microbe in alleviating drought and salinity stress as compared to AMF and *P. indica* inoculated plants in terms of membrane integrity.
 11. Under drought and salinity stress conditions, microbial colonization enhanced SOD activity in wheat and maize plants. With increasing levels of drought and salinity stress, PGPB and *P. indica*-inoculated wheat and maize plants displayed increased CAT activity. Conversely, AMF-inoculated maize and wheat plants exhibited decreased CAT activity in response to increased drought and salinity stress in comparison to control. Microbial inoculated wheat and maize plants displayed higher APX activity than uninoculated plants under all levels of drought and salinity. At each level of water stress, PGPB-inoculated plants exhibited significantly higher antioxidant enzyme activity than *P. indica* and AMF-inoculated plants. Microbial inoculation improved plant growth under drought stress because of the enhanced activity of the ROS-scavenging antioxidant enzyme (Tyagi et al. 2023). Thus, microbial inoculation reduces lipid peroxidation and membrane permeability in plants by increasing antioxidant enzyme activity. The higher activity of these antioxidant enzymes in microbial-inoculated plants may be due to better nutrient acquisition during drought and salinity stress. PGPB is the most efficient microbe in alleviating drought and salinity stress as compared to AMF and *P. indica* inoculated plants in terms of antioxidant enzyme activity.

12. The response of plants to drought and salinity stress was identified by the changes in ultrastructure. During severe drought and salinity, cells exhibited a substantial change in ultrastructure. Stress resulted in shrinkage of cytoplasm; an increase in the gap between the cell wall and plasma membrane; destacking of grana in the chloroplast, a decrease in the number of thylakoids; higher accumulation of plastoglobules. Microbial-inoculated wheat and maize plants showed reduced alterations in the plasma membrane, and chloroplast as compared to their uninoculated counterparts. The outcome of this study is microbial colonization prevents drought and salinity-induced ultrastructural changes. Several factors may be responsible for the reduction in ultrastructure damage in microbial inoculated plants – (i) production of osmolytes (ii) reduced lipid peroxidation, electrolyte leakage, and LOX activity; (iii) production of more enzymatic and non-enzymatic antioxidants. PGPB is the most efficient microbe in alleviating drought and salinity stress as compared to AMF and *P. indica* inoculated plants in terms of maintaining the cell ultrastructure.

Conclusion

In conclusion, the results of this study confirm that the PGPB consortium, AMF consortium, and *P. indica* inoculation significantly improved wheat and maize performance under drought and salinity-stress conditions. Our findings demonstrate that microbial inoculation protects wheat and maize plants from drought and salinity stress by enhancing physiological processes and osmolyte accumulation, leading to better growth and biomass than uninoculated plants. The higher osmolyte accumulation ensures osmotic adjustment and scavenges free radicals more effectively. Additionally, microbial colonization maintains favorable macronutrient and micronutrient levels than uninoculated plants, thereby mitigating drought and salinity-induced ionic imbalances. PGPB consortium, AMF consortium, and *P. indica* inoculation reduced lipid peroxidation, membrane permeability, and LOX activity and increased antioxidant enzymes (SOD, CAT, and APX) activity and accumulation of antioxidant metabolites (α -tocopherol, proline, carotenoids, polyamines) in wheat and maize plants. Microbial-inoculated plants, therefore, have a better antioxidant defense system for preventing oxidative damage, which makes them more drought and salinity resistant. Colonization

with the PGPB consortium, AMF consortium, and *P. indica* mitigated ultrastructural alterations caused by drought and salinity-induced ion toxicity, osmotic stress, and oxidative stress. Wheat and maize plants inoculated with microbes suffered less ultrastructural damage as a result of improved osmolyte accumulation, nutrient acquisition, and antioxidant defense system. The preservation of ultrastructure in microbial-inoculated plants indicated enhanced drought and salinity tolerance. According to this study, the PGPB consortium, AMF consortium, and *P. indica* have the potential to improve the yield and tolerance of wheat and maize to drought and salinity stress, making them an excellent choice for agricultural practice.

REFERENCES

- A Saga of Progress: Compendium of 50 Years of Achievements, Punjab Agricultural University, Ludhiana, 2012, pp 230.
- Aalipour H, Nikbakht A, Sabzalian MR (2023) Essential oil composition and total phenolic content in *Cupressus arizonica* G. in response to microbial inoculation under water stress conditions. *Scientific Reports* 13(1):1-11. <https://doi.org/10.1038/s41598-023-28107-z>
- Aazami MA, Maleki M, Rasouli F, Gohari G (2023) Protective effects of chitosan based salicylic acid nanocomposite (CS-SA NCs) in grape (*Vitis vinifera* cv.'Sultana') under salinity stress. *Scientific Reports* 17;13(1):883. <https://doi.org/10.1038/s41598-023-27618-z>
- Abbas S, Javed MT, Shahid M I, Haider MZ, Chaudhary HJ, Tanwir K, Maqsood, A (2020) *Acinetobacter* sp. SG-5 inoculation alleviates cadmium toxicity in differentially Cd tolerant maize cultivars as deciphered by improved physio-biochemical attributes, antioxidants and nutrient physiology. *Plant Physiology and Biochemistry*. 155, 815-827. <https://doi.org/10.1016/j.plaphy.2020.08.024>
- Abbas SR, Ahmad SD, Sabir SM, Shah AH (2014) Detection of drought tolerant sugarcane genotypes (*Saccharum officinarum*) using lipid peroxidation, antioxidant activity, glycine-betaine and proline contents. *Journal of soil science and plant nutrition* 14(1):233-243. <http://doi.org/10.4067/S0718-95162014005000019>
- Abdel Latef AA, Omer AM, Badawy AA, Osman MS, Ragaey MM (2021) Strategy of salt tolerance and interactive impact of *Azotobacter chroococcum* and/or *Alcaligenes faecalis* inoculation on canola (*Brassica napus* L.) plants grown in saline soil. *Plants* 10(1):110. <https://doi.org/10.3390/plants10010110>
- Abobatta WF (2019) Drought adaptive mechanisms of plants—a review. *Advances in Agriculture and Environmental Science* 2(1):42-45. <https://doi.org/10.30881/aaeoa.00021>

- Adejumo SA, Oniosun B, Akpoilih OA et al. (2021) Anatomical changes, osmolytes accumulation and distribution in the native plants growing on Pb-contaminated sites. *Environmental Geochemistry and Health* 43:1537-1549. <https://doi.org/10.1007/s10653-020-00649-5>
- Afrangan F, Kazemeini SA, Alinia M, Mastinu A (2023) *Glomus versiforme* and *Micrococcus yunnanensis* reduce the negative effects of salinity stress by regulating the redox state and ion homeostasis in *Brassica napus* L. crops. *Biologia* 17:1-13. <https://doi.org/10.1007/s11756-023-01479-3>
- Afridi MS, Javed MA, Ali S, De Medeiros FH, Ali B, Salam A, Marc RA, Alkhalifah DH, Selim S, Santoyo G (2022) New opportunities in plant microbiome engineering for increasing agricultural sustainability under stressful conditions. *Front. Plant Sci* 13, 1-22. <https://doi.org/10.3389/fpls.2022.899464>
- Afshar AS, Abbaspour H (2023) Mycorrhizal symbiosis alleviate salinity stress in pistachio plants by altering gene expression and antioxidant pathways. *Physiology and Molecular Biology of Plants* 29(2):263-276. <https://doi.org/10.1007/s12298-023-01279-8>
- Agnihotri R, Sahni S, Sharma MP, Gupta MM (2022) Facets of AM fungi in sequestering soil carbon and improving soil health. In: Rajpal VR, Singh I, Navi SS (eds) *Fungal diversity, ecology and control management*, Fungal Biology, Springer, Singapore, pp 327-344. https://doi.org/10.1007/978-981-16-8877-5_15
- Ahmed M, Abdel-Fattah GG, Holford P, Korany SM, Alsherif EA, Abdelgawad H, Ulhassan Z, Joško I, Ali B, Sheteiwy MS (2023) *Funneliformis constrictum* modulates polyamine metabolism to enhance tolerance of *Zea mays* L. to salinity. *Microbiological Research* 266:127254. <https://doi.org/10.1016/j.micres.2022.127254>
- Ait-El-Mokhtar M, Baslam M, Ben-Laouane R, Anli M, Boutasknit A, Mitsui T, Wahbi S, Meddich A (2020) Alleviation of detrimental effects of salt stress on date palm (*Phoenix dactylifera* L.) by the application of arbuscular mycorrhizal fungi and/or compost. *Frontiers in Sustainable Food Systems* 4:131. <https://doi.org/10.3389/fsufs.2020.00131>

- Akhilesh S, Basandrai AK, Kalia V (2000) Resistance in rabi maize to *Fusarium* stalk rot (*Fusarium moliniformae*) and common rust (*Puccinia sorghi*). *Crop Research (Hisar)* 20(2):221-225
- Alam MZ, Choudhury TR, Mridha MA (2023) Arbuscular Mycorrhizal Fungi Enhance Biomass Growth, Mineral Content, and Antioxidant Activity in Tomato Plants under Drought Stress. *Journal of Food Quality* 2023:2581608. <https://doi.org/10.1155/2023/2581608>
- Albqmi M, Selim S, Al-Sanea MM, Alnusaire TS, Almuhayawi MS, Jaouni SK, Hussein S, Warrad M, Sofy MR, AbdElgawad H (2023) Interactive Effect of Arbuscular Mycorrhizal Fungi (AMF) and Olive Solid Waste on Wheat under Arsenite Toxicity. *Plants* 12(5):1100. <https://doi.org/10.3390/plants12051100>
- Aldesuquy HS, Ibraheem FL, Ghanem HE (2018) Exogenously supplied salicylic acid and trehalose protect growth vigor, chlorophylls and thylakoid membranes of wheat flag leaf from drought-induced damage. *Journal of Agriculture and Forest Meteorology Research* 1(1):13-20.
- Alejandro S, Höller S, Meier B, Peiter E (2020) Manganese in plants: from acquisition to subcellular allocation. *Frontiers in plant science* 11:300. <https://doi.org/10.3389/fpls.2020.00300>
- Alhagri IA, Albeshry M (2023) Microwave-Assisted Digestion Using Dilute Nitric Acid and Hydrogen Peroxide for Multielement Determination in Wheat Flour by ICP-MS. *Journal of Chemistry* 2023: 2349838. <https://doi.org/10.1155/2023/2349838>
- Ali R, Gul H, Hamayun M, Rauf M, Iqbal A, Hussain A, Lee IJ (2022) Endophytic fungi controls the physicochemical status of maize crop under salt stress. *Pol. J. Environ Stud* 31:561-573. <https://doi.org/10.15244/pjoes/134540>
- Ali R, Hassan S, Shah D, Sajjad N, Bhat EA (2020) Role of polyamines in mitigating abiotic stress. In: Aryadeep Roychoudhury, Durgesh Kumar Tripathi (ed) *Protective chemical agents in the amelioration of plant abiotic stress: biochemical and molecular perspectives*, volume 22, Wiley, New York, pp 291-305. <https://doi.org/10.1002/9781119552154.ch13>

- Alori, E. T., Glick, B. R., and Babalola, O. O. (2017) Microbial phosphorus solubilization and its potential for use in sustainable agriculture. *Front. Microbiol* 8:971. <https://doi.org/10.3389/fmicb.2017.00971>
- Anjum SA, Xie X, Wang LC, Saleem MF, Man C, Lei W (2011) Morphological, physiological and biochemical responses of plants to drought stress. *African journal of agricultural research* 6(9):202632.
- Arnon DI (1949) Copper enzymes isolated chloroplasts, polyphenoloxidase in *Beta vulgaris*. *Plant Physiol* 24 (1):1-15. <https://doi.org/10.1104%2Fpp.24.1.1>
- Arora S, Jha PN (2023) Drought-tolerant *Enterobacter bugandensis* WRS7 induces systemic tolerance in *Triticum aestivum* L.(Wheat) under drought conditions. *Journal of Plant Growth Regulation* 42(12):7715-7730. <https://doi.org/10.1007/s00344-023-11044-6>
- Ashraf M, Foolad MR (2007) Roles of glycine betaine and proline in improving plant abiotic stress resistance. *Environ Exp Bot* 59(2):206-216. <https://doi.org/10.1016/j.envexpbot.2005.12.006>
- Aslani Z, Hassani A, Abdollahi Mandoulakani B, Barin M, Maleki R (2021) Effect of Plant Growth-Promoting Microorganisms Inoculation on some Growth and Physiological Parameters and Nutrients Content of Sage (*Salvia officinalis*) Under Salinity Stress Conditions. *Applied Soil Research* 9(3):104-122.
- Aslani Z, Hassani A, Mandoulakani BA, Barin A, Maleki A (2023) Effect of drought stress and inoculation treatments on nutrient uptake, essential oil and expression of genes related to monoterpenes in sage (*Salvia officinalis*). *Scientia Horticulturae* 309:111610. <https://doi.org/10.1016/j.scienta.2022.111610>
- Azcon-G de Aguilar, C., Azcón, R., and Barea, J. M. (1979) Endomycorrhizal fungi and Rhizobium as biological fertilisers for *Medicago sativa* in normal cultivation. *Nature* 279:325–327. <https://doi.org/10.1038/279325a0>

