

T.R.
GEBZE TECHNICAL UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY

**AN INVESTIGATION OF THE MYCORRHIZA-LIKE
GROWTH-PROMOTING AND STRESS TOLERANCE-
ENHANCING EFFECTS OF PIRIFORMOSPORA INDICA ON
BREAD AND DURUM WHEAT IN RELATION TO BORON
TOXICITY AND SALINITY STRESS**

OZGUR ALTUNDAS
A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
DEPARTMENT OF BIOTECHNOLOGY

GEBZE
2023

T.R.
GEBZE TECHNICAL UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY

**AN INVESTIGATION OF THE
MYCORRHIZA-LIKE GROWTH-
PROMOTING AND STRESS TOLERANCE-
ENHANCING EFFECTS OF
PIRIFORMOSPORA INDICA ON BREAD
AND DURUM WHEAT IN RELATION TO
BORON TOXICITY AND SALINITY STRESS**

OZGUR ALTUNDAS
A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
DEPARTMENT OF BIOTECHNOLOGY

THESIS SUPERVISOR
ASST. PROF. UMIT BARIS KUTMAN

GEBZE
2023

T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
BİYOTEKNOLOJİ ENSTİTÜSÜ

PIRIFORMOSPORA INDICA’NIN
EKMEKLİK VE MAKARNALIK BUĞDAY
ÜZERİNDEKİ MİKORİZA BENZERİ
BÜYÜMEYİ TEŞVİK EDİCİ VE STRES
TOLERANSINI ARTTIRICI ETKİLERİNİN
BOR TOKSİSİTESİ VE TUZLULUK STRESİ
BAĞLAMINDA ARAŞTIRILMASI

ÖZGÜR ALTUNDAŞ
YÜKSEK LİSANS TEZİ
BİYOTEKNOLOJİ ANABİLİM DALI

DANIŞMAN
DR. ÖĞR. ÜYESİ ÜMİT BARIŞ KUTMAN

GEBZE
2023



YÜKSEK LİSANS JÜRİ ONAY FORMU

GTÜ Biyoteknoloji Enstitüsü Yönetim Kurulu'nun 07/03/2023 tarih ve 2023/7 sayılı kararıyla oluşturulan jüri tarafından 06/03/2023 tarihinde tez savunma sınavı yapılan Özgür ALTUNDAŞ'ın tez çalışması Biyoteknoloji Anabilim Dalında YÜKSEK LİSANS tezi olarak kabul edilmiştir.

JÜRİ

ÜYE

(TEZ DANIŞMANI)

: Dr. Öğr. Üyesi Ümit Barış KUTMAN

ÜYE

: Doç. Dr. Müge İŞLETEN HOŞOĞLU

ÜYE

: Dr. Öğr. Üyesi Emre AKSOY

ONAY

Gebze Teknik Üniversitesi Fen Bilimleri Enstitüsü Yönetim Kurulu'nun

...../...../..... tarih ve/..... sayılı kararı.

SUMMARY

Nowadays, salinity stress and boron toxicity are classified as two major yield-limiting factors, and the prevalence of these factors have been increased and threatened sustainable wheat production due to climate change. Previous studies have been focused on investigating single or combined effects of salinity stress and boron toxicity but development of management strategies which may ameliorate destructive effects of these factors on wheat is limited.

The thesis aimed to reveal the physiological and morphological effects of *Piriformospora indica* on commercial durum and bread wheat grown under combined salinity stress and boron toxicity. In addition, interplay of simultaneous *Piriformospora indica* and arbuscular mycorrhizal fungi inoculation on wheat yield was investigated.

Piriformospora indica inoculation increased grain yield, shoot dry weight, specific activities of superoxide dismutase, ascorbate peroxidase, catalase, glutathione reductase, peroxidase of bread wheat by up to 36%, 22%, 28%, 24%, 36%, 7%, 11%, respectively. Furthermore, bread and durum wheat cultivars inoculated with *Piriformospora indica* had 40% lower Na and 66% lower malondialdehyde concentrations when compared to non-inoculated counterparts. The result of the thesis showed that *Piriformospora indica* enhanced wheat growth and conferred tolerance against combined salinity stress and boron toxicity by promoting antioxidant defence mechanisms, alleviating membrane injury, excluding excess Na⁺ and maintaining mineral homeostasis. Furthermore, grain yield promotion by co-inoculation with *Piriformospora indica* and arbuscular mycorrhizal fungi reached by up to 41% and 50% in bread and durum wheat cultivar, respectively. Thus, our data showed that *Piriformospora indica* inoculation protected wheat from combined salinity stress and boron toxicity.

Key Words: *Piriformospora indica*, Arbuscular mycorrhizal fungi, Salinity Stress, Boron Toxicity, Wheat

ÖZET

Günümüzde tuz stresi ve bor toksisitesi verimi sınırlayan iki ana faktör olarak sınıflandırılmaktadır ve bu faktörlerin yaygınlığı iklim değişikliği nedeniyle artmakta ve sürdürülebilir buğday üretimini tehdit etmektedir. Önceki çalışmalar, tuzluluk stresi ve bor toksisitesinin tek başına veya birleşik etkilerini belirlemeye odaklanmıştır, fakat B toksisitesinin ve tuzluluğun buğday üretimi üzerindeki olumsuz etkilerini giderebilmek için geliştirilmiş yönetim stratejileri kısıtlıdır.

Bu tez kapsamında, *Piriformospora indica*'nın tuz stresi ve bor toksisitesi altında yetiştirilen ticari makarnalık ve ekmeklik buğday üzerindeki fizyolojik ve morfolojik etkilerini araştırılmıştır. Ayrıca eş zamanlı *Piriformospora indica* ve arbüsküler mikorizal fungusların inokülasyonlarının buğdayın verimine etkileri ortaya konmuştur.

Tane verimi, gövde kuru ağırlığı ve süperoksit dismutaz, askorbat peroksidaz, katalaz, glutatyon redüktaz, peroksidaz enzimlerinin spesifik aktiviteleri sırasıyla %33, %22, %28, %24, %36, %7, %11 oranında *Piriformospora indica* inokülasyonu ile ekmeklik buğday çeşidinde artmıştır. Ayrıca, *Piriformospora indica* ile inoküle edilen ekmeklik ve makarnalık buğday çeşitleri edilmeyenlere kıyasla %40 ve %66 oranında daha düşük Na ve malondialdehit konsantrasyonuna sahipti. Bu tezin sonucu, *Piriformospora indica* inokülasyonunun antioksidan savunma mekanizmalarını geliştirerek, aşırı Na⁺ konsantrasyonunu düşürerek, hücre zarını koruyarak ve mineral dengesini sağlayarak buğdayın büyümesini artırdığını ve tuzluluk stresi ve bor toksisitesine karşı tolerans sağladığını göstermiştir. *Piriformospora indica* ve arbüsküler mikorizal fungusların eş zamanlı inokülasyonu sırasıyla %41 ve %50'ye varan oranlarda ekmeklik ve makarnalık buğdayın tane veriminde artış sağlamıştır. Böylece, elde ettiğimiz sonuçlar *Piriformospora indica* inokülasyonunun buğdayı kombine tuz stresinden ve bor toksisitesinden koruduğunu göstermiştir.

Anahtar Kelimeler: *Piriformospora indica*, Arbüsküler mikorizal funguslar, Tuz stresi, Bor Toksisitesi, Buğday

ACKNOWLEDGEMENTS

I would like to thank to my supervisor Asst. Prof. Dr. Ümit Barış KUTMAN for his scientific guidance, crucial advice, and vital critics. I will never forget his scientific attitude, professionalism, and efforts to encourage his students to act ethically. I would like to express my deep respect to Asst. Prof. Dr. Bahar YILDIZ KUTMAN for her contribution to my thesis.

I would like to thank jury members of my thesis committee Assoc. Prof. Dr. Müge İŞLETEN HOŞOĞLU and Asst. Prof. Dr. Emre AKSOY for their valuable contribution, scientific advice, and critics.

I would like to thank to Prof. Dr. Sedef TUNCA GEDİK, Prof. Dr. Filiz VARDAR and Res. Assist. Halil YILMAZ for their valuable contribution to my thesis.

I would like to thank to my lab-mates who worked with me in Kutman Lab between 2020-2022 namely Ece Sena TOPAL, Ali KAVAL, Ayşenur BAYRAK who listened to my complaint for hours, Ayşenur ÇATALER KARALAR, Burak TUNÇBAŞ, Elif Özlem GÜNÇALDI, Hande MUTLU DURAK, İlknur ÇAÇA, İlyas Zekeriya DİK, Kübra TUTKUN, Ömer Faruk TAŞTAN, Şükran DUMAN, Taşkın Kerem KOÇAK, Yağmur ARIKAN.

I wish to express my special thanks to Nurefşan CIRIK, Taha Yunus KAHRİMAN and Ece Sena TOPAL for encouraging me for earning a master's degree, being always supportive when I doubt my abilities due to imposter syndrome and convincing me to write my thesis, respectively.

I wish to thank Abdirraim NADİROV for his support and friendship.

I would like to give my special thanks to all members of my family especially to my grandmother for telling me fable, namely, “The Stork”.

Finally, I would like to acknowledge The Scientific and Technological Research Council of Turkey (TUBITAK) for supporting me by a scholarship and providing financial support to this thesis as a part of TUBITAK 1001 project (118Z984) with title “Investigation of the application potential and physiological effects of *Piriformospora indica* as a biological agent against boron toxicity in wheat”.

This thesis is dedicated to my horse Gece.