- Azeem M, Sultana R, Mahmood A, Qasim M, Siddiqui ZS, Mumtaz S, Javed T, Umar M, Adnan MY, Siddiqui MH (2023) Ascorbic and Salicylic Acids Vitalized Growth, Biochemical Responses, Antioxidant Enzymes, Photosynthetic Efficiency, and Ionic Regulation to Alleviate Salinity Stress in *Sorghum bicolor*. Journal of Plant Growth Regulation 1-14. <https://doi.org/10.1007/s00344-023-10907-2>
- Azizi M, Fard EM, Ghabooli M (2021) *Piriformospora indica* affect drought tolerance by regulation of genes expression and some morphophysiological parameters in tomato (*Solanum lycopersicum* L.). Scientia Horticulturae 287:110260. <https://doi.org/10.1016/j.scienta.2021.110260>
- Babalola, O. O., and Glick, B. R. (2012) The use of microbial inoculants in African agriculture: current practice and future prospects. J. Food Agric. Environ 10: 540–549.
- Badawy IH, Hmed AA, Sofy MR, Al-Mokadem AZ (2022) Alleviation of cadmium and nickel toxicity and phyto-stimulation of tomato plant l. by endophytic *Micrococcus luteus* and *Enterobacter cloacae*. Plants 11(15):2018. <https://doi.org/10.3390/plants11152018>
- Baltazar-Bernal O, Spinoso-Castillo JL, Mancilla-Álvarez E, Bello-Bello JJ (2022) Arbuscular mycorrhizal fungi induce tolerance to salinity stress in Taro plantlets (*Colocasia esculenta* L. Schott) during acclimatization. Plants 11(13):1780. <https://doi.org/10.3390/plants11131780>
- Bashir MA, Silvestri C, Coppa E, Brunori E, Cristofori V, Rugini E, Ahmad T, Hafiz IA, Abbasi NA, Nawaz Shah MK, Astolfi S (2021) Response of olive shoots to salinity stress suggests the involvement of sulfur metabolism. Plants 10(2):350. <https://doi.org/10.3390/plants10020350>
- Bates LS, Waldren RP, Teare ID (1973) Rapid determination of free proline for water-stress studies. Plant Soil 39:205-207. <https://doi.org/10.1007/BF00018060>
- Batool A, Akram NA, Cheng ZG, Lv GC, Ashraf M, Afzal M, Xiong JL, Wang JY, Xiong YC (2019) Physiological and biochemical responses of two spring wheat genotypes to non-hydraulic root-to-shoot signalling of partial and full root-zone drought stress. Plant Physiology and Biochemistry 139:11-20. <https://doi.org/10.1016/j.plaphy.2019.03.001>

- Begum N, Ahanger MA, Su Y, Lei Y, Mustafa NS, Ahmad P, Zhang L (2019) Improved drought tolerance by AMF inoculation in maize (*Zea mays*) involves physiological and biochemical implications. *Plants* 8(12):579. <https://doi.org/10.3390/plants8120579>
- Begum N, Ahanger MA, Zhang L (2020) AMF inoculation and phosphorus supplementation alleviates drought induced growth and photosynthetic decline in *Nicotiana tabacum* by upregulating antioxidant metabolism and osmolyte accumulation. *Environmental and Experimental Botany* 176:104088. <https://doi.org/10.1016/j.envexpbot.2020.104088>
- Begum N, Akhtar K, Ahanger MA, Iqbal M, Wang P, Mustafa NS, Zhang L (2021). Arbuscular mycorrhizal fungi improve growth, essential oil, secondary metabolism, and yield of tobacco (*Nicotiana tabacum* L.) under drought stress conditions. *Environmental Science and Pollution Research* 28:45276-45295. <https://doi.org/10.1007/s11356-021-13755-3>
- Begum N, Wang L, Ahmad H, Akhtar K, Roy R, Khan MI, Zhao T (2022) Co-inoculation of Arbuscular Mycorrhizal Fungi and the Plant Growth-Promoting Rhizobacteria Improve Growth and Photosynthesis in Tobacco Under Drought Stress by Up-Regulating Antioxidant and Mineral Nutrition Metabolism. *Microb Ecol* 83:971-988. <https://doi.org/10.1007/s00248-021-01815-7>
- Bhagat N, Raghav M, Dubey S et al (2021) Bacterial exopolysaccharides: Insight into their role in plant abiotic stress tolerance. *J. Microbiol. Biotechnol* 31(8): 1045-1059. <https://doi.org/10.4014/jmb.2105.05009>
- Bhardwaj AK, Chandra KK, Kumar R (2023). Water stress changes on AMF colonization, stomatal conductance and photosynthesis of *Dalbergia sissoo* seedlings grown in entisol soil under nursery condition. *Forest Science and Technology* 19(1):47-58. <https://doi.org/10.1080/21580103.2023.2167873>
- Bibi S, Ullah S, Hafeez A, Khan MN, Javed MA, Ali B, Din IU, Bangash SA, Wahab S, Wahid N, Zaman F (2022) Exogenous Ca/Mg quotient reduces the inhibitory effects of PEG induced osmotic stress on *Avena sativa* L. *Braz J Biol* 84: e264642. <https://doi.org/10.1590/1519-6984.264642>

- Bidalia A, Okram Z, Hanief M, Rao KS (2018) Assessment of tolerances in *Mitragyna parvifolia* (Roxb.) Korth. and *Syzygium cumini* Keels. seedlings to waterlogging. *Photosynthetica* 56(2):707-717. <https://doi.org/10.1007/s11099-017-0724-1>
- Birhane E, Bongers F, Damtew A et al (2023) Arbuscular mycorrhizal fungi improve nutrient status of *Commiphora myrrha* seedlings under drought. *Journal of Arid Environments* 209:104877. <https://doi.org/10.1016/j.jaridenv.2022.104877>
- Boutasknit A, Baslam M, Ait-El-Mokhtar M, Anli M, Ben-Laouane R, Douira A, El Modafar C, Mitsui T, Wahbi S, Meddich A (2020) Arbuscular mycorrhizal fungi mediate drought tolerance and recovery in two contrasting carob (*Ceratonia siliqua* L.) ecotypes by regulating stomatal, water relations, and (in) organic adjustments. *Plants* 9(1):80. <https://doi.org/10.3390/plants9010080>
- Bozzola JJ, Russell LD (1999) *Electron microscopy: principles and techniques for biologists*. Jones & Bartlett Learning.
- Brehelin C, Kessler F, van Wijk KJ (2007) Plastoglobules: versatile lipoprotein particles in plastids. *Trends Plant Sci* 12:260-266. <https://doi.org/10.1016/j.tplants.2007.04.003>
- Cao JL, He WX, Zou YN, Wu QS (2022) An endophytic fungus, *Piriformospora indica*, enhances drought tolerance of trifoliate orange by modulating the antioxidant defense system and composition of fatty acids. *Tree Physiology* 43(3):452-466. <https://doi.org/10.1093/treephys/tpac126>
- Chandra P, Enespa, Singh R (2020) soil salinity and its alleviation using Plant Growth–Promoting Fungi. *Agriculturally Important Fungi for Sustainable Agriculture: Volume 2: Functional Annotation for Crop Protection* 101-148. https://doi.org/10.1007/978-3-030-48474-3_4
- Chandrasekaran M, Chanratana M, Kim K, Seshadri S, Sa T (2019). Impact of arbuscular mycorrhizal fungi on photosynthesis, water status, and gas exchange of plants under salt stress—a meta-analysis. *Frontiers in Plant Science* 10:457. <https://doi.org/10.3389/fpls.2019.00457>

- Chen A, Gu M, Wang S, Chen J, Xu G (2018) Transport properties and regulatory roles of nitrogen in arbuscular mycorrhizal symbiosis. In *Seminars in Cell & Developmental Biology* Vol. 74, pp. 80-88, Academic Press. <https://doi.org/10.1016/j.semcd.2017.06.015>.
- Chiappero J, del Rosario Cappellari L, Alderete LG, Palermo TB, Banchio E (2019) Plant growth promoting rhizobacteria improve the antioxidant status in *Mentha piperita* grown under drought stress leading to an enhancement of plant growth and total phenolic content. *Industrial Crops and Products* 139:111553. <https://doi.org/10.1016/j.indcrop.2019.111553>
- Chourasiya D, Gupta MM, Sahni S, Oehl F, Agnihotri R, Buade R, Maheshwari HS, Prakash A, Sharma MP (2021) Unraveling the AM fungal community for understanding its ecosystem resilience to changed climate in agroecosystems. *Symbiosis* 84:295–310. <https://doi.org/10.1007/s13199-021-00761-9>
- de Bang TC, Husted S, Laursen KH et al (2021) The molecular–physiological functions of mineral macronutrients and their consequences for deficiency symptoms in plants. *New Phytologist* 229(5):2446-2469. <https://doi.org/10.1111/nph.17074>
- Desai S, Mistry J, Shah F, Chandwani S, Amaresan N, Supriya NR (2023) Salt-tolerant bacteria enhance the growth of mung bean (*Vigna radiata* L.) and uptake of nutrients, and mobilize sodium ions under salt stress condition. *International Journal of Phytoremediation* 25(1):66-73. <https://doi.org/10.1080/15226514.2022.2057419>
- Devarajan AK, Muthukrishnan G, Truu J et al (2021) The foliar application of rice phyllosphere bacteria induces drought-stress tolerance in *Oryza sativa* (L.). *Plants* 10(2):387. <https://doi.org/10.3390/plants10020387>
- Dhindsa RS, Plumb-Dhindsa P, Thorpe TA (1981) Leaf senescence: correlated with increased levels of membrane permeability and lipid peroxidation, and decreased levels of superoxide dismutase and catalase. *J Exp Bot* 32(1):93-101. <https://doi.org/10.1093/jxb/32.1.93>

- Diatta AA, Min D, Jagadish SK (2021) Drought stress responses in non-transgenic and transgenic alfalfa—Current status and future research directions. *Advances in Agronomy* 170:35-100. <https://doi.org/10.1016/bs.agron.2021.06.002>
- Dixit R, Bisht N, Misra S, Gupta SC, Chauhan PS (2023) *Bacillus* Consortia Modulate Transcriptional and Metabolic Machinery of *Arabidopsis* Plants for Salt Tolerance. *Current Microbiology* 80(2):77. <https://doi.org/10.1007/s00284-023-03187-2>
- Dobbelaere S, Vanderleyden J, Okon Y (2003) Plant growth promoting effects of diazotrophs in the rhizosphere. *CRC Crit Rev Plant Sci* 22:107–149
- Doderer A, Kokkelink I, Van Der Veen S, Valk BE, Schram AW, Douma AC (1992) Purification and characterization of two lipoxygenase isoenzymes from germinating barley. *Biochim Biophys Acta* 1120(1):97-104. <https://doi.org/10.1016/0167-48389290429-h>
- Dong OX, Ronald PC (2019) Genetic engineering for disease resistance in plants: recent progress and future perspectives. *Plant physiology* 180(1):26-38. <https://doi.org/10.1104/pp.18.01224>
- Dubey RS, Srivastava RK, Pessarakli M (2021) Physiological mechanisms of N absorption and assimilation in plants under stressful conditions. In: *Handbook of plant and crop physiology*, pp. 579-616, CRC Press.
- El-Dakak RA, Badr RH, Zeineldein MH, Swedan EA, Batrawy OE, Hassaballah AF, Hassan IA (2023) Effect of chilling and salinity stress on photosynthetic performance and ultrastructure of chloroplast in faba beans (*Vicia faba* L.) leaves. *Rendiconti Lincei Scienze Fisiche e Naturali* 34:447-456. <https://doi.org/10.1007/s12210-022-01131-3>
- Elgharably A, Nafady NA (2021) Inoculation with Arbuscular mycorrhizae, *Penicillium funiculosum* and *Fusarium oxysporum* enhanced wheat growth and nutrient uptake in the saline soil. *Rhizosphere* 18:100345. <https://doi.org/10.1016/j.rhisph.2021.100345>