TABLE of CONTENTS

	<u>Page</u>
SUMMARY	v
ÖZET	v
ACKNOWLEDGMENTS	vii
TABLE of CONTENTS	viii
LIST of ABBREVIATIONS and ACRONYMS	x
LIST of FIGURES	xii
LIST of TABLES	xvii
 1. INTRODUCTION	 1
1.1. Plant Microbe Interaction	3
1.1.1. Fungus	4
1.1.2. <i>Piriformospora indica</i>	6
1.1.3. Arbuscular Mycorrhizal Fungi	7
1.1.4. Interplay of Mycorrhizae and <i>Piriformospora indica</i>	8
1.2. Wheat	9
1.2.1. <i>Triticum aestivum</i> L.	10
1.2.2. <i>Triticum Durum</i>	11
1.3. Abiotic Stress	11
1.3.1. Salinity Stress	12
1.3.2. Effects of Salinity in Plant	13
1.4. Boron	15
1.4.1. Availability of Boron in Soil	15
1.4.2. Boron as Essential Micronutrient	16
1.4.3. Boron in Plant Cell Wall and Boron Deficiency	17
1.4.4. Boron Toxicity	19
1.4.5. Boron Toxicity Tolerance	21
1.4.5. Interaction Between Salinity and Boron Toxicity	21
1.5. The Questions Addressed in This Project	23
2. MATERIALS and METHODS	23
2.1. Materials	24
2.1.1. Plant Materials	24
2.1.2. Greenhouse	24
2.2. Methods	24
2.2.1. <i>Piriformospora indica</i> Culture and Inoculation Method	24
2.2.2. Co-cultivation of <i>Arabidopsis thaliana</i> with <i>Piriformospora indica</i>	25
2.2.3. Preparation of Mycorrhizal Inoculum	27
2.2.4. Soil and Transplantation Method	27
2.2.5. Calculation	28

2.2.6. Statistical Analysis	29
2.3. Experimental Designs	29
2.3.1. First Experiment	29
2.3.2. Second Experiment	31
2.3.3. Third Experiment	32
2.3.4. Fourth Experiment	32
2.3.5. Fifth Experiment	33
2.4. Analysis	34
2.4.1. Antioxidative Enzyme Activity and Total Protein Assays	34
2.4.2. Catalase Assay	35
2.4.3. Superoxide Dismutase Assay	35
2.4.4. Glutathione Reductase Assay	35
2.4.5. Ascorbate Peroxidase Assay	35
2.4.6. Peroxidase Assay	36
2.4.7. Estimation of Malondialdehyde	36
2.4.8. Determination of Glycine Betaine	37
2.4.9. Estimation of Proline	37
2.4.10. Phenylalanine Ammonia Lyase Activity	38
2.4.11. Relative Electrolyte Leakage Assay	38
2.4.12. Root Staining and Microscopic Observation	38
2.4.13. Polymerase Chain Reaction	39
2.4.14. Dry Weight, Tiller Number and Yield Parameters	40
2.4.15. Element Analysis	41
3. RESULTS	42
3.1. Results of First Experiment	42
3.2. Results of Second Experiment	47
3.3. Results of Third Experiment	57
3.4. Results of Fourth Experiment	63
3.5. Results of Fifth Experiment	85
4. DISCUSSION	104
5. CONCLUSION	109
REFERENCES	111
BIOGRAPHY	127

LIST of ABBREVIATIONS and ACRONYMS

<u>Abbreviations and acronyms</u>	<u>Explanations</u>
AMF	: Arbuscular Mycorrhizal Fungi
ANOVA	: Analysis of variance
APX	: Ascorbate peroxidase
ATP	: Adenosine triphosphate
B	: Boron
Ca	: Calcium
CA(NO ₃) ²	: Calcium nitrate
CAT	: Catalase
Cd	: Cadmium
Cl	: Chlorine
CO ₂	: Carbon dioxide
CRISPR	: Clustered regularly interspaced short palindromic repeats
Cu	: Cobber
dH ₂ O	: Distilled water
dS/m	: Decisiemens per metre
EC	: Electrical conductivity
g	: gram
GM	: Genetically modified
GR	: Glutathione reductase
GSH	: Reduced glutathione
GSSG	: Oxidized glutathione
H ₂ O ₂	: Hydrogen peroxide
H ₃ BO ₃	: Boric acid
HSD	: Honestly significant difference
ICP-OES	: Inductively coupled plasma optical emission spectrometry
K	: Potassium
K ₂ SO ₄	: Potassium sulfate
KCl	: Potassium chloride
Kg	: Kilogram

KH ₂ PO ₄	: Potassium dihydrogen phosphate
KI-I ₂	: Potassium iodide
K-P	: Potassium phosphate buffer
L	: Liter
MDA	: Malondialdehyde
Mg	: Milligram
Min	: Minute
Na	: Sodium
NaCl	: Sodium chloride
NADPH	: Nicotinamide adenine dinucleotide phosphate
NBT	: Nitro blue tetrazolium chloride
<i>n.s.</i>	: Non-significant
<i>n.d.</i>	: Non detected
P	: Phosphorus
POX	: Peroxidase
QTL	: Quantitative trait locus
REL	: Relative electrolyte leakage
RG-II	: Rhamnogalacturonan-II
rpm	: Revolutions per minute.
SOD	: Superoxide dismutase
TALEN	: Transcription activator-like effector nucleases
TBA	: Thiobarbituric acid
TCA	: Trichloroacetic acid

LIST OF FIGURES

<u>Figure No:</u>	<u>Page</u>
2.1: Co-cultivation of <i>A. thaliana</i> with <i>P. indica</i> and re-isolation of <i>P. indica</i> from roots of <i>A. thaliana</i> .	26
3.1: Observation of root colonization of <i>P. indica</i> and arbuscular mycorrhizal fungi.	42
3.2: Effects of single <i>P. indica</i> , single AMF, combined <i>P. indica</i> (+) AMF inoculation with low (20 mg P/kg soil) or adequate (100 mg P/kg of soil) P treatments on shoot biomass of 50-day-olds durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Different letters indicate significant differences according to the Tukey's protected HSD test at 95% confidence intervals. Bars represent means of five independent pot replicates each containing eight individual plants. Error bars represent standard deviation.	43
3.3: Effects of single <i>P. indica</i> , single AMF, combined <i>P. indica</i> (+) AMF inoculation on bread wheat (<i>Triticum aestivum</i> cv. Nusrat) plants grown in autoclaved soil under greenhouse conditions.	47
3.4: Effects of single <i>P. indica</i> , single AMF, combined <i>P. indica</i> (+) AMF inoculation on durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions.	48
3.5: Effects of single <i>P. indica</i> , single AMF, combined <i>P. indica</i> (+) AMF inoculation on thousand grain weight of bread wheat (<i>Triticum aestivum</i> Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions.	50
3.6: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on shoot dry weight of 32-day-olds bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot	57

replicates each containing five individual plants. Error bars represent standard deviation.

- 3.7: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on B and Na concentration of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. 58
- 3.8: Observation of effects of root colonization of *P. indica* on 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) plants grown under different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies in non-autoclaved soil under greenhouse conditions. 63
- 3.9: Observation of effects of root colonization of *P. indica* on 32-day-olds bread wheat (*Triticum durum* cv. Saricanak-98) plants grown under different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies in non-autoclaved soil under greenhouse conditions. 64
- 3.10: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on tiller number and shoot dry weight of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing four individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. 66

- 3.11: Microscopic observation of roots of 32-days-old wheat plants grown in 67
absence and presence of *P. indica* inoculation. Chlamydospores of *P. indica* are showed by white arrows. Roots of wheats were stained by trypan blue. Scale bars (horizontal black lines) indicates magnification.
- 3.12: PCR amplification result of total DNA samples extracted from roots of 68
32-days-old-wheat plants which were inoculated with or without *P. indica*. PCR products were loaded into wells of 1.5 % agarose gel and run at 90 V for 2 hours. Except ladder, the remaining well was loaded with 25 µl PCR product. DNA ladder was loaded with 5 µl. M: 100bp DNA Ladder; N: Negative control; 1: non-inoculated wheat sample; 2-3-4-5-6: *P. indica* inoculated wheat sample. Amplification products of *EF-1 alpha (tef)* gene produced bands at 220 bp.
- 3.13: Effects of different salinity treatments (control: No salt treatment; 69
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on Malondialdehyde (MDA) concentration of 32-day-olds leaves of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing four individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels × B supply × Inoculation) is significant.
- 3.14: Effects of different salinity treatments (control: No salt treatment; 70
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on Relative Electrolyte Leakage of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing four individual plants. Error bars represent

standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.

- 3.15: Effects of different salinity treatments (control: No salt treatment; 72
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic
(20 mg/kg B) B supplies on shoot B and Na concentration of 32-day-
olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat
(*Triticum durum* cv. Saricanak-98) plants which were inoculated with
or without *P. indica* and grown in non-autoclaved soil under
greenhouse conditions. Bars represent means of four independent pot
replicates each containing four individual plants. Error bars represent
standard deviation. Different letters indicate significant differences
between means of treatments group according to Tukey's protected
HSD test. Letters appear above the bar if the interaction of the three
variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.
- 3.16: Observation of effects of *P. indica* colonization on morphology of 98- 85
day-olds bread wheat (*Triticum aestivum* cv. Nusrat) plants grown
under different salinity treatments (control: No salt treatment;
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic
(20 mg/kg B) B supplies in non-autoclaved soil under greenhouse
conditions.
- 3.17: Observation of effects of *P. indica* colonization on morphology of 98- 86
day-olds durum wheat (*Triticum durum* cv. Saricanak-98) plants grown
under different salinity treatments (control: No salt treatment;
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic
(20 mg/kg B) B supplies in non-autoclaved soil under greenhouse
conditions.
- 3.18: Effects of different salinity treatments (control: No salt treatment; 87
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic
(20 mg/kg B) B supplies on thousand grain weight of 32-day-olds
bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum
durum* cv. Saricanak-98) plants which were inoculated with or without

P. indica and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.

- 3.19: Effects of different salinity treatments (control: No salt treatment; 90
 moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on grain B and Na concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.
- 3.20: Effects of different salinity treatments (control: No salt treatment; 92
 moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on grain B and Na concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation.