- EN ISO 20483 (2006) AN 300 the determination of nitrogen according to Kjeldahl using block digestion and steam distillation. The International Organization for Standardization, Geneva, Switzerland.
- Etesami H, Jeong BR, Glick BR (2021) Contribution of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and silicon to P uptake by plant. *Frontiers in Plant Science* 12:699618. <https://doi.org/10.3389/fpls.2021.699618>
- Etesami H, Maheshwari DK (2018) Use of plant growth promoting rhizobacteria (PGPRs) with multiple plant growth promoting traits in stress agriculture: Action mechanisms and future prospects. *Ecotoxicology and environmental safety* 156:225-1246. <https://doi.org/10.1016/j.ecoenv.2018.03.013>
- Etesami H, Mirsyed Hosseini H, Alikhani HA (2014) In planta selection of plant growth promoting endophytic bacteria for rice (*Oryza sativa* L.). *Journal of soil science and plant nutrition* 14(2):491-503. <http://dx.doi.org/10.4067/S0718-95162014005000039>
- Evelin H, Giri B, Kapoor R (2013) Ultrastructural evidence for AMF mediated salt stress mitigation in *Trigonella foenum-graecum*. *Mycorrhiza* 23:71-86. <https://doi.org/10.1007/s00572-012-0449-8>
- Evelin, H., Giri, B., and Kapoor, R. (2012) Contribution of *Glomus intraradices* inoculation to nutrient acquisition and mitigation of ionic imbalance in NaCl-stressed *Trigonella foenum-graecum*. *Mycorrhiza* 22:203–217. <https://doi.org/10.1007/s00572-011-0392-0>
- Fadiji AE, Santoyo G, Yadav AN et al (2022) Efforts towards overcoming drought stress in crops: Revisiting the mechanisms employed by plant growth-promoting bacteria. *Front. Microbiol* 13:962427. <https://doi.org/10.3389/fmicb.2022.962427>
- Farooq M, Wahid A, Kobayashi NS, Fujita DB, Basra SM (2009) Plant drought stress: Effects, mechanisms and management. *Agronomy for sustainable development* 29(1):185-212 <https://doi.org/10.1051/agro:2008021>

- Ferdosi MF, Shoaib A, Habib S, Khan KA (2021) Modulation of salt-induced stress impact in *Gladiolus grandiflorus* L. by exogenous application of salicylic acid. Scientific Reports 11(1):15597. <https://doi.org/10.1038/s41598-021-95243-9>
- Ferreira C, Simioni C, Schmidt ÉC, Ramlov F, Maraschin M, Bouzon ZL (2017) The influence of salinity on growth, morphology, leaf ultrastructure, and cell viability of the seagrass *Halodule wrightii* Ascherson. Protoplasma 254:1529-1537. <https://doi.org/10.1007/s00709-016-1041-4>
- Ferreira JC, Paschoalin VM, Panek AD, Trugo LC (1997) Comparison of three different methods for trehalose determination in yeast extract. Food Chem 60: 251-254. [https://doi.org/10.1016/S0308-8146\(96\)00330-5](https://doi.org/10.1016/S0308-8146(96)00330-5)
- Founoune H, Duponnis R, Ba AM, Ei Bouami F (2002) Influence of the dual arbuscular endomycorrhizal/ectomycorrhizal symbiosis on the growth of *Acacia holosericea* (A. Cunn.ex G. Don) in glasshouse conditions. Annals of Forest Science 59:93–98.
- Fraj H, Werbrouck SP (2023) Constant and Intermittent Contact with the Volatile Organic Compounds of *Serendipita indica* Alleviate Salt Stress In Vitro *Ocimum basilicum* L. Applied Sciences 13(3):1776. <https://doi.org/10.3390/app13031776>
- Fresno DH, Solé-Corbatón H, Munné-Bosch S (2023) Water stress protection by the arbuscular mycorrhizal fungus *Rhizoglyphus irregularis* involves physiological and hormonal responses in an organ-specific manner. Physiologia Plantarum 175(1):e13854. <https://doi.org/10.1111/ppl.13854>
- Gao X, Kuyper TW, Zou C, Zhang F, Hoffland E (2007) Mycorrhizal responsiveness of aerobic rice genotypes is negatively correlated with their zinc uptake when nonmycorrhizal. Plant and Soil 290:283-291. <https://doi.org/10.1007/s11104-006-9160-x>
- Garcia NS, Fu F, Sedwick PN, Hutchins DA (2015) Iron deficiency increases growth and nitrogen-fixation rates of phosphorus-deficient marine cyanobacteria. The ISME journal 9(1):238-45. <https://doi.org/10.1038/ismej.2014.104>

- Garg N, Manchanda G (2009) Role of arbuscular mycorrhizae in the alleviation of ionic, osmotic and oxidative stresses induced by salinity in *Cajanus cajan* (L.) Millsp. (pigeonpea). *Journal of Agronomy Crop Science* 195:110–123.
- Ghabooli M, Kaboosi E (2022) Alleviation of the adverse effects of drought stress using a desert adapted endophytic fungus and glucose in tomato. *Rhizosphere* 21:100481. <https://doi.org/10.1016/j.rhisph.2022.100481>
- Ghabooli M, Khatabi B, Ahmadi FS, Sepehri M, Mirzaei M, Amirkhani A, JorrínNovo JV, Salekdeh GH (2013) Proteomics study reveals the molecular mechanisms underlying water stress tolerance induced by *Piriformospora indica* in barley. *Journal of proteomics* 94:289301. <https://doi.org/10.1016/j.jprot.2013.09.017>
- Ghorbani A, Omran VO, Razavi SM, Pirdashti H, Ranjbar M (2019) *Piriformospora indica* confers salinity tolerance on tomato (*Lycopersicon esculentum* Mill.) through amelioration of nutrient accumulation, K⁺/Na⁺ homeostasis and water status. *Plant Cell Reports* 38:1151-1163. <https://doi.org/10.1007/s00299-019-02434-w>
- Giovannetti M, Mosse B (1980) An evaluation of techniques for measuring vesicular arbuscular mycorrhizal infection in roots. *New phytologist* 84(3):489-500.
- Giovannini L, Palla M, Agnolucc, M et al (2020) Arbuscular mycorrhizal fungi and associated microbiota as plant biostimulants: Research strategies for the selection of the best performing inocula. *Agronomy* 10(1):106. <https://doi.org/10.3390/agronomy10010106>
- Gómez-Sagasti MT, López-Pozo M, Artetxe U, Becerril JM, Hernández A, García-Plazaola JI, Esteban R (2023) Carotenoids and their derivatives: a “Swiss Army knife-like” multifunctional tool for fine-tuning plant-environment interactions. *Environmental and Experimental Botany* 20:105229. <https://doi.org/10.1016/j.envexpbot.2023.105229>
- Gopalakrishnan T, Kumar L (2021) Linking long-term changes in soil salinity to paddy land abandonment in Jaffna Peninsula, Sri Lanka. *Agriculture* 11(3):211. <https://doi.org/10.3390/agriculture11030211>

- Gopalakrishnan T, Kumar L, Mikunthan T (2020) Assessment of spatial and temporal trend of groundwater salinity in Jaffna Peninsula and its link to paddy land abandonment. *Sustainability* 12(9):3681. <https://doi.org/10.3390/su12093681>
- Goussi R, Manaa A, Derbali W, Cantamessa S, Abdelly C, Barbato R (2018) Comparative analysis of salt stress, duration and intensity, on the chloroplast ultrastructure and photosynthetic apparatus in *Thellungiella salsuginea*. *Journal of Photochemistry and Photobiology B: Biology* 183:275-287. <https://doi.org/10.1016/j.jphotobiol.2018.04.047>
- Grieve CM, Grattan SR (1983) Rapid assay for determination of water soluble quaternary ammonium compounds. *Plant Soil* 70(2):303-307. <https://doi.org/10.1007/BF02374789>
- Grobelak A, Hiller J (2017) Bacterial siderophores promote plant growth: Screening of catechol and hydroxamate siderophores. *International journal of phytoremediation* 19(9):825-833. <https://doi.org/10.1080/15226514.2017.1290581>
- Gunasekera D, Ratnasekera D (2023) Chapter 19-Advancement in mitigating the effects of drought stress in wheat. In: Khan MK, Pandey A, Hamurcu H, Gupta OP, Gezgin S (eds) *Abiotic Stresses in Wheat*. Academic Press, Cambridge, United States, pp 297-311. <https://doi.org/10.1016/B978-0-323-95368-9.00023-0>
- Guo H, Cui YN, Pan YQ et al (2020) Sodium chloride facilitates the secretohalophyte *Atriplex canescens* adaptation to drought stress. *Plant Physiology and Biochemistry* 150:99-108. <https://doi.org/10.1016/j.plaphy.2020.02.018>
- Hachiya T, Sakakibara H (2017) Interactions between nitrate and ammonium in their uptake, allocation, assimilation, and signaling in plants. *Journal of Experimental Botany* 68(10):2501-2512. <https://doi.org/10.1093/jxb/erw449>
- Haghighi TM, Saharkhiz MJ, Kavoosi G et al (2023) Adaptation of *Glycyrrhiza glabra* L. to water deficiency based on carbohydrate and fatty acid quantity and quality. *Scientific Reports* 13(1):1766. <https://doi.org/10.1038/s41598-023-28807-6>
- Hao S, Wang Y, Yan Y (2021) A review on plant responses to salt stress and their mechanisms of salt resistance. *Horticulturae* 7(6):132. <https://doi.org/10.3390/horticulturae7060132>

- Hari NS, Jindal J, Malhi NS (2008) Resistance of Cry1Ab maize to spotted stemborer *Chilo partellus* (Lepidoptera: Crambidae) in India. International Journal of Tropical Insect Science 27:223-228. <https://doi.org/10.1017/S1742758407850983>
- Hasanuzzaman M, Bhuyan MB, Nahar K et al (2018) Potassium: a vital regulator of plant responses and tolerance to abiotic stresses. Agronomy 8(3):31. <https://doi.org/10.3390/agronomy8030031>
- Hashem A, Abd-Allah EF, Alqarawi AA et al (2016) Bioremediation of adverse impact of cadmium toxicity on *Cassia italica* Mill by arbuscular mycorrhizal fungi. Saudi J. Biol. Sci 23(1):39-47. <https://doi.org/10.1016/j.sjbs.2015.11.007>
- Hashem A, Alqarawi AA, Radhakrishnan R, Al-Arjani AB, Aldehaish HA, Egamberdieva D, Abd_Allah EF (2018) Arbuscular mycorrhizal fungi regulate the oxidative system, hormones, and ionic equilibrium to trigger salt stress tolerance in *Cucumis sativus* L. Saudi journal of biological sciences 25(6):1102-1114. <https://doi.org/10.1016/j.sjbs.2018.03.009>
- Hashemi M, Behboodian B, Karimi E et al (2022) *Azotobacter chroococcum* inoculation under low drought stress condition improves *Trachyspermum ammi* seeds' essential oil bioactivity. Biochemical Systematics and Ecology 105:104537. <https://doi.org/10.1016/j.bse.2022.104537>
- Hassan MU, Chattha MU, Ullah A, Khan I, Qadeer A, Aamer M, Khan AU, Nadeem F, Khan TA (2019) Agronomic biofortification to improve productivity and grain Zn concentration of bread wheat. Int. J. Agric. Biol 21:615-620. <https://doi.org/10.17957/IJAB/15.0936>
- Heath RL, Packer L (1968) Photoperoxidation in isolated chloroplasts: I. kinetics and stoichiometry of fatty acid peroxidation. Arch Biochem Biophys 125 (1):189-198. <https://doi.org/10.1016/0003-98616890654-1>
- Hidri R, Mahmoud OM, Farhat N, Cordero I, Pueyo JJ, Debez A, Barea JM, Abdelly C, Azcon R (2019) Arbuscular mycorrhizal fungus and rhizobacteria affect the physiology and performance of *Sulla coronaria* plants subjected to salt stress by mitigation of ionic imbalance. Journal of Plant Nutrition and Soil Science 182(3):451-462.

- Hiscox JD, Israelstam GFA (1979) Method for the extraction of chlorophyll from leaf tissue without maceration. *Can J Bot* 57(12):1332-1334. <https://doi.org/10.1139/b79-163>
- Huang S, Gill S, Ramzan M, Ahmad MZ, Danish S, Huang P, Al Obaid S, Alharbi SA (2023) Uncovering the impact of AM fungi on wheat nutrient uptake, ion homeostasis, oxidative stress, and antioxidant defense under salinity stress. *Scientific Reports* 13(1):8249. <https://doi.org/10.1038/s41598-023-35148-x>
- Huang S, Gill S, Ramzan M, Ahmad MZ, Danish S, Huang P, Al Obaid S, Alharbi SA (2023) Uncovering the impact of AM fungi on wheat nutrient uptake, ion homeostasis, oxidative stress, and antioxidant defense under salinity stress. *Scientific Reports* 13(1):8249. <https://doi.org/10.1038/s41598-023-35148-x>
- Huang WT, Xie YZ, Chen XF, Zhang J, Chen HH, Ye X, Guo J, Yang LT, Chen LS (2021) Growth, mineral nutrients, photosynthesis and related physiological parameters of Citrus in response to nitrogen deficiency. *Agronomy* 11(9):1859. <https://doi.org/10.3390/agronomy11091859>
- Hussain S, Khalid MF, Hussain M, Ali MA, Nawaz A, Zakir I, Fatima Z, Ahmad S (2018) Role of micronutrients in salt stress tolerance to plants. *Plant nutrients and abiotic stress tolerance* 2018:363-376. https://doi.org/10.1007/978-981-10-9044-8_15
- Ilyas N, Mumtaz K, Akhtar N et al (2020) Exopolysaccharides producing bacteria for the amelioration of drought stress in wheat. *Sustainability* 12(21):76-88. <https://doi.org/10.3390/su12218876>
- Inbaraj MP (2021) Plant-microbe interactions in alleviating abiotic stress - a mini review. *Front. Agron* 3 <https://doi.org/10.3389/fagro.2021.667903>
- Iqbal M, Naveed M, Sanaullah M, Brtnicky M, Hussain MI, Kucerik J, Holatko J, Mustafa A (2023) Plant microbe mediated enhancement in growth and yield of canola (*Brassica napus* L.) plant through auxin production and increased nutrient acquisition. *Journal of Soils and Sediments* 23(3):1233-1249. <https://doi.org/10.1007/s11368-022-03386-7>

- Iqbal U, Jamil N, Ali I, Hasnain S (2010) Effect of zinc-phosphate-solubilizing bacterial isolates on growth of *Vigna radiata*. *Annals of microbiology* 60(2):243-248. <https://doi.org/10.1007/s13213-010-0033-4>
- Israel A, Langrand J, Fontaine J et al (2022) Significance of Arbuscular Mycorrhizal Fungi in Mitigating Abiotic Environmental Stress in Medicinal and Aromatic Plants: A Review. *Foods* 11(17):2591. <https://doi.org/10.3390/foods11172591>
- Jalili F, Khavazi K, Pazira E et al (2009) Isolation and characterization of ACC deaminase-producing fluorescent pseudomonads, to alleviate salinity stress on canola (*Brassica napus* L.) growth. *J. Plant Physiol* 166:667-674. <https://doi.org/10.1016/j.jplph.2008.08.004>
- Jarrett U, Miller S, Mohtadi H (2023) Dry spells and global crop production: A multi-stressor and multi-timescale analysis. *Ecological Economics* 203:107627. <https://doi.org/10.1016/j.ecolecon.2022.107627>
- Jing Z, Liu C, Qi R, Ren P (2018) Many-body effect determines the selectivity for Ca²⁺ and Mg²⁺ in proteins. *Proceedings of the National Academy of Sciences* 115(32):E7495-74501.
- Jofre MF, Mammana SB, Lopez Appiolaza M et al (2023) Melatonin production by rhizobacteria native strains: Towards sustainable plant growth promotion strategies. *Physiologia Plantarum* 175(1):e13852.
- Jyoti K, Ramesh K, Kumar RS, Sain D, Bilal B, Kamboj OP, Usha N, Yadav AK (2013) Response of maize (*Zea mays* L.) hybrids to excess soil moisture stress at different growth stages. *Research on Crops* 14(2):439-443.
- Kakabouki I, Stavropoulos P, Roussis I, Mavroeidis A, Bilalis D (2023) Contribution of Arbuscular Mycorrhizal Fungi (AMF) in Improving the Growth and Yield Performances of Flax (*Linum usitatissimum* L.) to Salinity Stress. *Agronomy* 13(9):2416. <https://doi.org/10.3390/agronomy13092416>
- Karim S, Alezzawi M, Garcia-Petit C et al (2014). A novel chloroplast localized Rab GTPase protein CPRabA5e is involved in stress, development, thylakoid biogenesis and vesicle transport in *Arabidopsis*. *Plant molecular biology* 84:675-692. <https://doi.org/10.1007/s11103-013-0161-x>

- Karima B, Amima H, Ahlam M, Zoubida B, Benoît T, Yolande D, Anissa LH (2023) Native Arbuscular Mycorrhizal Inoculum Modulates Growth, Oxidative Metabolism and Alleviates Salinity Stresses in Legume Species. *Current Microbiology* 80(2):66. <https://doi.org/10.1007/s00284-022-03145-4>
- Karimi R, Noori A (2022) *Streptomyces rimosus* rhizobacteria and *Glomus mosseae* mycorrhizal fungus inoculation alleviate salinity stress in grapevine through morphophysiological changes and nutritional balance. *Scientia Horticulturae* 305:111433. <https://doi.org/10.1016/j.scienta.2022.111433>
- Karimi R, Noori A (2017) *Streptomyces rimosus* rhizobacteria and *Glomus mosseae* mycorrhizal fungus inoculation alleviate salinity stress in grapevine through morphophysiological changes and nutritional balance. *Scientia Horticulturae* 305:111433. <https://doi.org/10.1016/j.scienta.2022.111433>
- Karimzadeh J, Alikhani HA, Etesami H, Pourbabaei AA (2021) Improved phosphorus uptake by wheat plant (*Triticum aestivum* L.) with rhizosphere fluorescent Pseudomonads strains under water-deficit stress. *Journal of Plant Growth Regulation* 40:162-178. <https://doi.org/10.1007/s00344-020-10087-3>
- Karnovsky MJ (1965) A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy. *J Cell Biol* 27:137-138.
- Khan MA, Sahile AA, Jan R, Asaf S, Hamayun M, Imran M, Adhikari A, Kang SM, Kim KM, Lee IJ (2021) Halotolerant bacteria mitigate the effects of salinity stress on soybean growth by regulating secondary metabolites and molecular responses. *BMC Plant Biology* 21(1):15. <https://doi.org/10.1186/s12870021029373>
- Khan SW, Yaseen T, Naz F et al (2019) Influence of arbuscular mycorrhizal fungi (AMF) inoculation on growth and mycorrhizal dependency of (*Lens culinaris* L.) varieties. *Int. J. Bioorganic Chem* 4:47.
- Khan V, Umar S, Iqbal N (2023) Palliating Salt Stress in Mustard through Plant-Growth-Promoting Rhizobacteria: Regulation of Secondary Metabolites, Osmolytes, Antioxidative Enzymes and Stress Ethylene. *Plants* 12(4):705. <https://doi.org/10.3390/plants12040705>

- Khan Y, Yang X, Zhang X, Yaseen T, Shi L, Zhang T (2021) Arbuscular mycorrhizal fungi promote plant growth of *Leymus chinensis* (Trin.) Tzvelev by increasing the metabolomics activity under nitrogen addition. *Grassland Science* 67(2):128-138. <https://doi.org/10.1111/grs.12299>.
- Khandelwal S, Parwez S, Mehra M (2023) Climate change: effects on health and nutrition. In *Encyclopedia of Human Nutrition (Fourth Edition)*, Academic Press, pp. 369-378. <https://doi.org/10.1016/B978-0-12-821848-8.00131-1>
- Khazaei Z, Esmailpour B, Estaji A (2020) Ameliorative effects of ascorbic acid on tolerance to drought stress on pepper (*Capsicum annuum* L) plants. *Physiology and Molecular Biology of Plants* 26(8):1649-1662. <https://doi.org/10.1007/s12298-020-00846-7>
- Kim SE, Lee CJ, Ji CY et al (2019) Transgenic sweetpotato plants overexpressing tocopherol cyclase display enhanced α -tocopherol content and abiotic stress tolerance. *Plant Physiology and Biochemistry* 144:436-444. <https://doi.org/10.1016/j.plaphy.2019.09.046>
- Kiran S, Furtana GB, Ellialtioglu ŞŞ (2022) Physiological and biochemical assay of drought stress responses in eggplant (*Solanum melongena* L.) inoculated with commercial inoculant of *Azotobacter chroococum* and *Azotobacter vinelandii*. *Scientia Horticulturae* 305:111394. <https://doi.org/10.1016/j.scienta.2022.111394>
- Kleczkowski LA, Igamberdiev AU (2021) Magnesium Signaling in Plants. *Int J Mol Sci.* 22(3) :1159. <https://doi.org/10.1093/jxb/erz241>
- Kloepper JW, Leong J, Teintze M, Schroth MN (1980) Enhanced plant growth by siderophores produced by plant growth-promoting rhizobacteria. *Nature* 286(5776):885-896. <https://doi.org/10.1038/286885a0>
- Kobayashi T, Nishizawa NK (2012) Iron uptake, translocation, and regulation in higher plants. *Annual review of plant biology* 63:131-152. <https://doi.org/10.1146/annurev-arplant-042811-105522>

- Komatsu KJ, Esch NL, Bloodworth KJ et al (2023) Rhizobial diversity impacts soybean resistance, but not tolerance, to herbivory during drought. *Basic and Applied Ecology* 66:31-39. <https://doi.org/10.1016/j.baae.2022.12.004>
- Kotula L, Clode PL, Jimenez JD, Colmer TD (2019) Salinity tolerance in chickpea is associated with the ability to ‘exclude’ Na from leaf mesophyll cells. *Journal of Experimental Botany*, 70(18), 4991-5002. <https://doi.org/10.1093/jxb/erz241>
- Kour D, Rana KL, Yadav AN et al (2020) Amelioration of drought stress in Foxtail millet (*Setaria italica* L.) by P-solubilizing drought-tolerant microbes with multifarious plant growth promoting attributes. *Environmental Sustainability* 3(1):23-34. <https://doi.org/10.1007/s42398-020-00094-1>
- Krestini EH, Rusmawati U, Susilawati A (2020) Effectiveness of microbial consortium on growth, yield, and intensity of withered disease (*Fusarium oxysporum* Schelecht) on garlic plants. In: *BIO Web of Conferences*, Vol. 20. EDP Science, pp. 3009
- Kumar N (2015a) Effect of copper mining dust on the soil and vegetation in India: a critical review *Int. J. Mod. Sci. Eng. Technol* 2(2):73-76.
- Kumar R, Kaul J, Dubey RB, Singode A, GK C, Manivannan A, Debnath MK (2015b) Assessment of drought tolerance in maize (*Zea mays* L.) based on different indices. *SABRAO Journal of Breeding & Genetics* 47 (3) 291-298.
- Kumar S, Kumar S, Mohapatra T (2021) Interaction between macro-and micro-nutrients in plants. *Frontiers in Plant Science* 12:665583. <https://doi.org/10.3389/fpls.2021.665583>
- Li Y, Wang X, Chen X, Lu J, Jin Z, Li J (2023) Functions of arbuscular mycorrhizal fungi in regulating endangered species *Heptacodium miconioides* growth and drought stress tolerance. *Plant Cell Reports* 42(12):1967-1986. <https://doi.org/10.1007/s00299-023-03076-9>
- Liang BB, Wang WJ, Fan XX, Kurakov AV, Liu YF, Song FQ, Chang W (2021) Arbuscular mycorrhizal fungi can ameliorate salt stress in *Elaeagnus angustifolia* by improving leaf photosynthetic function and ultrastructure. *Plant Biology* 23:232-241. <https://doi.org/10.1111/plb.13164>