LIST of TABLES

<u>Table No:</u>	<u>Page</u>
2.1: Physiochemical characteristics of soil used in this thesis.	28
2.2: Inoculum application method that was followed in the first and second experiments.	30
2.3: Amplification conditions and components of specific segment of <i>EF-1-alpha (tef)</i> gene by PCR.	40
3.1: Effects of single <i>P. indica</i> , single AMF, combined <i>P. indica</i> (+) AMF inoculation with low (20 mg P/kg soil) or adequate (100 mg P/kg of soil) P treatments on shoot concentration of selected micronutrients of 50-day-olds durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of five independent replicates each containing eight individual plants.	44
3.2: Effects of single <i>P. indica</i> , single AMF, combined <i>P. indica</i> (+) AMF inoculation with low (20 mg P/kg soil) or adequate (100 mg P/kg of soil) P treatments on shoot concentration of selected macronutrients of 50-day-olds durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of five independent replicates each containing eight individual plants.	45
3.3: Effects of single <i>P. indica</i> , single AMF, combined <i>P. indica</i> (+) AMF inoculation on grain yield, straw dry weight, and harvest index parameters of bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.	49
3.4: Effects of single <i>P. indica</i> , single AMF, combined <i>P. indica</i> (+) AMF inoculation on grain macronutrient concentration of bread wheat	51

- (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.
- 3.5: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation on grain micronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants. 53
- 3.6: Effects of single and co-inoculation of *P. indica*, single AMF on straw macronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants. 54
- 3.7: Effects of single and co-inoculation of *P. indica*, single AMF on straw micronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants. 55
- 3.8: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on macronutrient concentrations of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants. 60
- 3.9: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on micronutrient concentrations of 32-day- 62

olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

- 3.10: Effects of different salinity treatments (control: No salt treatment; 73
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on macronutrient concentration of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.
- 3.11: Effects of different salinity treatments (control: No salt treatment; 74
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on micronutrient concentration of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.
- 3.12: Effects of different salinity treatments (control: No salt treatment; 76
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on macronutrient concentration of 32-day-olds durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.
- 3.13: Effects of different salinity treatments (control: No salt treatment; 78
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on micronutrient concentration of 32-day-olds durum wheat (*Triticum durum* cv. Saricanak-98) plants which

were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

- 3.14: Effects of different salinity treatments (control: No salt treatment; 79
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on leaves Glycine Betaine and Proline concentration of 32-day-olds bread (*Triticum aestivum* cv. Nusrat) durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.
- 3.15: Effects of different salinity treatments (control: No salt treatment; 81
moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on leaves Phenylalanine ammonia-lyase (PAL) activity and Trolox equivalent antioxidant capacity (TEAC) of 32-day-olds bread (*Triticum aestivum* cv. Nusrat) durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.
- 3.16: Effects of inoculation with *P. indica* on specific activities of 82
superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POX) enzymes of leaves of 32-days-old bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

- 3.17: Effects of inoculation with *P. indica* on specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POX) enzymes of leaves of 32-days-old durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants. 84
- 3.18: Effects of inoculation with *P. indica* on grain yield, straw dry weight and harvest index of bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants. 88
- 3.19: Effects of inoculation with *P. indica* on grain yield, straw dry weight, and harvest index of durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants. 89
- 3.20: Effects of inoculation with *P. indica* on selected grain macronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants. 94

3.21:	Effects of inoculation with <i>P. indica</i> on selected grain micronutrient concentration of bread wheat (<i>Triticum aestivum</i> cv. Nusrat) plants grown at adequate (1 mg B kg ⁻¹ soil) or toxic (20 mg B kg ⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.	95
3.22:	Effects of inoculation with <i>P. indica</i> on selected grain macronutrient concentration of durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown at adequate (1 mg B kg ⁻¹ soil) or toxic (20 mg B kg ⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.	96
3.23:	Effects of inoculation with <i>P. indica</i> on selected grain micronutrient concentration of durum wheat (<i>Triticum durum</i> cv. Saricanak-98) plants grown at adequate (1 mg B kg ⁻¹ soil) or toxic (20 mg B kg ⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.	97
3.24:	Effects of inoculation with <i>P. indica</i> on selected straw macronutrient concentration of bread wheat (<i>Triticum aestivum</i> cv. Nusrat) plants grown at adequate (1 mg B kg ⁻¹ soil) or toxic (20 mg B kg ⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.	98
3.25:	Effects of inoculation with <i>P. indica</i> on selected straw micronutrient concentration of bread wheat (<i>Triticum aestivum</i> cv. Nusrat) plants grown at adequate (1 mg B kg ⁻¹ soil) or toxic (20 mg B kg ⁻¹ soil) B	99

supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

- 3.26: Effects of inoculation with *P. indica* on selected straw macronutrient concentration of durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants. 101
- 3.27: Effects of inoculation with *P. indica* on selected straw micronutrient concentration of durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants. 103

1. INTRODUCTION

Countries mainly target to supply required amount of nutritional and healthy food for maintaining higher quality of life to society while eliminating any kind of possible environmental hazards which may be originated from agricultural practices at the same time [Timsina, 2018]. However, future projections show that 60% production enhancement is needed to meet food demand by 2050 but annual yield improvement indicate that target improvement rate may not be not achievable [Horton et al., 2021]. Another projection demonstrated that global food production should be increased 70% in 2050 when compared to 2005 [Doering and Sorensen, 2018]. Furthermore, meeting future crop production target mainly relies on sustainable and efficient production of just a few stable crops including wheat [Ray et al., 2013]. Among 275 crops, wheat alone contribute almost 16% to daily calories intake [Tilman et al., 2011].

Thus, like Green Revolution boost food production and maximizing yield during the middle of the 20th century [Harwood, 2019] we need a new touchstone to cope with the bottlenecks which limit modern agricultural production such as climate change and environmental stress factors. Therefore, developing innovative and sustainable solutions to major challenges of global wheat production is utmost importance for global food security.

Climate change has resulted from industrialization. Post-industrialization period raise level of greenhouse gasses in the atmosphere because industrialization mainly rely on usage of fossil fuels [Rakshit et al., 2020]. Climate change causes significant alterations of temperature and rainfall pattern while atmospheric conditions have also fluctuated such as CO₂ concentration [Chaudhry et al., 2022]. Rapid changes in climate brings into question whether plants' ability to adapt their environment thanks to plasticity and genetic changes will be enough or not. The answer is uncertain [Shaw and Etterson, 2012].

Although climate change offer various pros and cons for plant production but it is certain that severity of abiotic and biotic stress factors are extremely affected from climate change and prevalence of abiotic stresses are increasing [Rehaman et al., 2021], [Raza et al., 2018], [Agrimonti et al., 2021]. Rosenzweig et al., [2014]

demonstrate that climate change causes overall yield reduction of crops all around the world. For instance, rising of global temperature because of climate change lead to yield reduction and it also trigger different abiotic stress factors such as salinity [Chaudhry et al., 2022]. In addition, abiotic stress factors occur together in the field so plants must deal with a combination of abiotic stress factors under field conditions [Lawas et al., 2019a], [Lawas et al., 2019b]. The combination of abiotic stresses is the major reason of yield losses because their simultaneous effects causes high incidence rate of stress [Choudhury et al., 2016], [Suzuki et al., 2014], [Mittler, 2006]. Therefore, improving tolerance levels of crops in agriculture against abiotic stresses has significant importance. Studies aim to enhance tolerance levels of crops against abiotic stress factors by using various methods. Godoy et al., [2021] pointed out the methods as listed below:

- i. Cisgenic, transgenic and intragenic techniques (GM techniques).
- ii. Classic and modern breeding techniques.
- iii. Genome engineering/editing techniques (TALEN, CRISPR etc.).
- iv. Biostimulant application.

Above mentioned techniques offer different advantages and disadvantages. For instance, usage of crops that are modified by GM techniques are limited because of health and safety concern in many areas such as Europe and abiotic stress mechanism include multiple genes thus GM techniques may not be sufficient to transfer the numerous genes from donor to recipient plants [Vij and Tyagi, 2007], [Zimny et al., 2019], [Godoy et al., 2021].

On the other hand, crops that are produced by using classical breeding techniques seem to be healthier and safer, but production processes require too much time and available germplasm may not contain desired genes [Anwar and Kim, 2020], [Lohani et al., 2022].

Genome editing techniques are used to modify the target genome without needing addition of a novel genes into the genomes but these techniques are also regulated with the same way GM crops are regulated in many countries such as New Zealand and the Europe [Ishii, 2018]. Therefore, it seems that feasibility of these methods to meet demand of tolerant cultivar development is limited.

Biostimulants are defined by European Biostimulant Industry Council (EBIC) as a substance and/or microorganism which applied crops or soil to enhance growth

and development of plants irrespective to their composition [Ricci et al., 2019]. In addition to definition of EBIC, biostimulant is also described as a material which is able to promote plant development and survival ability under unsuitable environmental conditions and it may be delivered by microorganism [du Jardin, 2015]. Biostimulator promotes plant growth such as fungi. Fungi colonize in plant roots and establish relationship with plants such as arbuscular mycorrhizal fungi (AMF) and *Piriformospora indica* (*P. indica*), which belong to the phylum *Glomeromycota* and *Basidiomycota*, respectively [Moreira et al., 2015].

Finally, sustaining enough foods to substantially rising global population for meeting global food demand while minimizing any kind of environmental risks associated with modern and/or conventional agricultural methods is required to development holistic approaches.

Therefore, the first aim of this thesis was to reveal effects of *P. indica* colonization on physiology of durum and bread wheat which exposed to salinity stress and boron toxicity. The second purpose of this thesis was to investigate interactive effects of AMF and *P. indica* on vegetative and generative development in different P levels and control conditions, respectively. In this way, the thesis aimed to investigate efficacy and efficiency of fungi treatments for boosting plant's ability to survive in an environment which affected with and without abiotic stresses.

1.1. Plant Microbe Interaction

Microbes have potent to design numerous plant metabolic activity like an architect. Microbes contributed to plant evolutionary processes by increasing their fitness throughout the history and the contribution is termed as microbe mediated adaptation [Petipas et al., 2021]. Plant fitness is enhanced by microbe mediated adaptation in two ways; (i) microbe mediated local adaptation and (ii) microbe mediated adaptive plasticity [Petipas et al., 2021].

Microbes are one of the factor to affect amount of plant diversity in particular region by favouring certain plants (positive plant-soil feedback) and make them dominant species or disfavouring plant species so they cause plant diversity (negative plant-soil feedback) [Raynaud et al., 2021]. Because of the great interaction ability between microbe and plant, it seem to be one of the promising approach to accelerate

and sustain agricultural production when protecting environment. Therefore, beneficial microorganisms are used as stress alleviating tool and crop quality enhancer [Schikora et al., 2020]. In addition, certain endophytes support particular plant species in their natural habitat so that these plant species provide an advantages against other intra and interspecies to become more dominant [Y. Zhou et al., 2018].