- Liang D, Ni ZY, Xia H et al (2019) Exogenous melatonin promotes biomass accumulation and photosynthesis of kiwifruit seedlings under drought stress. *Sci. Hortic* 246:34-43. <https://doi.org/10.1016/j.scienta.2018.10.058>
- Liu CY, Hao Y, Wu XL, Dai FJ, Abd-Allah EF, Wu QS, Liu SR (2023a) Arbuscular mycorrhizal fungi improve drought tolerance of tea plants via modulating root architecture and hormones. *Plant Growth Regulation* 4:1-10. <https://doi.org/10.1007/s10725-023-00972-8>
- Liu F, Ma H, Liu B, Du Z, Ma B, Jing D (2023b) Effects of Plant Growth-Promoting Rhizobacteria on the Physioecological Characteristics and Growth of Walnut Seedlings under Drought Stress. *Agronomy* 13(2):290. <https://doi.org/10.3390/agronomy13020290>
- Liu H, Wang Y, Tang M, Chen H (2021) Arbuscular mycorrhiza protects the ultrastructure of mesophyll cells and photochemical activity of *Lycium barbarum* under salt stress. *Pak. J. Bot* 53(1):329-335. DOI: [http://dx.doi.org/10.30848/PJB2021-1\(41\)](http://dx.doi.org/10.30848/PJB2021-1(41))
- Liu Q, Ying SH, Feng MG, Jiang XH (2009) Physiological implication of intracellular trehalose and mannitol changes in response of entomopathogenic fungus *Beauveria bassiana* to thermal stress. *Antonie Van Leeuwenhoek* 95:65-75. <https://doi.org/10.1007/s10482-008-9288-1>
- Liu Y, Guo Z, Shi H (2022) Rhizobium Symbiosis Leads to Increased Drought Tolerance in Chinese Milk Vetch (*Astragalus sinicus* L.). *Agronomy* 12(3):725. <https://doi.org/10.3390/agronomy12030725>
- Liu Y, Lu J, Cui L, Tang Z, Ci D, Zou X, Zhang X, Yu X, Wang Y, Si T (2023c) The multifaceted roles of Arbuscular Mycorrhizal Fungi in peanut responses to salt, drought, and cold stress. *BMC Plant Biology* 23(1):1-19. <https://doi.org/10.1186/s12870-023-04053-w>
- Liu Y, Wei X (2021) Dark septate endophyte improves the drought-stress resistance of *Ormosia hosiei* seedlings by altering leaf morphology and photosynthetic characteristics. *Plant Ecol* 222:761-771. <https://doi.org/10.1007/s11258-021-01135-3>

- López-Galiano MJ, García-Robles I, González-Hernández AI et al (2019) Expression of miR159 is altered in tomato plants undergoing drought stress. *Plants* 8(7):201. <https://doi.org/10.3390/plants8070201>
- Mahreen N, Yasmin S, Asif M et al (2023) Mitigation of water scarcity with sustained growth of Rice by plant growth promoting bacteria. *Frontiers in Plant Science* 14:1081537. <https://doi.org/10.3389/fpls.2023.1081537>
- Marasco R, Rolli E, Ettoumi B, Vigani G, Mapelli F, Borin S, Abou-Hadid AF, El-Behairy UA, Sorlini C, Cherif A, Zocchi G (2012) A drought resistance-promoting microbiome is selected by root system under desert farming. *PloS one* 7(10):e48479. <https://doi.org/10.1371/journal.pone.0048479>
- Marcé M, Brown DS, Capell T et al (1995) Rapid high-performance liquid chromatographic method for the quantification of polyamines as their dansyl derivatives: application to plant and animal tissues. *J Chromatogr B* 666(2):329-335. <https://doi.org/10.1016/0378-43479400586-T>
- Marschner, H. (1995) *Mineral Nutrition of Higher Plants*, 2nd Ed. Academic Press, New York, NY, USA.
- Masrahi AS, Alasmari A, Shahin MG, Qumsani AT, Oraby HF, Awad-Allah MM (2023) Role of Arbuscular Mycorrhizal Fungi and Phosphate Solubilizing Bacteria in Improving Yield, Yield Components, and Nutrients Uptake of Barley under Salinity Soil. *Agriculture* 13(3):537. <https://doi.org/10.3390/agriculture13030537>
- Meng F, Feng N, Zheng D, Liu M, Zhang R, Huang X, Huang A, Chen Z (2023) Exogenous Hemin alleviates NaCl stress by promoting photosynthesis and carbon metabolism in rice seedlings. *Scientific Reports* 13(1):3497. <https://doi.org/10.1038/s41598-023-30619-7>
- Minocha R, Shortle WC, Long SL et al (1994) A rapid and reliable procedure for extraction of cellular polyamines and inorganic ions from plant tissues. *J Plant Growth Regul* 13(4):187-193. <https://doi.org/10.1007/BF00226036>

- Miranda V, Silva-Castro GA, Ruiz-Lozano JM et al (2023) Fungal Endophytes Enhance Wheat and Tomato Drought Tolerance in Terms of Plant Growth and Biochemical Parameters. *Journal of Fungi* 9(3):384. <https://doi.org/10.3390/jof9030384>
- Miransari M (2013) Soil microbes and the availability of soil nutrients. *Acta physiologiae plantarum* 35(11):3075-3084. <https://doi.org/10.1007/s11738-013-1338-2>
- Mirzabaev A, Kerr RB, Hasegawa T et al (2023) Severe climate change risks to food security and nutrition. *Climate Risk Management* 39:100473 <https://doi.org/10.1016/j.crm.2022.100473>
- Mokabel S, Olama Z, Ali S, El-Dakak R (2022) The role of plant growth promoting rhizosphere microbiome as alternative biofertilizer in boosting *Solanum melongena* L. Adaptation to salinity stress. *Plants* 11(5):659. <https://doi.org/10.3390/plants11050659>
- Moreno-Galván AE, Cortés-Patiño S, Romero-Perdomo F et al (2020) Proline accumulation and glutathione reductase activity induced by drought-tolerant rhizobacteria as potential mechanisms to alleviate drought stress in *Guinea* grass. *Appl. Soil Ecol* 147:103367 <https://doi.org/10.1016/j.apsoil.2019.103367>
- Mori Y (2015) Functional polymeric membrane in agriculture. In: Cirillo G, Spizzirri UG, Iemma F (eds) *Functional Polymers in Food Science: From Technology to Biology*. John Wiley & Sons, USA, Volume 2, pp 33–45. <https://doi.org/10.1002/9781119108580.ch3>
- Mousavi SS, Karami A, Saharkhiz MJ, Etemadi M, Ravanbakhsh M (2022b) Microbial amelioration of salinity stress in endangered accessions of Iranian licorice (*Glycyrrhiza glabra* L.). *BMC Plant Biology* 22(1):1-17. <https://doi.org/10.1186/s12870-022-03703-9>
- Mousavi SS, Karami A, Saharkhiz MJ, Etemadi M, Zarshenas MM (2022a) Evaluation of metabolites in Iranian Licorice accessions under salinity stress and *Azotobacter* sp. inoculation. *Scientific Reports* 12(1):15837. <https://doi.org/10.1186/s12870-022-03703-9>

- Nakano Y, Asada K (1981) Hydrogen peroxide is scavenged by ascorbate-specific peroxidase in spinach-chloroplasts. *Plant Cell Physiol* 22:867-880.
- Naraian, R., and Kumari, S. (2018) "Microbial production of organic acids," in: *Microbial Functional Foods and Nutraceuticals*, eds V. K. Gupta, H. Treichel, V. Shapaval, L. A. de Oliveira, and M. G. Tuohy, New York, NY: John Wiley & Sons Ltd. <https://doi.org/10.1002/9781119048961.ch5>
- Naservafaei S, Sohrabi Y, Moradi P (2023) Effects of Exogenous Application of 24-Epibrassinolide on Photosynthesis Parameters, Grain Yield, and Protein of Dragon's Head (*Lallemantia iberica*) Under Drought Stress Conditions. *Journal of Plant Growth Regulation* 18:1-13 <https://doi.org/10.1007/s00344-023-10910-7>
- Ndiate NI, Zaman QU, Francis IN, Dada OA, Rehman A, Asif M, Goffner D, Kane A, Liqun C, Haider FU (2022) Soil amendment with arbuscular mycorrhizal fungi and biochar Improves salinity tolerance, growth, and lipid metabolism of common wheat (*Triticum aestivum* L.). *Sustainability* 14(6):3210. <https://doi.org/10.3390/su14063210>
- Nemeskeri E, Horvath KZ, Andryei B et al (2022) Impact of plant growth-promoting rhizobacteria inoculation on the physiological response and productivity traits of field-grown tomatoes in Hungary. *Horticulturae* 8(7):641. <https://doi.org/10.3390/horticulturae8070641>
- Niki E (2021) Lipid oxidation that is, and is not, inhibited by vitamin E: Consideration about physiological functions of vitamin E. *Free Radical Biology and Medicine* 176:1-15. <https://doi.org/10.1016/j.freeradbiomed.2021.09.001>
- Noreen S, Faiz S, Akhter MS, Shah KH (2019) Influence of foliar application of osmoprotectants to ameliorate salt stress in sunflower (*Helianthus annuus* L.). *Sarhad J. Agric* 35:1316-1325. <https://doi.org/10.17582/journal.sja/2019/35.4.1316.1325>
- Nuccio ML, Paul M, Bate NJ, Cohn J, Cutler SR (2018) Where are the drought tolerant crops? An assessment of more than two decades of plant biotechnology effort in crop improvement. *Plant science* 273:110-119.

- Numan M, Bashir S, Khan Y, Mumtaz R, Shinwari ZK, Khan AL, Khan A, Ahmed AH (2018) Plant growth promoting bacteria as an alternative strategy for salt tolerance in plants: a review. *Microbiological research* 209:21-32. <https://doi.org/10.1016/j.micres.2018.02.003>
- Oliveira TC, Cabral JS, Santana LR, Tavares GG, Santos LDS, Paim TP, Müller C, Silva FG, Costa AC, Souchie EL, Mendes GC (2022) The arbuscular mycorrhizal fungus *Rhizophagus clarus* improves physiological tolerance to drought stress in soybean plants. *Scientific Reports* 12(1):9044. <https://doi.org/10.1038/s41598-022-13059-7>
- Ortiz N, Armada E, Duque E, Roldán A, Azcón R (2015) Roldan, A.; Azcon, R. Contribution of arbuscular mycorrhizal fungi and/or bacteria to enhancing plant drought tolerance under natural soil conditions: Effectiveness of autochthonous or allochthonous strains. *J. Plant Physiol* 174:87-96. <https://doi.org/10.1016/j.jplph.2014.08.019>
- Osorio Vega NW (2007) A review on beneficial effects of rhizosphere bacteria on soil nutrient availability and plant nutrient uptake. *Revista Facultad Nacional de Agronomía Medellín* 60(1):3621-3643.
- Ostadi A, Javanmard A, Amani Machiani M, Sadeghpour A, Maggi F, Nouraein M, Morshedloo MR, Hano C, Lorenzo JM (2022) Co-application of TiO₂ nanoparticles and arbuscular mycorrhizal fungi improves essential oil quantity and quality of sage (*Salvia officinalis* L.) in drought stress conditions. *Plants* 11(13):1659. <https://doi.org/10.3390/plants11131659>
- Oviya G, Rangasamy A, Ariyan M, Krishnamoorthy R, Senthilkumar M, Gopal NO, Thiyageshwari S, Meena S, Vincent S (2023) Halotolerant Nodule Rhizobial and Passenger Endophytes Alleviates Salinity Stress in Groundnut (*Arachis hypogaea* L.). *Journal of Plant Growth Regulation* 17:1-16. <https://doi.org/10.1007/s00344-023-10919-y>
- Pan J, Peng F, Tedeschi A, Xue X, Wang T, Liao J, Zhang W, Huang C (2020) Do halophytes and glycophytes differ in their interactions with arbuscular mycorrhizal fungi under salt stress? A meta-analysis. *Botanical studies* 61:1-13.

- Pan J, Peng F, Xue X, You Q, Zhang W, Wang T, Huang C (2019) The growth promotion of two salt-tolerant plant groups with PGPR inoculation: a meta-analysis. *Sustainability* 11(2):378
- Pandey GK, Sanyal SK (2021) Calcium-from Nutrition to Signaling. In: *Functional Dissection of Calcium Homeostasis and Transport Machinery in Plants*, Springer Briefs in Plant Science. Springer, Cham, pp 1-9. https://doi.org/10.1007/978-3-030-58502-0_1
- Pandey SN (2006) Accumulation of heavy metals (Cd, Cr, Cu, Ni and Zn) in *Raphanus sativus* L. and *Spinacia oleracea* L. plants irrigated with industrial effluent. *Journal of environmental biology* 37(2):381-394.
- Pandey, S.N. (2018). Biomolecular Functions of Micronutrients Toward Abiotic Stress Tolerance in Plants. In: Hasanuzzaman, M., Fujita, M., Oku, H., Nahar, K., Hawrylak-Nowak, B. (eds) *Plant Nutrients and Abiotic Stress Tolerance*. Springer, Singapore. https://doi.org/10.1007/978-981-10-9044-8_6
- Parihar M, Rakshit A, Rana K, Tiwari G, Jatav SS (2020) The effect of arbuscular mycorrhizal fungi inoculation in mitigating salt stress of pea (*Pisum Sativum* L.). *Communications in Soil Science and Plant Analysis* 51(11):1545-1559. <https://doi.org/10.1080/00103624.2020.1784917>
- Patel MK, Kumar M, Li W, Luo Y, Burritt DJ, Alkan N, Tran LS (2020) Enhancing salt tolerance of plants: From metabolic reprogramming to exogenous chemical treatments and molecular approaches. *Cells* 9(11):2492. <https://doi.org/10.3390/cells9112492>
- Pathania S, Bajaj A, Mavi MS, Choudhary OP (2023) Comprehensive evaluation, analysis of mechanisms and the prediction of salinity tolerance in pomegranate. *Scientia Horticulturae* 313:111918. <https://doi.org/10.1016/j.scienta.2023.111918>
- Pequeno DNL, Hernández-Ochoa IM, Reynolds M et al (2021) Climate impact and adaptation to heat and drought stress of regional and global wheat production. *Environ. Res. Lett* 16(5):054070. <https://doi.org/10.1088/1748-9326/abd970>

- Pereira S, Singh S, Oliveira RS, Ferreira L, Rosa E, Marques G (2020) Coinoculation with rhizobia and mycorrhizal fungi increases yield and crude protein content of cowpea (*Vigna unguiculata* (L.) Walp.) under drought stress. *Landbauforschung J Sust Org Agric Syst* 70:5665. <https://doi.org/10.3220/LBF1607613362000>
- Phillips JM, Hayman DS (1970) Improved procedures for clearing roots and staining parasitic and vesicular-arbuscular mycorrhizal fungi for rapid assessment of infection. *Transactions of the British mycological Society* 55(1):158-161. [https://doi.org/10.1016/S0007-1536\(70\)80110-3](https://doi.org/10.1016/S0007-1536(70)80110-3)
- Puccio G, Ingraffia R, Mercati F, Amato G, Giambalvo D, Martinelli F, Sunseri F, Frenda AS (2023) Transcriptome changes induced by Arbuscular mycorrhizal symbiosis in leaves of durum wheat (*Triticum durum* Desf.) promote higher salt tolerance. *Scientific Reports* 13(1):116. <https://doi.org/10.1038/s41598-022-26903-7>
- Rabhi M, Barhoumi Z, Ksouri R, Abdely C, Gharsalli M (2007) Interactive effects of salinity and iron deficiency in *Medicago ciliaris*. *Comptes Rendus Biologies* 330(11):779-788. <https://doi.org/10.1016/j.crv.2007.08.007>
- Rabie GH, Almadini AM (2005). Role of bioinoculants in development of salttolerance of *Vicia faba* plants under salinity stress. *African Journal of Biotechnology* 4:210–222.
- Rahmani V, Movahhedi Dehnavi M, Balouchi H et al (2023) Silicon Can Improve Nutrient Uptake and Performance of Black Cumin Under Drought and Salinity Stresses. *Communications in Soil Science and Plant Analysis* 54(3):297-310. <https://doi.org/10.1080/00103624.2022.2112590>
- Rajput S, Sengupta P, Kohli I, Varma A, Singh PK, Joshi NC (2022) Role of *Piriformospora indica* in inducing soil microbial communities and drought stress tolerance in plants. In: Singh H and Vaishnav A (eds) *New and future developments in microbial biotechnology and bioengineering, Sustainable Agriculture: Microorganisms as Biostimulants*. Elsevier, Amsterdam, pp 93–110. <https://doi.org/10.1016/B978-0-323-85163-3.00003-X>

- Ramakrishna W, Rathore P, Kumari R et al (2020). Brown gold of marginal soil: Plant growth promoting bacteria to overcome plant abiotic stress for agriculture, biofuels and carbon sequestration. *Science of the Total Environment* 711:135062. <https://doi.org/10.1016/j.scitotenv.2019.135062>
- Raza A, Mehmood SS, Tabassum J, Batool R (2019) Targeting plant hormones to develop abiotic stress resistance in wheat. In *Wheat production in changing environments*, (eds.) Mirza Hasanuzzaman, Kamrun Nahar, and Md. Amzad Hossain, Singapore: Springer, pp. 557–577.
- Razaq, M., Zhang, P., and Shen, H. L. (2017) Influence of nitrogen and phosphorous on the growth and root morphology of *Acer mono*. *PLoS ONE* 12:e0171321. doi: 10.1371/journal.pone.0171321 Rilling, J. I., Acuna, J. J., Nannipieri, P., Cassan, F., Maruyama, F., and Jorquera, M.
- Reshna OP, Beena R, Joy M, Viji MM, Roy S (2022) Elucidating the effect of growth promoting endophytic fungus *Piriformospora indica* for seedling stage salinity tolerance in contrasting rice genotypes. *Journal of Crop Science and Biotechnology* 25(5):583-598. <https://doi.org/10.1007/s12892-022-00153-5>
- Riyazuddin R, Nisha N, Singh K et al (2022) Involvement of dehydrin proteins in mitigating the negative effects of drought stress in plants. *Plant Cell Reports* 41(3):519-533. <https://doi.org/10.1007/s00299-021-02720-6>
- Roy S, Chakraborty AP, Chakraborty R (2021) Understanding the potential of root microbiome influencing salt-tolerance in plants and mechanisms involved at the transcriptional and translational level. *Physiologia Plantarum* 173(4):1657-1681. <https://doi.org/10.1111/ppl.13570>
- Ru C, Wang K, Hu X, Chen D, Wang W, Yang H (2022) Nitrogen modulates the effects of heat, drought, and combined stresses on photosynthesis, antioxidant capacity, cell osmoregulation, and grain yield in winter wheat. *Journal of Plant Growth Regulation* 42(3):1681-1703. <https://doi.org/10.1007/s00344-022-10650-0>

- Sadasivam S, Manickam A (2008) Biochemical methods, 3rd edn. New Age International Publishers, New Delhi India.
- Saddique MA, Ali Z, Khan AS, Rana IA, Shamsi IH (2018) Inoculation with the endophyte *Piriformospora indica* significantly affects mechanisms involved in osmotic stress in rice. *Rice* 11(1):112. <https://doi.org/10.1186/s1228401802261>
- Samaddar S, Chatterjee P, Choudhury AR, Ahmed S, Sa T (2019) Interactions between *Pseudomonas* spp. and their role in improving the red pepper plant growth under salinity stress. *Microbiological research* 219:66-73. <https://doi.org/10.1016/j.micres.2018.11.005>
- Saravi HB, Gholami A, Pirdashti H, Firouzabadi MB, Asghari H, Yaghoobian Y (2022) Improvement of salt tolerance in *Stevia rebaudiana* by co-application of endophytic fungi and exogenous spermidine. *Industrial Crops and Products* 177:114443. <https://doi.org/10.1016/j.indcrop.2021.114443>
- Sardans J, Peñuelas J (2021) Potassium control of plant functions: Ecological and agricultural implications. *Plants* 10(2):419. <https://doi.org/10.3390/plants10020419>
- Selim S, Akhtar N, Hagagy N, Alanazi A, Warrad M, El Azab E, Elamir MY, Al-Sanea MM, Jaouni SK, Abdel-Mawgoud M, Shah AA (2022) Selection of newly identified growth-promoting *Archaea haloferax* species with a potential action on cobalt resistance in maize plants. *Frontiers in Plant Science* 13:872654. <https://doi.org/10.3389/fpls.2022.872654>
- Semenova GA (2002) The thylakoid membrane in a wide pH range. *Journal of plant physiology* 159(6):613-625. <https://doi.org/10.1078/0176-1617-0632>
- Shah MN, Wright DL, Hussain S et al (2023) Organic fertilizer sources improve the yield and quality attributes of maize (*Zea mays* L.) hybrids by improving soil properties and nutrient uptake under drought stress. *Journal of King Saud University-Science* 35(4):102570.

- Sharma A, Shahzad B, Kumar V et al (2019) Phytohormones regulate accumulation of osmolytes under abiotic stress. *Biomolecules* 9(7):285. <https://doi.org/10.3390/biom9070285>
- Sharma P, Kaushal S, Baishya R (2023) Biofertilizers Alleviate Salinity Stress in Medicinally Important Plant-Catharanthus roseus (L.) G. Don by Enhancing Morphological and Photosynthetic Attributes. *International Journal of Ecology and Environmental Sciences* 9(4):387-397. <https://doi.org/10.55863.2023.2742>
- Sharma S, Anand G, Singh N, Kapoor R (2017) Arbuscular mycorrhiza augments arsenic tolerance in wheat (*Triticum aestivum* L.) by strengthening antioxidant defense system and thiol metabolism. *Frontiers in plant science* 8:906. <https://doi.org/10.3389/fpls.2017.00906>
- Sharma SB, Sayyed RZ, Trivedi MH, and Gobi TA (2013) Phosphate solubilizing microbes: sustainable approach for managing phosphorus deficiency in agricultural soils. *Springerplus* 2:587–600. <https://doi.org/10.1186/2193-1801-2-587>
- Sheteiwy MS, Ulhassan Z, Qi W, Lu H, AbdElgawad H, Minkina T, Sushkova S, Rajput VD, El-Keblawy A, Joško I, Sulieman S (2022) Association of jasmonic acid priming with multiple defense mechanisms in wheat plants under high salt stress. *Frontiers in Plant Science* 13:886862. <https://doi.org/10.3389/fpls.2022.886862>
- Shu S, Yuan Y, Chen J et al (2015) The role of putrescine in the regulation of proteins and fatty acids of thylakoid membranes under salt stress. *Scientific reports* 5(1):1-16. <https://doi.org/10.1038/srep14390>
- Siddique S, Naveed M, Yaseen M et al (2022) Exploring potential of seed endophytic bacteria for enhancing drought stress resilience in maize (*Zea mays* L.). *Sustainability* 14(2):673. <https://doi.org/10.3390/su14020673>
- Silva S, Dias MC, Silva A (2023) Potential of MgO and MgCO₃ nanoparticles in modulating lettuce physiology to drought. *Acta Physiologiae Plantarum* 45(2):1-9. <https://doi.org/10.1007/s11738-022-03507-2>

- Silva S, Santos C, Serodio J et al (2018) Physiological performance of drought-stressed olive plants when exposed to a combined heat–UV-B shock and after stress relief. *Functional Plant Biology* 45(12):1233-1240. <https://doi.org/10.1071/FP18026>
- Simard RR (1993) Ammonium Acetate Extractable Elements. In: Martin, R., Carter, S., (eds), *Soil Sampling and Methods of Analysis*. Lewis Publisher, Florida, USA pp. 39-43.
- Singh M, Sharma JG, Giri B (2023a) Augmentative Role of Arbuscular Mycorrhizal Fungi, *Piriformospora indica*, and Plant Growth-Promoting Bacteria in Mitigating Salinity Stress in Maize (*Zea mays* L.). *Journal of Plant Growth Regulation* 19:1-21. <https://doi.org/10.1007/s00344-023-11177-8>
- Singh M, Sharma JG, Giri B (2023b) Microbial inoculants improve growth in *Zea mays* L. under drought stress by up-regulating antioxidant, mineral acquisition, and ultrastructure modulations. *Symbiosis* 91(1):55-77. <https://doi.org/10.1007/s13199-023-00945-5>
- Singh M, Tiwari N (2021) Microbial amelioration of salinity stress in HD 2967 wheat cultivar by up-regulating antioxidant defense. *Communicative & Integrative Biology* 114(1):136-150. <https://doi.org/10.1080/19420889.2021.1937839>
- Sinha SK, Sinha AK, Thakur D, Lakra A, Tripathi AK (2019) Maize Research in Chhattisgarh: Status and Progress. *Int. J. Curr. Microbiol. Appl. Sci* 8:2210-2230. <https://doi.org/10.20546/ijcmas.2019.803.265>
- Solórzano-Acosta R, Toro M, Zúñiga-Dávila D (2023) Plant Growth Promoting Bacteria and Arbuscular Mycorrhizae Improve the Growth of *Persea americana* var. Zutano under Salt Stress Conditions. *Journal of Fungi* 9(2):233. <https://doi.org/10.3390/jof9020233>
- Soni G, Savalia SG (2023) Effect of simulated soil salinity conditions and varieties of pigeon pea (*Cajanus cajan* L.) on growth, yield and yield attributes. *The Pharma Innovation Journal* 12(1):101-104.