In 2019, top ten important questions which drive microbe-plant interaction studies was selected by over three hundred attendees in International Congress on Molecular Plant-Microbe Interactions (IC-MPMI) in Glasgow, Scotland. One of these question was “how does abiotic stress influence plant-microbe interactions” [Harris et al., 2020]. Firstly, factors which are altering because of global change generally decrease soil microbial diversity [Y. Yang et al., 2021]. Secondly, climate change may disrupt plant-microbe interaction [Rudgers et al., 2020]. Therefore, current knowledge indicates that abiotic stress factors become more destructive, and soil microbial diversity is also negatively affected from rapid changes of environmental conditions. So, studying combined abiotic stress factors become more important especially these days microbial diversity is hindering by climate change and plant-microbe interaction are disrupted due to microplastics.

1.1.1. Fungus

Nowadays, true fungi are classified into nine lineages, namely, Opisthosporidia, *Chytridiomycota*, *Neocallimastigomycota*, *Blastocladiomycota*, *Zoopagomycota*, *Mucoromycota*, *Glomeromycota*, *Ascomycota* and *Basidiomycota* [Naranjo-Ortiz and Gabaldón, 2019]. In addition, based on estimation over 2 million fungi exist in the Earth [Lücking et al., 2021]. Fungi perform different function for ecosystem such as degrading residues, establishing beneficial relationship with other organism and causing some diseases [Zabel and Morrell, 2020]. Fungi and plant interaction mainly formed as three different forms, namely, biotrophy, necrotrophy and hemibiotrophy [Zuccaro et al., 2011]. Fungi help plants to continue their life while interacting with plant in different form such as endophyte and mycorrhiza [Naranjo-Ortiz and Gabaldón, 2019].

Even though, there are numerous advantages of using fungi as a tool to ameliorate detrimental effects of abiotic stresses and to support sustainable agricultural

production, output of academic researches generally do not turn into industrial product [Hyde et al., 2019]. However, the history of plant and fungi interaction is dated for over 400 million years especially with endophyte and mycorrhiza [Krings et al., 2007]. Endophyte promote plant growth under stress conditions and ameliorate harm of stress by indirectly triggering defence system and directly producing some pathogen suppressor [Lu et al., 2021]. The result of a meta-analysis which examined 209 articles had been conducted to obtain a comprehensive result which had aimed to figure out how endophytes affect plants under drought stress, salinity stress and nitrogen (N) deficiency conditions by Rho et al., [2018] confirmed that endophytes promote plant growth and development.

Additionally, AMF enhances crop quality and quantity. Moreover, AMF helps plant to acquire minerals from soil especially Phosphorous (P) because these minerals are finite source, it is important to integrate beneficial fungus in agricultural production to create environmental friendly and sustainable agricultural ecosystem [Igiehon and Babalola, 2017]. Therefore, AMF may use to decrease the amount of fertilizer and water usages because AMF reshape root architecture to uptake more water and minerals from soil even under water limited conditions due to accumulation of salts in soil [Acosto et al., 2020].

Besides the existence of technological advancement in plant science, production and utilization of AMF under field conditions is not fully achieved because of challenges which arise from strain purification and contamination problems during the production of inoculum [Igiehon and Babalola, 2017]. Furthermore, fungal endophyte are able to support plant development via different ways, optimum conditions for growth of fungal endophyte for certain plant species need to be determined and which endophytes fungus is more capable to boost yield of which plant species should be pointed out [Chitarra et al., 2021]. Probably, missing information that is required to develop industrial products to cope with abiotic stresses by using beneficial fungus is the main reason behind the failure to turn an idea into a novel industrial product.

1.1.2. *Piriformospora indica*

P. indica is an endophytic fungus that lives at least some period of its life cycle together with plants without causing any harm. In literature, the name *Serendipita indica* is started to use more widely instead of *P. indica* in recent studies [Fraj and Werbrouck, 2023], [Mehri et al., 2023]. However, in this thesis, the name *P. indica* is preferred.

P. indica belongs to class, order and family of *Basidiomycota*, *Sebacinales* and *Sebacinaceae* respectively [Chitarra et al., 2021], [Hosseini et al., 2017]. In literature, the first experiment which reveals growth-promoting effects of *P. indica* colonization in root of different plant species had done by Varma et al., [1999] and the study demonstrated that the fungus is able to colonize in various host species.

In colonized plants with *P. indica*, chlamydospores and fungal hyphae are observed in cortical, rhizodermal and root hair cells [Deshmukh et al., 2006]. Zuccaro et al., [2011] suppose that broad host range of *P. indica* is originated from in addition to colonize both living and death cell, ability to suppress or minimize microbe-associated molecular pattern (MAMP) triggered immunity (MTI). Furthermore, in contrast to others symbiont, *P. indica* has some characteristics which is commonly present in saprophyte such as expansions of cell wall degrading enzymes [Zuccaro et al., 2011]. Thus, these features may be the reason of why the fungus is predominant in most soil.

Previous studies demonstrated that *P. indica* is able to enhance tolerance against many abiotic and biotic stress factors which restrict proper development of plants such as arsenic (As) toxicity in rice [Mohd et al., 2017], salt stress in barley [Becker et al., 2005], cold (freezing) stress in *Arabidopsis* [Jiang et al., 2021], cadmium (Cd) toxicity in tobacco [Su et al., 2021], [Hui et al., 2015], fungal diseases in onion [Roylawar et al., 2021], combined drought and mechanical stress in wheat [Hosseini et al., 2017], zinc deficiency in wheat [Jahandideh Mahjen Abadi et al., 2021], copper (Cu) toxicity in Proso millet [Saman and Sepehri, 2021] etc. The results of the above-mentioned studies obviously indicated that inoculation with *P. indica* ameliorate harmful effects of biotic and abiotic factors.

In addition to enhance plant tolerance against stress factors, *P. indica* also promotes plant growth and development even under stress conditions [Li et al., 2021].

Another important feature of *P. indica* is increasing number and length of adventitious root formation and this feature may be integrated to plant propagation system to improve plant production [Druege et al., 2007]. Moreover, the fungus helps plant to acquire nutrients by solubilizing of P from inorganic sources by lowering the pH [Ngwene et al., 2016]. Finally, Padash et al., [2016] revealed that zinc (Zn) concentration of *P. indica* inoculated lettuce leaf significantly increased with Zn supply. Based on this results, Verma et al., [2021] propose that *P. indica* may be integrated to genetic and agronomic biofortification programmed to tackle with hidden hunger.

To date, several studies have focused on the underlying physiological mechanism of plants that is triggered by *P. indica* inoculation to acquire nutrients, tolerate stress factors, promote growth and development, produce secondary metabolites, and enhance photosynthesis efficiency. Data from previous studies have illustrated that *P. indica* protect plants from stress factors by preventing membrane damages [Jiang et al., 2021], [L. Li et al., 2017]; increasing antioxidant enzyme activity such as catalase (Cat), improving photosynthesis efficiency and jasmonic acid (JA) accumulation [Li et al., 2021]; elevating heavy metal toxicity by cell wall adsorption, vacuolization or immobilization [Mohd et al., 2017]; avoiding translocation of toxic minerals from root to shoot [Becker et al., 2005], [Mohd et al., 2017]; increasing proline and decreasing hydrogen peroxide levels [Nanda and Agrawal, 2018].

1.1.3. Arbuscular Mycorrhizal Fungi

AMF belongs to the phylum *Glomeromycota* [Schüßler et al., 2001]. AMF are obligate biotroph fungus so they cannot survive without host. Obligate biotrophic lifestyle may be the result of deficiency of genes which are vital to proper functioning of certain metabolic pathways such as nitrate and sulfate assimilation pathways. That's why, they are obligate to interact with plant for obtaining required bioavailable form of nitrogen (N) and sulfur (S) [Duplessis et al., 2011], and carbon (C) [Denison and Kiers, 2011]. AMF are predominantly found in most ecosystem and they associate with over 70% vascular plant species. Plants are rewarded from the association by

enlargement of their roots and taking nutrients [Christie et al., 2004]. Plants and AMF have been dating mutualistically for 450 million years [Denison and Kiers, 2011].

Because AMF are obligate biotrophs, they remain in dormancy when they do not associate with plant root. This association starts with germination of chlamydospores in soil. Following germination, primary germ tube development by fungi is promoted via releasing root exudates and strigolactones from plants [Begum et al., 2019]. Further, extensively branched structure, namely arbuscules or coils, forms to provide nutrient exchange in parenchyma cortex of host. Moreover, external fungal hyphae seek environment to take up nutrients [Begum et al., 2019], [Denison and Kiers, 2011]. AMF are symbiotic fungi which do not live without host and they colonize in host tissues such as root cortex [Scheublin et al., 2010]. AMF is particularly important to functional uses of finite sources in soil. For instance, P concentration in soil was more drastically changed when plant inoculated with AMF because AMF enhanced P absorption [Van Der Heijden et al., 1998]. In addition, AMF enhances plant tolerance against heavy metals [Christie et al., 2004].

Additionally, AMF supports plant growth under stressful environmental conditions by enhancing multi mechanism that increases efficiency of photosynthesis and water uptake [Begum et al., 2019]. Data from the study of Bowles et al., [2016] revealed that AMF enhanced crop yield and usage efficiency of water under water limited conditions in tomato grown under field conditions. This findings may be particularly important to offer an alternative solution to growth of crops under drought conditions. Rouphael et al., [2015] reviewed the literature and proposed that AMF are promising biostimulant candidate to boost horticultural production because of the ability of the fungus to participate in solubilization, uptake and transport of nutrients by reforming root architecture.

1.1.4. Interplay of Mycorrhizae and *Piriformospora indica*

Interaction between AMF and *P. indica* have been investigated in previous studies. Researcher hypothesis that promotion of plant growth and development may be greater when plant inoculated with AMF and *P. indica*. To test the hypothesis, L. Yang et al., [2021] inoculated trifoliate orange (*Poncirus trifoliata*) with single AMF, single *P. indica* and dual AMF and *P. indica*. The study showed that single AMF

inoculation is better than single *P. indica* and simultaneous *P. indica* and AMF inoculation at the same time in terms of positive effects on growth and nutrient uptake.