- Srinivasa Rao Ch, Prabhakar M, Maheswari M, Srinivasa Rao M, Sharma L, Srinivas K, Prasad JVNS, Rama Rao CA, Vanaja M, Ramana DVB, Gopinath KA, Subba Rao AVM, Rejani R, Bhaskar S, Sikka AK, Alagusundaram K (2016) National Innovations in Climate Resilient Agriculture (NICRA), Research Highlights 2015-16. Central Research Institute for Dryland Agriculture, Hyderabad. Pp 112.
- Srinivasan R, Yandigeri MS, Kashyap S, Alagawadi AR (2012) Effect of salt on survival and P-solubilization potential of phosphate solubilizing microorganisms from salt affected soils. Saudi J. Biol. Sci 19:427–434. <https://doi.org/10.1016/j.sjbs.2012.05.004>
- Sujkowska-Rybkowska M, Rusaczek A, Kasowska D, Gediga K, Banasiewicz J, Stepkowski T, Bernacki MJ (2022) Potential of Rhizobia Nodulating *Anthyllis vulneraria* L. from Ultramafic Soil as Plant Growth Promoting Bacteria Alleviating Nickel Stress in *Arabidopsis thaliana* L. International Journal of Molecular Sciences 23(19):11538. <https://doi.org/10.3390/ijms231911538>
- Taiz L, and Zeiger E (2006) Plant Physiology. Sunderland, MA: Sinaur Associates.
- Tang L, Xiao L, Chen E, Lei X, Ren J, Yang Y, Xiao B, Gong C (2023) Magnesium transporter CsMGT10 of tea plants plays a key role in chlorosis leaf vein greening. Plant Physiology and Biochemistry 17:107842. <https://doi.org/10.1016/j.plaphy.2023.107842>
- Teranishi Y, Tanaka A, Osumi M et al (1974) Catalase activities of hydrocarbon-utilizing *Candida* yeasts. Agric Biol Chem 38(6):1213-1220. <https://doi.org/10.1080/00021369.1974.10861301>
- Timmusk S, Abd El-Daim IA, Copolovici L et al (2014) Drought-tolerance of wheat improved by rhizosphere bacteria from harsh environments: enhanced biomass production and reduced emissions of stress volatiles. PloS one 9(5):e96086. <https://doi.org/10.1371/journal.pone.0096086>
- Tisarum R, Theerawitaya C, Samphumphuang T, Polispitak K, Thongpoem P, Singh HP, Cha-Um S (2020) Alleviation of salt stress in upland rice (*Oryza sativa* L. ssp. indica cv. Leum Pua) using arbuscular mycorrhizal fungi inoculation. Frontiers in Plant Science 11:348. <https://doi.org/10.3389/fpls.2020.00348>

- Tsai HJ, Shao KH, Chan MT et al (2020) *Piriformospora indica* symbiosis improves water stress tolerance of rice through regulating stomata behavior and ROS scavenging systems. *Plant Signal Behav* 15(2):1722447. <https://doi.org/10.1080/15592324.2020.1722447>
- Tsonev T, Cebola Lidon FJ (2012) Zinc in plants-an overview. *Emirates Journal of Food & Agriculture (EJFA)* 24(4).
- Tyagi J, Mishra A, Kumari S et al (2023) Deploying a microbial consortium of *Serendipita indica*, *Rhizophagus intraradices*, and *Azotobacter chroococcum* to boost drought tolerance in maize. *Environmental and Experimental Botany* 206:105142. <https://doi.org/10.1016/j.envexpbot.2022.105142>
- Tyagi J, Shrivastava N, Sharma AK et al (2021) Effect of *Rhizophagus intraradices* on growth and physiological performance of Finger Millet (*Eleusine coracana* L.) under drought stress. *Plant Science Today* 8(4):912-923. <https://doi.org/10.14719/pst.2021.8.4.1240>
- Ullah A, Farooq M (2022) The challenge of drought stress for grain legumes and options for improvement. *Archives of Agronomy and Soil Science* 68(11):1601-1618. <https://doi.org/10.1080/03650340.2021.1906413>
- Ullah I, Khan AR, Park GS, Lim JH, Waqas M, Lee IJ, Shin JH (2013) Analysis of phytohormones and phosphate solubilization in *Photorhabdus* spp. *Food Science and Biotechnology* 22:25-31. <https://doi.org/10.1007/s10068-013-0044-6>
- Umar UD, Ahmed N, Zafar MZ, Rehman A, Naqvi SA, Zulfiqar MA, Malik MT, Ali B, Saleem MH, Marc RA (2022) Micronutrients foliar and drench application mitigate mango sudden decline disorder and impact fruit yield. *Agronomy* 12(10):2449. <https://doi.org/10.3390/agronomy12102449>
- van Der Heijden MG, Martin FM, Selosse MA et al (2015) Mycorrhizal ecology and evolution: the past, the present, and the future. *New phytologist* 205(4):1406-1423. <https://doi.org/10.1111/nph.13288>

- Vishnupradeep R, Bruno LB, Taj Z, Karthik C, Challabathula D, Kumar A, Freitas H, Rajkumar M (2022) Plant growth promoting bacteria improve growth and phytostabilization potential of *Zea mays* under chromium and drought stress by altering photosynthetic and antioxidant responses. *Environmental Technology & Innovation* 25:102154. <https://doi.org/10.1016/j.eti.2021.102154>
- Wang H, An T, Huang D, Liu R, Xu B, Zhang S, Deng X, Siddique KH, Chen Y (2021) Arbuscular mycorrhizal symbioses alleviating salt stress in maize is associated with a decline in root-to-leaf gradient of Na⁺/K⁺ ratio. *BMC Plant Biology* 21(1):1-15. <https://doi.org/10.1186/s12870-021-03237-6>
- Wang J, Fu Z, Ren Q, Zhu L, Lin J, Zhang J, Cheng X, Ma J, Yue J (2019) Effects of arbuscular mycorrhizal fungi on growth, photosynthesis, and nutrient uptake of *Zelkova serrata* (Thunb.) Makino seedlings under salt stress. *Forests* 10(2):186. <https://doi.org/10.3390/f10020186>
- Wang K, Li J, Yi Y, Lv B, Wu Y, Wang C, Li H, Li Y, Liu Y, Cai X, Meng X, Jiang X, Zheng X, Zhou Z, Bu W (2022) Polyamine-activated carbonyl stress strategy for oxidative damage therapy. *Nano Today* 42:101355. <https://doi.org/10.1016/j.nantod.2021.101355>
- Wang Y, Wang M, Li Y, Wu A, Huang J (2018) Effects of arbuscular mycorrhizal fungi on growth and nitrogen uptake of *Chrysanthemum morifolium* under salt stress. *PLoS One* 13(4):e0196408. <https://doi.org/10.1371/journal.pone.0196408>
- Wang Y, Zou YN, Shu B, Wu Q (2023) Deciphering molecular mechanisms regarding enhanced drought tolerance in plants by arbuscular mycorrhizal fungi. *Scientia Horticulturae* 308:111591. <https://doi.org/10.1016/j.scienta.2022.111591>
- Waqar A, Bano A, Ajmal M (2022) Effects of PGPR bioinoculants, hydrogel and biochar on growth and physiology of soybean under drought stress. *Communications in Soil Science and Plant Analysis* 53(7):826-847. <https://doi.org/10.1080/00103624.2022.2028818>

- Wen-Ya Ma, Qiu-Yun Qin, Ying-Ning Zou, Kamil Kuca, Bhoopander Giri, Qiang-Sheng Wu, Abeer Hashem, Al-Bandari Fahad Al-Arjani, Khalid F. Almutairi, Elsayed Fathi Abd_Allah and Yong-Jie Xu (2022) Arbuscular mycorrhiza induces low oxidative burst in drought-stressed walnut through activating antioxidant defense systems and heat shock transcription factor expression. *Frontiers in Plant Science* 13:1089420. <http://doi.org/10.3389/fpls.2022.1089420>
- Woodson JD (2022) Control of chloroplast degradation and cell death in response to stress. *Trends in Biochemical Sciences* 47(10):851-864. <https://doi.org/10.1016/j.tibs.2022.03.010>
- Xu Z, Pehlivan N, Ghorbani A, Wu C (2022) Effects of *Azorhizobium caulinodans* and *Piriformospora indica* co-inoculation on growth and fruit quality of tomato (*Solanum lycopersicum* L.) under salt stress. *Horticulturae* 8(4):302. <https://doi.org/10.3390/horticulturae8040302>
- Yadav S, Modi P, Dave A, Vijapura A, Patel D, Patel M (2020) Effect of abiotic stress on crops. In *Sustainable Crop Production*, IntechOpen: London, UK, pp. 3–24. <https://doi.org/10.5772/intechopen.88434>
- Yaghoubian I, Antar M, Ghassemi S, Modarres-Sanavy SA, Smith DL (2022) The Effects of Hydro-Priming and Colonization with *Piriformospora indica* and *Azotobacter chroococcum* on Physio-Biochemical Traits, Flavonolignans and Fatty Acids Composition of Milk Thistle (*Silybum marianum*) under Saline Conditions. *Plants* 11(10):1281. <https://doi.org/10.3390/plants11101281>
- Yan W, Zhong Y, Shangguan Z (2016) A meta-analysis of leaf gas exchange and water status responses to drought. *Scientific Reports* 6(1):20917. <https://doi.org/10.1038/srep20917>
- Yang Y, Zha F, Zhang J, Zhu J, Dong S (2014) Effect of *Piriformospora indica* on yield, quality and physiological changes of waterlogging *Brassica chinensis*. *Hubei Agric. Sci* 53:35169.

- Yasin NA, Akram W, Khan WU, Ahmad SR, Ahmad A, Ali A (2018) Halotolerant plant-growth promoting rhizobacteria modulate gene expression and osmolyte production to improve salinity tolerance and growth in *Capsicum annum* L. Environmental Science and Pollution Research 25:23236-23250. <https://doi.org/10.1007/s11356-018-2381-8>
- Yasmeen S, Wahab A, Saleem MH, Ali, B., Qureshi KA, Jaremko M (2022) Melatonin as a Foliar Application and Adaptation in Lentil (*Lens culinaris* Medik.) Crops under Drought Stress. Sustainability 14(24):16345. <https://doi.org/10.3390/su142416345>
- Yu J, Fan N, Hao T, Bian Y, Zhuang L, Li Q, Yang Z (2023) Ethionine-mitigation of drought stress associated with changes in root viability, antioxidant defense, osmotic adjustment, and endogenous hormones in tall fescue. Plant Growth Regulation 21:1-14. <https://doi.org/10.1007/s10725-022-00944-4>
- Zahra N, Al Hinai MS, Hafeez MB, Rehman A, Wahid A, Siddique KH, Farooq M (2022) Regulation of photosynthesis under salt stress and associated tolerance mechanisms. Plant Physiology and Biochemistry, 178, 55-69. <https://doi.org/10.1016/j.plaphy.2022.03.003>
- Zai XM, Fan JJ, Hao ZP, Liu XM, Zhang WX (2021) Effect of co-inoculation with arbuscular mycorrhizal fungi and phosphate solubilizing fungi on nutrient uptake and photosynthesis of beach palm under salt stress environment. Scientific Reports 11(1):5761. <https://doi.org/10.1038/s41598-021-84284-9>
- Zhang B, Shi F, Zheng X, Pan H, Wen Y, Song F (2023) Effects of AMF Compound Inoculants on Growth, Ion Homeostasis, and Salt Tolerance-Related Gene Expression in *Oryza sativa* L. Under Salt Treatments. Rice 16(1):1-18. <https://doi.org/10.1186/s12284-023-00635-2>
- Zhang D, Wang X, Zhang Z, Li C, Xing Y, Luo Y, Li D, Ma Z, Cai H (2022b) Symbiotic System Establishment between *Piriformospora indica* and *Glycine max* and Its Effects on the Antioxidant Activity and Ion-Transporter-Related Gene Expression in Soybean under Salt Stress. International Journal of Molecular Sciences 23(23):14961. <https://doi.org/10.3390/ijms232314961>