In addition, Moreira et al., [2015] revealed that co-inoculation with mycorrhiza and *P. indica* enhance P and N content under 80 mg/kg P treatment but under higher P treatment than 80 mg/kg control plants exhibited greater P and N content in pine apple plantlets. Furthermore, Khalvandi et al., [2021] investigated whether co-inoculation with *P. indica* and mycorrhiza protect peppermint from the destructive effects of salinity. The result of the study showed that co-inoculation was more promising to protect plant from salinity damages in terms of lowering severity of membrane electrolyte leakage, enhancing chlorophyll a and b contents and enhancing essential oil content when compared to single inoculation. Finally, co-inoculation resulted with greater shoot dry weight when compared to non-inoculation and single inoculation. Generally, previous research findings demonstrate that co-inoculation with AMF and *P. indica* enhanced plant growth in different species.

1.2. Wheat

Wheat was domesticated in Fertile Crescent, about 10,000 years ago and since that wheat has been cultivated in all around the world [Sousa et al., 2021]. Ancient cultivated wheats are diploid (einkorn) and tetraploid (emmer) [Shewry, 2009]. Even though there are numerous wheat varieties, durum wheat (*Triticum durum*) ($2n = 4x = 28$) and bread wheat (*Triticum aestivum* L.) ($2n = 6x = 42$) are seemed to be the most economically important amongst other species nowadays [Sousa et al., 2021]. Paste and bread are made up by using *Triticum durum* and *Triticum aestivum* L., respectively [Walkowiak et al., 2020]. *Triticum aestivum* L. is grown more widely than *Triticum durum* [Shewry and Hey, 2015b].

Wheat is an important carbohydrate, protein and fiber sources for human diet [Hossain et al., 2021], [Shewry and Hey, 2015]. The estimated total wheat production of the World in 2022/2023 was 774.8 million tons. While European Union, China and India are projected as the top three wheat producers, Indonesia, Egypt and Turkey were listed as the top three wheat importers in 2022/2023 [Sowell and Swearingen, 2022].

Current wheat production rate is not sufficient to meet future wheat demand of rising global populations and situation getting worst due to biotic and abiotic stresses

which are aggravated by climate change [Hossain et al., 2021]. In addition, current wheat varieties have been developed by breeding program based on their yield potential so they are particularly susceptible to abiotic and biotic stresses [Sousa et al., 2021]. Furthermore, breeders have introduced yield related traits into wheat breeding programs for maximizing yield potential of wheats to sustain global food demand throughout the history but considering yield as a primary target may cause reduction of nutritional values of modern wheat cultivars when compared to ancient wheat [Arzani and Ashraf, 2017], [Biel et al., 2021], [Fan et al., 2008], [Poudel et al., 2021],[Velu et al., 2014], [Rawat et al., 2013]. So, the nutritional value of wheat cultivars is particularly important for countries whose daily diets mainly included products made from processed wheat. People who live in these countries take nearly 50% of their energy intake by consuming wheat-processed commodities [Peter R. Shewry, 2018], [Cakmak et al., 2010]. *Triticum aestivum* L.

1.2.1. *Triticum aestivum* L.

Bread wheat (*Triticum aestivum* L.) is also known as common wheat is one of the most cultivated wheat species which was artificially produced by hybridizing tetraploid emmer and diploid goat grass. Hybridization dated back to the 8,000 years ago [Brenchley et al., 2012].

Bread wheat is more tolerant against salinity stress when compared to durum wheat because bread wheats is better to limit translocation of Na^+ from roots to shoots and exclude of Na^+ from leaves. In addition, the exclusion of Na^+ is mainly controlled by *Kna1* locus in bread wheat [Byrt et al., 2014]. *Kna1* locus takes place in chromosome 4D and it maintains high K^+/Na^+ ratio [Colmer et al., 2006]. In addition, *Nax1* and *Nax2* are the genes which confer exclusion of Na^+ from xylem in roots. However, *Nax1* is differentiated from *Nax2* by excluding Na^+ not only from the roots but also leaf sheaths [James et al., 2006].

Sustainable crop production depends on growing high yielding cultivars. Yield is determined by genotype (G), environment (E) and interaction between genotype and environment (G x E) [Balakrishnan et al., 2016]. Thus, it is vital to consider the G of wheat and their performance under different environmental conditions to select the most suitable wheat cultivars [Nehe et al., 2019]. For instance, G that can produce

relatively high yield even under salinity stress but cannot tolerate B toxicity may not be a good candidate for growing in a region significantly affected from salinity and B toxicity [R. Reid, 2010].

In literature, durum wheat is pronounced as more stress tolerant than bread wheat and thus under non-suitable environmental conditions for wheat production durum wheat produces more yield while bread wheat is offered to plant in optimal conditions. However, Marti and Slafer [2014] have shown that durum wheat was outweighed bread wheat under suitable environmental conditions.

Wheat is listed among plant species which can tolerate high B concentration in soil. Wheat and barley have broad range for B concentration from 10 to 130 mg B kg⁻¹ dry weight. In addition to differences among plant species, even within wheat species and genotypes tolerance levels of B is vary [Nable et al., 1997], [Torun et al., 2006]. Moreover, germplasm of bread wheat has higher tolerance accession than durum wheat [Nable et al., 1997]. In addition, bread wheat confers higher tolerance to B toxicity than durum wheat and 10 mg B kg⁻¹ in soil causes B toxicity in wheat [Turan et al., 2018].

1.2.2. *Triticum durum*

Durum wheat is commonly cultivated in semiarid climates and it is mainly used to made paste . Durum wheat is especially important for developing countries [Cakmak et al., 2010]. Before the first durum wheat cultivars was introduced into market in 1931, local varieties had been planted. Development of durum wheat cultivars in Turkey were started with establishment of “Seed Improvement Station.” Later, developed durum wheat cultivars were released such as Kunduru 1149 [Xynias et al., 2020]. Five percent of cultivated wheat is durum wheat. Durum wheat is used to make paste and quality of paste is determined by the gluten protein in wheat grains [Arzani and Ashraf, 2017]. Durum is a good source for lutein.[Peter R. Shewry, 2018]. Lutein is the main form of carotenoids and lutein concentration of durum wheat is greater than bread wheat [Peter R. Shewry and Hey, 2015].

1.3. Abiotic Stress

In crop physiology, abiotic stress is defined as any growth- and/or yield-limiting conditions caused by non-optimal parameters in the non-living environment [Tenhaken, 2015a] . Abiotic and biotic stresses affect quality of the crops. Thus, previous studies have been aimed to reveal quantitative trait locus (QTL) which confer tolerance against abiotic and biotic stresses in durum and bread wheat. Soriano et al., [2021] showed that molecular mechanism which provide tolerance to abiotic stresses is more sophisticated than biotic stresses because greater number of genes contribute to abiotic stress traits than biotic stress. Numerous abiotic stress factors such as drought, salinity, heat, cold, deficiency and toxicity of nutrition significantly affect plant production [Tenhaken, 2015], [Zhu, 2016]. Plant have evolved to cope with abiotic stress factors by undergoing physiological, morphological, biochemical and molecular changes [He et al., 2018], [Nadarajah, 2020], [R. Zhou et al., 2017]. Some of the strategies which plants are adopted to alleviate destructive impacts of abiotic stresses are scavenging reactive oxygen species (ROS) and increasing photosynthesis efficiency [Dahro et al., 2016]; reducing oxidative membrane injury [Jiang et al., 2020]; changing permeability of cuticle layer [Bourdenx et al., 2011]; adjustment of stomatal conductance [Marchin et al., 2022]; reshaping hormone homeostasis [Bhanbhro et al., 2020]; adopting differential transpiration strategies [Sinha et al., 2022].

1.3.1. Salinity Stress

Salinity is one of the well-known limiting factors of agricultural production and it poses a threat to 20% of agricultural areas worldwide [Mukhopadhyay et al., 2021]. Detrimental effects of salinity causes significant yield reduction especially in arid and semi-arid regions [Chen et al., 2021] and predominance of saline soil in the World is expected to be raised due to climate change [Roy et al., 2014], [Mukhopadhyay et al., 2021]. Soil salinization is caused by natural phenomenon such as weathering and anthropogenic practices such as usages of irrigation waters which has high salt index and improper application of fertilizers [Maathuis et al., 2014], [Mukhopadhyay et al.,

2021]. Soil is described as saline when the electrical conductivity of soil equal to 4 dSm⁻¹ or more [Maathuis et al., 2014].

1.3.2. Effects of Salinity in Plant

Accumulation of soluble salts in soil cause soil salinity. These soluble salts are particularly formed in the form of certain ionic compound such as sodium (Na⁺), calcium (Ca²⁺), magnesium (Mg²⁺), potassium (K⁺), chloride (Cl⁻), sulphate (SO₄²⁻), bicarbonate (HCO₃⁻), carbonate (CO₃²⁻), and nitrate (NO₃⁻) [Sahab et al., 2021]. Although soil salinity may be caused by accumulation of different salt compound in soil, NaCl is the main reason for the soil salinity [Hongqiao et al., 2021] because NaCl is one of the most soluble salt and it is predominantly found in most saline soil [Munns and Tester, 2008], [Ludwiczak et al., 2021].

Salt is one of the important ingredients of human diet because of the essentiality of Na as an electrolyte [Wilck et al., 2019]. However, it is not an essential mineral for plant metabolism so when the plants are exposed to the high amount of NaCl, their metabolism is impaired by directly osmotic and ionic stress and indirectly secondary stresses such as oxidative stress. Even though NaCl is pronounced chiefly because of its harmful effects on plant in literature, its promotion to plant growth when it is used at low levels is also shown [Hongqiao et al., 2021].

Salinity decreases yield potential of plant and causes yield losses due to osmotic and ionic stress [Aycan et al., 2021]. Osmotic stress is described as reduction of water potential of cell due to different kind of stress factors such as salinity [Rout and Das, 2013]. On the other hand, ionic stress is defined as the disruption of mineral homeostasis in plant [Y. Zhang et al., 2018]. Osmotic stress and ionic stress are two following steps of salinity stress. Shoot growth of plants are reduced due to osmotic stress firstly. Further, early senescence of older leaves is observed as a secondary effects of the salinity stress [Roy et al., 2014], [Mukhopadhyay et al., 2021].

Detrimental effects of salinity stress are originated from Na⁺ instead of Cl⁻. Additionally, level of cytoplasmic Na⁺ is more critical than the concentration of Na⁺ inside the cells [Maathuis et al., 2014]. Thus Na⁺ toxicity is the major reason of yield reduction behind the salinity stress. Toxicity is explained by the concentration of minerals which may lead to 10% reduction in yield of a particular plant species [White

and Brown, 2010]. Na^+ toxicity mainly affects cell wall, K^+/Na^+ ratio, enzyme activities, photosynthesis, ion homeostasis, Ca^{+2} levels [Zhang et al., 2018], [Maathuis et al., 2014], [Zhao et al., 2021].

Salinity tolerance mechanisms of plants rely on ionic tolerance and osmotic tolerance. Ionic tolerance is achieved by two ways. These are exclusion of toxic levels of ions or increasing tolerance levels of tissues against ions by compartmentalization [Roy et al., 2014]. Compartmentalization provides a physiological barrier to prevent damages that is caused by ionic stress in plants. Na^+ levels in cytoplasm is decreased to non-toxic levels by transferring excess Na^+ into vacuole. Compartmentalization is achieved by Na^+/H^+ antiporters. These transporters have critical function to cope with salt stress [Zhao et al., 2021].

In addition to compartmentalization, guttation seems to be another feature of plants which is used to eliminate excess Na^+ from leaves but the concentration of the removing Na^+ from leaves is considerably lower [Maathuis et al., 2014]. Under salinity stress expression of *SOS1* is upregulated [Shi et al., 2000] and *SOS1* enhances exchange capacity between Na^+ and H^+ in this way SOS pathway regulated Na^+ homeostasis [Zhao et al., 2021].

Plants are aiming to tolerate excess levels of Na^+ in their tissues by accumulating compatible solutes and activating certain enzymes which are responsible for the ameliorating harmful effects of the reactive oxygen species (ROS) [Roy et al., 2014]. Another mechanism to tolerate toxic levels of Na^+ in tissues is maintaining high K^+/Na^+ ratio. For this, 30,50-cyclic guanosine monophosphate (cGMP) decrease K^+ uptake and lower K^+ efflux [Maathuis et al., 2014]. Moreover, sustaining high K^+/Na^+ is required to exclusion of Na^+ and the exclusion mechanism is triggered by expression of two genes namely *Nax1* and *Nax2* in wheat leaves [James et al., 2011], [James et al., 2008].

Osmotic tolerance is another important feature of plant against salinity stress. However, the mechanism behind osmotic tolerance is not completely illustrated yet [Munns and Tester, 2008], [Rajendran et al., 2009]. In addition, the gene which confers tolerance to osmotic stress is not known yet [Roy et al., 2014]. However, plants especially halophytes can accumulate osmolyte which are defined as a compound which has low-molecular-weight such as proline, glycine betaine under salinity stress to achieve osmotic tolerance [Yu et al., 2020].

In addition, osmotic tolerance is responsible for the adjustment of conductance of stomata and leaf growth [Rajendran et al., 2009]. Moreover, high stomatal conductance (g_s) provide tolerance to osmotic stress and promote plant growth under saline conditions [James et al., 2008].

1.4. Boron

B takes place in group 13 in periodic Table and B has features of metal and non-metal together [Bolaños et al., 2004], [Brdar-Jokanović, 2020]. Valence electron number of B is three. Besides B does not find abundantly in star and gas cloud (cosmic abundance), it is commonly exist in soil and ocean [Bolaños et al., 2004]. Additionally, B formed relatively higher number of compound when compared to remaining elements [Z. Zhang et al., 2017].

1.4.1. Availability of Boron in Soil

B has a quite narrow range between deficiency and toxicity zone [R. J. Reid et al., 2004]. The toxicity and deficiency of the B is strongly dependent on the levels of the B in the soil. B is predominantly found in soil with different forms namely, boric acid (H_3BO_3), borate anion $[B(OH)_4^-]$ and minerals of borosilicate [Vera et al., 2019], [Pereira et al., 2021], [García-Sánchez et al., 2020]. Almost 96% of B is found as H_3BO_3 [Bolaños et al., 2004]. Even though B concentration of soil is ranged between 2-200 $mg\ kg^{-1}$, the bioavailability of the B is low in soil [García-Sánchez et al., 2020]. Only between 1-3% of total soil B is in the proper form for taken up by plants [Brdar-Jokanović, 2020]. Although soil solution has a broad range B concentration, B concentration which is not neither cause deficiency nor toxicity in plant is between 0.3-1 $mg\ kg^{-1}$. However, generally soil solution has higher average B concentration (10 $mg\ kg^{-1}$) [Tripathi et al., 2015]. H_3BO_3 is the available form of the B for the plants because of its quite soluble nature. However, it may disappear in soil rapidly due to the rainfall and improper irrigation. Therefore, B deficiency and toxicity are observed in mainly rainfall and arid/semi-arid regions, respectively [Yoshinari and Takano, 2017]. Additionally, soil texture and structure affect availability of the B. For instance,

soil which has sandy texture with high Al^{+3} concentration decreases availability of B for plants [Chormova and Fry, 2016].

Prevalence of B toxicity is lower than B deficiency. Turkey, Australia, Russia are listed among the countries which have high B concentration in their soil [Brdar-Jokanović, 2020]. Excess B in soil does not only cause damage to the plants but also lowering N availability for microbes and plants. Therefore, monitoring B concentration in water which is used to irrigate crops is important for eliminating B originated hazards in soil and plant. 1 mg L^{-1} B concentration in irrigation water does not pose any threat to both soil and plant [Vera et al., 2019].

1.4.2. Boron as Essential Micronutrient

Plant needs 17 essential minerals for completing their lifespan [White and Brown, 2010]. While Boron (B) is classified as beneficial for human health because of its contribution to proper development of bones and functioning of brain [Nielsen, 2014], B is listed among 17 essential minerals and categorized as an essential micronutrients for plants [Funakawa and Miwa, 2015]. Micronutrients are needed by plants relatively at lower degree when compared to primary nutrients [Hänsch and Mendel, 2009]. Although B has a unique function for plant development and growth, the importance of the B is relatively newly understood by plant nutritionists. In early 20th century B was recognized as critical for the development of *Vicia faba* L. [Pereira et al., 2021]. Warington [1923] was the first researcher who revealed that B is an essential micronutrient for plants in the history. Despite its high importance for plant survival, providing sufficient B supply to plants is considerably difficult task because in the absence of perfectly optimized B supply, B treatment may easily causes toxicity and it should be not forgotten that management of the B toxicity is more challenging than B deficiency [Takano et al., 2008].

1.4.3. Boron in Plant Cell Wall and Boron Deficiency

Plant cell wall (CW) contain cellulose, hemicellulose, glycoproteins, pectic polysaccharides, phenolics and water [Houston et al., 2016], [Jonsson et al., 2022], [Holland et al., 2020]. Pectin polysaccharides is the most abundant component of the cell wall with over 90% contribution to cell wall structure [Houston et al., 2016]. Cell wall component may be synthesized in different apparatus of cell. For example, while production of cellulose is took place in plasma membrane, pectin and hemicellulose are manufactured in Golgi [Dora et al., 2022]. CW is an alive barrier which has diverse function such as protecting plant against external damages and pathogen attacks, resisting turgor pressure (dP) and shaping the cell, providing flexibility [Tenhaken, 2015], [Jonsson et al., 2022], [Houston et al., 2016], [Holland et al., 2020]. Additionally, plant cell wall's composition may differentiate between tissues and even neighbouring the similar cell [Dora et al., 2022], [Houston et al., 2016].

Pectin is known for its acidic polysaccharide nature. Pectin polysaccharides are abundantly embedded in the primary cell wall, and they are responsible for providing porosity and communication of the cell with the internal and external environment (an intracellular signalling [Houston et al., 2016]. Additionally, major pectic polysaccharides are listed as homogalacturonan (HG), rhamnogalacturonan- I (RG-I) and II (RG-II) [Rui and Dinneny, 2020]. Formation of a link between the pectic monomers is described by using the term “crosslink”. The crosslink between HG and RG-II to form pectic networks are formed by Ca^{2+} and borate, respectively [Funakawa and Miwa, 2015], [Rui and Dinneny, 2020].

Dimerization of RG-II makes possible with the existence of adequate B in soil. The dimerization is important for connection and mechanical force of cells. Additionally, B generally is needed less amount by monocot when compared to dicots because cell wall of monocot has lower pectin composition than dicots [Funakawa and Miwa, 2015]. Finally, insufficient formation of pectic polysaccharides in cell wall due to failure of RG-II dimerization cause irreparable damages to plant due to swelling of cell wall with raising thickness and porosity [Tenhaken, 2015].

B deficiency causes irreversible damages to physiology and metabolism of plant. Plants are affected by B deficiency in both vegetative and reproductive stages [Camacho-Cristóbal et al., 2011]. However, plants during reproductive stages is more

susceptible to B deficiency than vegetative stage [Rerkasem and Jamjod, 2004], [Subedi et al., 1998], [Qin et al., 2021]. Additionally, because the most important function of the B is supporting proper functioning of plant cell wall by establishing a bridge between the pectic polysaccharides chain, under B deficiency numerous properties of plant cell wall is significantly damaged [Camacho-Cristóbal et al., 2018].

B deficiency symptoms may exhibit different patterns among plant species. However, the symptoms of B deficiency are firstly observed in young leaves with interveinal chlorosis and necrosis in plants which has low phloem mobility [Shireen et al., 2018]. On the other hand, plant which has high B phloem mobility thanks to ability to synthesize sugar alcohol exhibit B deficiency symptoms in their old leaves due to retranslocation of B from old leaves to newly emerging young leaves [Wimmer and Eichert, 2013].