- Zhang H, Zhu J, Gong Z, Zhu JK (2022a) Abiotic stress responses in plants. *Nature Reviews Genetics* 23(2):104-119. <https://doi.org/10.1038/s41576-021-00413-0>
- Zhang K, Zhang Y, Sun J, et al (2021) Deterioration of orthodox seeds during ageing: Influencing factors, physiological alterations and the role of reactive oxygen species. *Plant Physiology and Biochemistry* 158:475-485. <https://doi.org/10.1016/j.plaphy.2020.11.031>
- Zhao B, Liu Q, Wang B, Yuan F (2021) Roles of phytohormones and their signaling pathways in leaf development and stress responses. *Journal of Agricultural and Food Chemistry* 69(12):3566-3584. <https://doi.org/10.1021/acs.jafc.0c07908>
- Zhao C, Zhang H, Song C, Zhu JK, Shabala S (2020) Mechanisms of plant responses and adaptation to soil salinity. *The innovation* 1(1):100017. <https://doi.org/10.1016/j.xinn.2020.100017>
- Zhu J, Cai D, Wang J et al (2021) Physiological and anatomical changes in two rapeseed (*Brassica napus* L.) genotypes under drought stress conditions. *Oil Crop Science* 6(2):97-104. <https://doi.org/10.1016/j.ocsci.2021.04.003>
- Zwiazek JJ, Blake TJ (1991) Early detection of membrane injury in black spruce (*Picea mariana*). *Canadian journal of forest research* 21(3):401-404. <https://doi.org/10.1139/x91-050>

LIST OF PUBLICATIONS

Plant Growth Regulation
https://doi.org/10.1007/s10725-023-01059-0

ORIGINAL PAPER



Microbial inoculants alter resilience towards drought stress in wheat plants

Madhullika Singh^{1,2} · Jai Gopal Sharma¹ · Bhoopander Giri²

Received: 27 May 2023 / Accepted: 28 July 2023
© The Author(s), under exclusive licence to Springer Nature B.V. 2023

Abstract

Microbes play crucial roles in enhancing plant growth by forming symbiotic relationships, promoting nutrient uptake, and stimulating overall plant health in various habitats. The present study aimed to investigate the role of *Piriformospora indica*, arbuscular mycorrhiza fungi (AMF), and plant growth-promoting bacteria (PGPB) in alleviating drought stress in the *Triticum aestivum* HD-2967 cultivar. In a completely randomized design experiment, plants were subjected to different water regimes of 75% and 35% field capacity (FC) under greenhouse conditions. Under different water regimes, microbial inoculation significantly enhanced the morphological, physico-biochemical, and ultrastructural characteristics of the wheat plants. Plants inoculated with PGPB, *P. indica*, and AMF showed increased shoot and root length, shoot and root biomass, leaf area, photosynthetic rate, transpiration rate, stomatal conductance, and internal CO₂ as compared to uninoculated plants under all water regimes. The PGPB, *P. indica*, and AMF-inoculated wheat plants accumulated higher content of glycine betaine, total sugars, trehalose, proline, putrescine, spermidine, carotenoids, proteins, α -tocopherol, and a decrease in lipid peroxidation, relative membrane permeability, and lipoxygenase enzyme activity as compared to uninoculated plants. Besides, microbes-inoculated wheat plants showed a higher level of antioxidant enzymes viz., superoxide dismutase, catalase, and ascorbate peroxidase than uninoculated plants. Microbial inoculation helped wheat plants to overcome water stress-induced deficiency of macro- (Ca²⁺, Mg²⁺, and K⁺) and micronutrient (Cu, Mn²⁺, Fe, and Zn²⁺), and reduced damage to the cell ultrastructure (plasma membrane and chloroplasts). Comparing the potential of microbial inoculants to increase growth and nutritional, biochemical, physiological, and ultrastructural changes, the PGPB-inoculated wheat plants showed greater drought resilience followed by AMF and *P. indica* inoculated plants.

Keywords Antioxidant defense · Cell ultrastructure · Nutrient acquisition · Osmoregulation · Physiological response

Introduction

Drought is one of the major abiotic stresses that adversely affects agricultural production and threatens food security worldwide (Guo et al. 2020; Mirzabaei et al. 2023). A decline in rainfall and an increasing frequency of dry spells are contributing to drought stress (Jarrett et al. 2023). As a consequence, about 45% of agricultural fields are

experiencing continuous drought conditions (Yadav et al. 2020) that eventually negatively affect plant growth by decreasing transpiration and photosynthesis rates (Naservafaei et al. 2023), osmolytes (Haghighi et al. 2023) and nutrient uptake (Shah et al. 2023). Under drought stress, plants face the problem of increased electrolyte leakage, lipid peroxidation, and reactive oxygen species (ROS) production (Yu et al. 2023). Nevertheless, prolonged drought may result in irreparable damage to plant growth, resulting in reduced crop yields. However, to overcome the problem, the plant needs to maintain better osmotic adjustment, antioxidant defense mechanisms, stomatal closure, and increased root-shoot ratios (López-Galiano et al. 2019).

In 2021, global wheat production was reported to be 770 million thousand tonnes; nevertheless, wheat productivity is vulnerable to drought stress, and climate change is projected to cause a 1.9% reduction in world wheat production

Communicated by Jupei Shen.

✉ Bhoopander Giri
bhoopg@yahoo.com

¹ Department of Biotechnology, Delhi Technological University, Delhi, India

² Department of Botany, Swami Shradhanand College, University of Delhi, Delhi, India

Published online: 21 August 2023

Springer

Symbiosis
<https://doi.org/10.1007/s13199-023-00945-5>



Microbial Inoculants Improve growth in *Zea mays* L. under drought stress by up-regulating antioxidant, mineral acquisition, and ultrastructure modulations

Madhulika Singh^{1,2} · Jai Gopal Sharma¹ · Bhoopander Giri²

Received: 5 July 2023 / Accepted: 28 September 2023
 © The Author(s), under exclusive licence to Springer Nature B.V. 2023

Abstract

Drought-induced stress poses a formidable impediment to agricultural productivity, compromising crop growth and yield. While the utilization of arbuscular mycorrhizal fungi (AMF) consortium, plant growth promoting bacteria (PGPB) consortium, and *Piriformospora indica* inoculum shows promise in mitigating drought effects, a rigorous scientific assessment of their relative efficacy is lacking. This study thus investigates the comparative effects of *P. indica*, AMF consortium, and PGPB consortium inoculation on nutrient assimilation, ultrastructural adjustments, metabolic responses, and antioxidant defenses in drought-exposed maize plants. A completely randomized block design was employed, subjecting plants to varying drought stress levels (75%, 55%, 45%, and 35% of soil field capacity (FC)) in a greenhouse. The study analyzed multiple aspects, including growth parameters, nutrient levels, physiological responses, osmolyte content, antioxidant defense mechanisms, and leaf ultrastructure. Inoculation with AMF, *P. indica*, and PGPB yielded substantial enhancements in growth metrics like plant height, biomass accrual, leaf area expansion, stomatal conductance, photosynthetic activity, internal CO₂ dynamics, and transpiration rate, particularly evident across all drought stress intensities. Notably, microbial treatments counteracted nutrient deficiencies induced by drought, with augmented levels of osmolytes (total sugars, trehalose, glycine betaine), proteins, attenuated lipoxygenase enzyme (LOX) activity, ion leakage, and lipid peroxidation relative to non-inoculated plants. Elevated enzymatic activity of antioxidants, including ascorbate peroxidase (APX), catalase (CAT), and superoxide dismutase (SOD), in addition to elevated non-enzymatic antioxidants (proline, α -tocopherol, polyamines), was evident in microbial-inoculated plants. Furthermore, microbial inoculation ameliorated drought-induced ultrastructural alterations. Noteworthy disparities in drought resilience were observed among inoculants, with PGPB displaying the highest robustness, followed by AMF and *P. indica*. This research underscores the substantial potential of microbial inoculants in fostering sustainable agriculture practices in drought-challenged contexts.

Keywords Antioxidants · Cell ultrastructure · Minerals · Osmolyte · Photosynthesis · Polyamines

1 Introduction

Drought, being a prominent abiotic stressor, presents a substantial peril to worldwide food security and agricultural production (Mirzabaev et al. 2023). Decreased rainfall and prolonged dry periods contribute to drought stress, affecting

nearly 45% of agricultural fields (Yadav et al. 2020; Jarrett et al. 2023). These environmental conditions adversely impact plant growth by reducing nutrient accumulation (Shah et al. 2023), osmolyte content (Haghighi et al. 2023), photosynthesis, and transpiration rates (Naservafaei et al. 2023). Drought-stressed plants face challenges such as ion leakage, reactive oxygen species (ROS) generation, and lipid peroxidation (Yu et al. 2023) leading to irreversible damage and decreased crop yields. However, plants can employ strategies like antioxidant defense mechanisms, stomatal closure, and osmotic adjustment to mitigate these effects (López-Galiano et al. 2019). Addressing drought stress is crucial for sustaining agricultural productivity and ensuring food availability.

Bhoopander Giri
 bhoopg@yahoo.com

¹ Department of Biotechnology, Delhi Technological University, Delhi, India

² Department of Botany, Swami Shradhdhanand College, University of Delhi, Delhi, India

Published online: 27 October 2023

Springer



Augmentative Role of Arbuscular Mycorrhizal Fungi, *Piriformospora indica*, and Plant Growth-Promoting Bacteria in Mitigating Salinity Stress In Maize (*Zea mays* L.)

Madhulika Singh^{1,2} · Jai Gopal Sharma¹ · Bhoopander Giri²

Received: 26 June 2023 / Accepted: 4 November 2023
 © Springer Science+Business Media, LLC, part of Springer Nature 2023

Abstract

Salinity is a significant abiotic factor that has adverse effects on crop growth and productivity. The objective of this current study was to investigate the potential of *Piriformospora indica*, arbuscular mycorrhizal fungi (AMF), and plant growth-promoting bacteria (PGPB) in alleviating salinity stress in the Maharaja BIO 9637 hybrid maize variety. The experiment employed a completely randomized design, where plants were subjected to two salinity levels: 0 and 200 mM NaCl (corresponding to electrical conductivity values of 0.02 and 17.1 mS cm⁻¹, respectively) under greenhouse conditions. The findings of this study demonstrate that microbial inoculation significantly enhanced plant growth, biomass, photosynthetic efficiency, photosynthetic pigment concentration, osmolyte accumulation, and nutrient uptake compared to control plants under salinity stress. Moreover, microbe-colonized plants exhibited reduced levels of malondialdehyde, membrane permeability, and lipoxigenase activity. The microbe-colonized maize plants also displayed elevated levels of antioxidant enzymes, viz. superoxide dismutase (SOD), catalase (CAT), and ascorbate peroxidase (APX). However, AMF colonization did not affect CAT activity and decreased at high salinity levels. Remarkably, microbe-colonized plants exhibited less damage at the ultrastructural level than control plants, irrespective of the salinity levels. Henceforth, PGPB, *P. indica*, and AMF possess substantial promise as feasible choices for advancing sustainable agricultural methodologies in situations marked by environmental stress.

Keywords Antioxidant enzymes · Chloroplast · Nutrient uptake · Plasma membrane · Stomatal conductance

Abbreviations

AMF	Arbuscular mycorrhizal fungi
PGPB	Plant-growth promoting bacteria
ROS	Reactive oxygen species
HPLC	High-performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
LOX	Lipoxigenase
APX	Ascorbate peroxidase
CAT	Catalase
SOD	Superoxide dismutase
MDA	Malondialdehyde

Handling Editor: Vijay Pratap Singh.

Bhoopander Giri
 bhoopg@yahoo.com

¹ Department of Biotechnology, Delhi Technological University, Delhi, India

² Department of Botany, Swami Shradhanand College, University of Delhi, Delhi, India

Introduction

The global population is expected to reach 9 billion by 2050 (Khandelwal et al. 2023), necessitating a 70% increase in food production to meet demand. Salinity stress adversely affects plant growth, productivity, and yield (Soni and Savalia 2023), with an estimated 50% of global agricultural land anticipated to be impacted by salinity by 2050 (Gopalakrishnan and Kumar 2021). Salinity issues result from various factors, including reduced rainfall, high evaporation rates, weathering processes, excessive fertilizer use, and improper irrigation practices (Gopalakrishnan et al. 2020; Zhao et al. 2020).

Plant exposure to salinity induces significant alterations in morphology, physiology, biochemistry, and ultrastructural characteristics, hampering growth and development (El-Dakak et al. 2023). Salinity also negatively impacts enzyme activity, stomatal conductance, photosynthesis, and reactive oxygen species (ROS) production. These ROS, byproducts of metabolic pathways, cause damage to cellular

Published online: 19 December 2023

Springer

CONFERENCES