B deficiency is widely observed in soil and the deficiency causes detrimental harmful effects to sustainable agricultural production due to yield losses. For instance, water status of plant indirectly effected from B deficiency because root and vascular tissues of plant which grown under B deficient conditions are damaged [Wimmer and Eichert, 2013]. Additionally, B deficiency disrupt numerous plant metabolism by triggering production of reactive oxygen species (ROS), causing secondary metabolism malfunction, changing the nitrogen (N) fixation and assimilation [Camacho-Cristóbal et al., 2011]. Specifically, it is known that B deficiency decreases nitrate content in tobacco [Camacho-Cristóbal et al., 2018]. M. Li et al., [2017] showed that in addition to reduction of stomatal conductance in cotton grown under B deficient condition, it also suffered from significant reduction in the rate of photosynthesis and transpiration as well. Furthermore, plants which are not able to uptake sufficient amount of B not only affected from direct consequences of B deficiency but also become more susceptible to other abiotic stresses [Wimmer and Eichert, 2013].

The plant reproductive system is susceptible to B deficiency because B has a unique function in the development of pollen. Thus, under B deficient conditions two distinct units of plant reproductive structure namely, pollen and anther, are developed improperly and germination of pollen does not occur [Subedi et al., 1998]. Additionally, yield of many cereal crops such as wheat, rice and maize are reduced due to failure of grain set formation. Failure of grain set development is the direct

consequence of B deficiency which inhibit pollen tube elongation [Camacho-Cristóbal et al., 2018]. Inability to develop grain set because of male sterility which is originated from B deficiency is recorded even in wheat although wheat require less amount of B and it causes significant yield reduction [Rerkasem and Jamjod, 2004].

1.4.4. Boron Toxicity

Boron toxicity is considered one of the major limiting factors in agricultural production worldwide due to the presence of the soil which has high B concentration [Robert and Fitzpatrick, 2009]. In contrast to deficiency of B, toxicity of B is more commonly observed than B deficiency in soil. The reason behind the predominant existence of B toxicity in specifically arid and semi-arid region is the disappearance of water from soil and thus B become more concentrated [Riaz et al., 2021]. B toxicity may be occurred different reasons in addition to natural existence in soil such as improper fertilizer practices and usages of high B concentrated water for irrigation. United State (US) and Turkey are the well-known examples of the regions where B toxicity detrimentally affects agricultural production [Brdar-Jokanović, 2020]. To overcome B toxicity in plants in California, plants which are able to accumulate excess B in their tissues were planted to make the soil more cultivable [Nable et al., 1997]. B toxicity exhibit different pattern in terms of its time and severity of its harmful effects because of the distribution of B in the soil. If the soil B is most widely occupied in deeper section of soil, plant roots do not encounter with excess B during the early growing period. Thus, symptoms of toxicity will be identifiable in late growing stages [Brdar-Jokanović, 2020]. The severe effects of B toxicity in plants such as wheat has been manifested in literature. Studies show that there is not a positive correlation between soil B levels and plant B concentration [Brdar-Jokanović, 2020].

B toxicity may be identified by the appearance of necrosis and chlorosis in margin of mature leaves. In contrast to shoot, generally B toxicity in roots is not observed. Additionally, B toxicity affects young shoot due to retranslocation in plants which has high phloem mobility of B [García-Sánchez et al., 2020]. Furthermore, management of B toxicity is not easy task because among species and even genotypes of the same plant species may exhibit different tolerance levels to B toxicity. Further, switch from B deficiency to B toxicity in plants are only caused by existence of even

very small quantity of B [Al-Huqail et al., 2020]. Therefore, planting tolerant cultivars against B toxicity seem to be one of the most suitable management strategies for eliminating possible yield and quality losses due to B toxicity. Tolerance against B toxicity rely on prevention of uptake of toxic levels of B by root in plant [Robert J. Reid and Fitzpatrick, 2009], [Riaz et al., 2021].

Under B toxicity, numerous plant physiological and morphological features are significantly affected. Riaz et al., [2021] reveal that while chlorophyll and protein content in cell wall of rice which grown under B toxicity were significantly reduced, membrane damage was significantly increased due to B toxicity. Additionally, decreases in number of spike, thousand-seed weight and stem height were reported due to B toxicity [Brdar-Jokanović, 2020]. Similar to findings of Riaz et al., [2021], Wu et al., [2019] also shown that B toxicity in trifoliata orange caused significant decrease in protein content while increasing carbohydrate content. Furthermore, Shah et al., [2017] studied how B toxicity affect different physiological and morphological parameters of citrange orange plants. Result demonstrated that B toxicity decreased transpiration rate, stomatal conductance, chlorophyll content, plant growth, carotenoid content and activities of major antioxidant enzymes. Additionally, metabolic function of ATP, NADP and NADPH may be degraded due to B toxicity [Takano et al., 2008]. The plausible explanation of the degradation process is explained by unique chemical nature of B which makes it quite reactive. Thus, B disrupts metabolic function of B ATP, NADP NADPH by interacting with ribose moieties of these molecules [Kabu and Akosman, 2013].

Diverse management strategies to B toxicity are offered in literature. For example, Sonmez et al., [2009] found out that mycorrhiza inoculation in wheat decreased shoot and root B concentration but did not enhance yield. Melatonin treatment also protects wheat from detrimental effects of B toxicity by activating plant defense mechanisms and enhance plant growth [Al-Huqail et al., 2020].

1.4.5. Boron Toxicity Tolerance

Tolerance to B toxicity in plants rely on different mechanism. One of the mechanisms which provide tolerance against B toxicity in plant is avoidance of B uptake from the roots [Reid and Fitzpatrick, 2009]. So, relatively lower concentration of B in roots of tolerant genotypes limit B concentration in shoot due to limiting translocation of B [R. Reid, 2007], [Nable et al., 1997]. BOR family is responsible for the exclusion of toxic B from roots [Yoshinari and Takano, 2017]. Specially, accumulation of BOR4 decrease shoot and root B concentration under B toxicity in *Arabidopsis thaliana* (*A. thaliana*). NIP5;1 is a gene which responsible for B uptake. Under B toxic conditions NIP5;1 is downregulated to constrain B uptake. Additionally, expression levels of BOR homologs in wheat and barley genotypes are higher than non-tolerant genotypes [Takano et al., 2008]. Moreover, efflux seem to be the dominant tolerance mechanism against B toxicity [Princi et al., 2015].

The distribution of B between tissues defines whether plants can tolerate excess B or not. For example, a plant which has B mobility in phloem seem to be susceptible to B toxicity. On the other hand, immobility of B in phloem is considered a tolerance mechanism which aims to avoid B damage to considerably more critical sites such as seed while accumulating B in the margin of old leaves [Nable et al., 1997]. Furthermore, cellular basis of B toxicity is not completely illustrated yet but tolerant genotypes remove toxic levels of B from cytoplasm to cell wall which can tolerate high levels of B [Reid and Fitzpatrick, 2009].

1.4.6. Interaction Between Salinity Stress and Boron Toxicity

Studies that focus on interaction of B and salinity is limited up to date. Usually, researchers have been aimed to estimate the effects of B and salinity independently. Even though certain studies have been conducted to illustrate the combined effects of the mentioned stress factors, there is no consensus about it [Smith et. al., 2010]. Therefore, examination of these two commonly observed stress factors is critical for tackling field scenarios that contain both of stress factors. For instance, Smith et al., 2010 found that while high salinity treatment decreases shoot B concentration when broccoli plants were exposed to high level of B, low B concentration did not reduce

by increasing salinity treatments. On the contrary to finding of Smith et al., 2010, in wheat plants B concentration was become higher when plant grown under both salinity and B toxicity when compared to the alone B toxicity and salinity stress [Wimmer et al., 2003]. A comprehensive review was published by Pandey et al., [2019] and the review clearly shown that interaction of these stresses' factors may significantly retard growth in some studies but in the others combination of these stresses may not significantly reduces growth or other growth-related parameters when compared to alone ones.

In contrast to elevated detrimental effects of the B toxicity together with salinity stress in plantae, severity of salinity stress is reduced or alleviated by B treatment by maintaining plant homeostasis [Rahman et al., 2021]. So, it is critical that to understand the interaction mechanism between B and NaCl which may get worse consequences of salinity stress or make it less severe based on the concentration of B. This is more important for these days where climate change significantly affect crop production and world warming is making situation get worse worst day by day worldwide.

Sustaining global food security, dealing with crop losses due to one of the two abiotic stresses known as salinity and B toxicity, protecting soil health and fertility with more sustainable and environmentally friendly solutions are required. In this thesis, we were aiming to come up with an idea which directly offer a solution to above mentioned threatening problems. Our idea came from the fact that ability of beneficial microorganism to support plants ecosystem and live with them by establishing mutualist relationships. Then, we thought that investigating possibilities of usage of beneficial microorganism to get rid of harmful consequences of B toxicity and salinity stress together and separate.

1.5.7. The Questions Addressed in This Project

The aim of this thesis was to evaluate the usage potential of *P. indica* as a tool to ameliorate the detrimental effects of combined B toxicity and salinity stress on crop yield. Therefore, more than one consecutive experiment was conducted. The question that intended to answer in these experiments are listed below:

- i. How do AMF and *P. indica* inoculation affect the yield and mineral homeostasis of wheat plants grown under low and high P supply in autoclaved soil?
- ii. What impact do AMF and *P. indica* inoculation have on the yield and mineral concentration of wheat plants grown under autoclaved soil?
- iii. What are the consequences associated with salinity stress and B toxicity in wheat?
- iv. How effective is the usage of *P. indica* as an inoculant to ameliorate yield-losing and quality-decreasing effects of salinity stress and B toxicity in wheat plants?

2. MATERIALS AND METHODS

2.1. Materials

2.1.1. Plant Materials

In this thesis, two wheat cultivars namely *Triticum durum* cv. Saricanak-98 and *Triticum aestivum* cv. Nusrat were used as plant materials. Based on experimental design, number of seed that sowed pots, number of pots, the duration of experiment, starting and ending time of experiment and treatments were changed. All experiments were conducted in greenhouse and pots were irrigated with distilled water (dH₂O).

2.1.2. Greenhouse

Wheat plants were grown in Venlo type greenhouse under natural daylight. Greenhouse is located at Gebze Technical University and the latitude and longitude of greenhouse are 40°48'23.5" N, 29°21'46.2"E respectively. The greenhouse has capability to kept temperatures between 12-30 °C by heating and evaporative cooling system. Plants were grown under natural daylight and temperature of greenhouse was maintained according to seasons. For spring and summer seasons temperature was kept between 20-23/15-18 °C for day/night. The day/night temperature in fall and winter was arranged between 23-26/18-21 °C.

2.2. Methods

2.2.1. *Piriformospora indica* Culture and Inoculation Method

P. indica was kindly provided by Prof. Dr. Ralf Oelmüller, University of Jena, Germany. *P. indica* culture was prepared in petri dishes by following the protocols of Hill and Kafer [2001] on Hill & Käfer medium (KM). Grown *P. indica* spores were

transferred into Erlenmeyer flask which had 200 ml liquid medium and located into growth chamber and incubated at 28 °C, 200 rpm.

The duration of incubation and concentration were differentiated from one experiment to another, detail of inoculation methods and number of spores etc., are explained in respective experiment in below. In all experiments, Erlenmeyer flasks contained liquid *P. indica* culture were centrifugated and chlamydospore/mycelium suspension was collected in the day when experiments were conducted. After removing the supernatant, pellets that contain mycelium and chlamydospores were collected and weighted.

2.2.2. Co-cultivation of *Arabidopsis thaliana* with *Piriformospora indica* and Re-isolation

In literature, to sustain fresh and required amount of inoculant for on-going experiment sub-culture of *P. indica* was performed several times. However, multiple sub-cultures of the fungus may decrease its colonization and proliferation ability in host. Therefore, roots of *Arabidopsis thaliana* Columbia (*A. thaliana*) were inoculated with *P. indica* and two-weeks later from inoculation, *P. indica* spores were isolated from the roots. *A. thaliana* seeds were sterilized and then sowed in Murashige and Skoog (MS) medium for 14 days. Nylon membrane was used in agar plate to increase the percentage of germination. At the same time *P. indica* spores were proliferated in KM. 14 days later from the starting date of inoculation of *P. indica* and sowing of *A. thaliana* seeds on culture media, co-cultivation of *P. indica* and *A. thaliana* was performed. Mycelial discs were taken from the petri dishes used for *P. indica* proliferation and depositing on roots of *A. thaliana*. After inoculation of *A. thaliana* roots with *P. indica*, plates were transferred to growth chamber. Duration of co-inoculation was changed based on the growth rate of everyone's plants in plates. Co-cultivated *A. thaliana* were taken from petri dishes and transferred to autoclaved magenta box to sustain further growth. When the hairs of roots of *A. thaliana* reached the highest density and cover surface of the media, inoculated roots were cut and sterilized. KM was inoculated with sterilized roots. Mycelium and chlamydospore of *P. indica* were proliferated in KM. Obtained newly fresh culture was used for sub-culturing. Co-cultivation and re-isolation processes were described in Figure 2.1

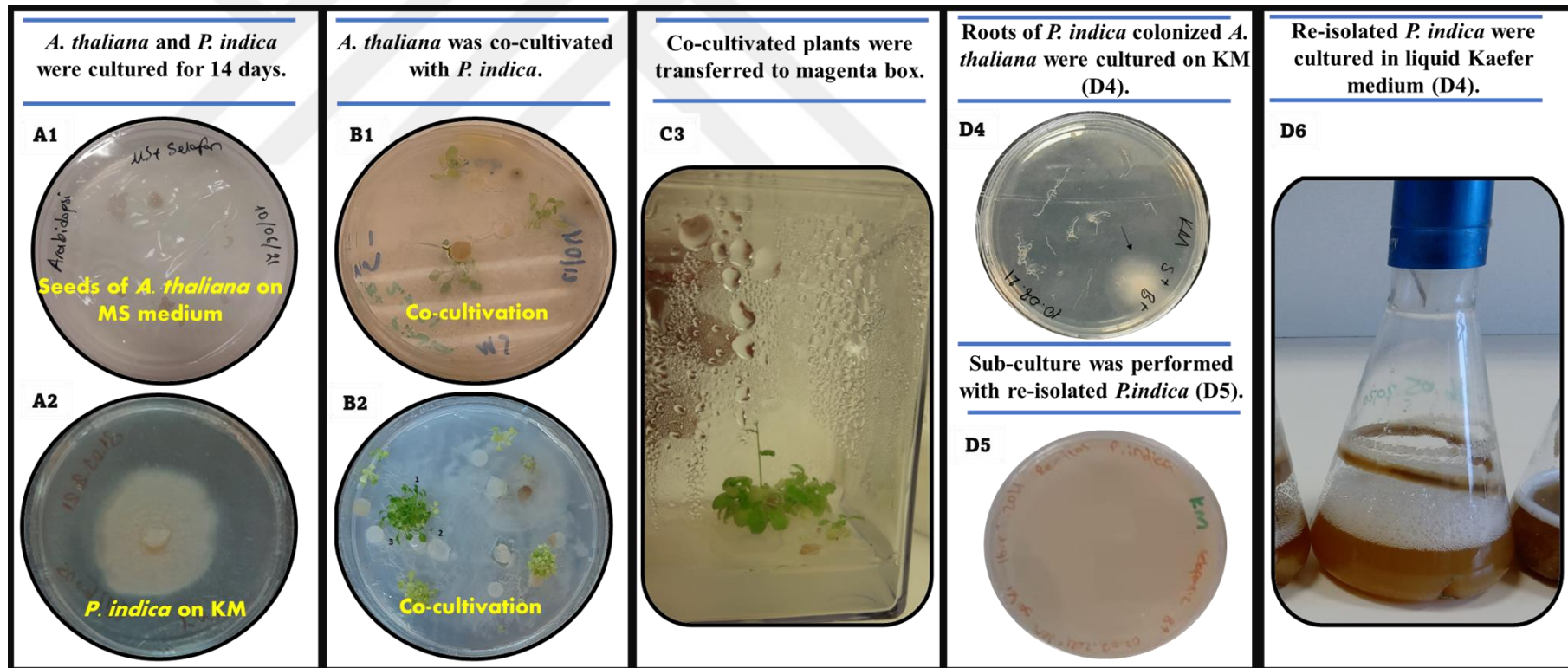


Figure 2. 1: Co-cultivation of *A. thaliana* with *P. indica* and re-isolation of *P. indica* from roots of *A. thaliana* were performed.

2.2.3. Preparation of Mycorrhizal Inoculum

In this thesis, a commercial product was manufactured by Shubhodaya brand was used as a mycorrhizal biofertilizer which contain two different species namely, *Glomus intradices* and *Glomus proliferum*. According to manual of commercial “Shubhodaya mikoriza” product, the weight of the product is 50 g and it contained 1×10^5 mycorrhizal propagules.

In mycorrhiza treated pots, previously dissolved 0.5 g mycorrhizal biofertilizer in 100 ml dH₂O was applied roots of wheat plant. Mock treated pots were treated with the same way with treatment groups but given mycorrhiza inoculant was autoclaved before treatment at 121 °C for 30 minutes (min). In this way, mock treatment was achieved to avoid any kind background effects which may be caused by nutritional features of mycorrhizae. Detail of inoculation methods were explained for each experiment in below.

2.2.4. Soil Preparation and Transplantation

Experimental soil was prepared by sifting before basal fertilizer applied to remove large materials such as rock. Autoclaved and non-autoclaved soil was used based on the experimental design. Soil sterilization was achieved by autoclaving the soil at 121 °C for 30 minutes to minimize microbial activity in soil. In all experiments, N, P, B and CI were given in the form of Ca (NO₃)₂4H₂O, KH₂PO₄, H₃BO₃ and NaCl at different concentrations according to experimental design, respectively. Basal fertilizer treatment increased soil salinity 3 dS/m.

All fertilizer were uniformly mixed with soil before transplantation. Wheat seedlings were grown for 2 or 3 days in a growth chamber under long day conditions (16 and 8 h of day and night period, respectively). Temperature and relative humidity for day and night period were 25/20 °C and 60/70 %, respectively. Number of seedlings which were transplanted to pots were determined based on the experimental design. The information about the pots number, plant number in each pot, starting and ending date of experiments were explained for every experiment in “Experimental Design” section.

Table 2. 1: Physiochemical characteristics of soil used in this thesis.

Soil Attributes		
Characteristic	Unit	Values
Soil Texture	-	Loam
Salt	%	0.02
Lime	%	3.19
Organic Matter	%	0.96
pH	-	7.67
P (P ₂ O ₅)	Kg/daa	5.57
K (K ₂ O)	Kg/daa	25.39
Ca	%	0.44
Mg	%	0.01
Fe	mg/kg	8.55
Zn	mg/kg	1.26
Cu	mg/kg	6.83
Mn	mg/kg	21.17
Na	mg/kg	59.00

In all experiments, 2.5 kg experimental soil were mixed with 125 g perlite. Perlite and soil mixed uniformly to enhance permeability of soil, preventing formation of soil compaction, and maintaining proper development of roots and increasing the chance of microbial colonization. The characteristics of soil which was used as a substrate in this thesis shown in Table 2.1.

2.2.5. Calculation

Mineral concentrations were calculated by multiplying the result of ICP-OES measurements with the dilution factor. Dilution factors were determined by division of total sample volume (ml) to dry weight of digested sample and mineral contents of plants were described by multiplying mineral concentrations with the total respective plant weight.

2.2.6. Statistical Analysis

In this thesis statistical analysis was performed by JMP software. 95% was defined as a confidence interval ($p \leq 0.05$). Obtained data were subjected to analysis of variance (ANOVA) to reveal effects of the factors whether significant or not at 95% confidence interval. Two-way ANOVA in the first and second experiment, three-way ANOVA in the third, fourth and fifth experiments were performed. The means of the treatments were used to assess significant differences among treatments by Tukey's honestly significant difference (HSD) tests if ANOVA result was significant.

2.3. Experimental Designs

In all experiments were conducted in this thesis was completed by using the same soil. Full factorial experimental design was employed to investigate different combinations of the treatments. Number of pot replicate and number of plants in each pot were changed based on the experimental design. According to experimental design, soil was autoclaved or not.

2.3.1. First Experiment

In this experiment, two different P levels were used. 20 mg/kg P (low P) and 100 mg/kg P (adequate P) were P levels. Before transplantation, N, P, B and CI were given to pots. $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ used as N and Ca sources. KH_2PO_4 was used as P sources. Pots were exposed to 1 and 10 ppm B and CI respectively. In this experiment, soil was autoclaved at 121 °C for 30 minutes.

There were four different microbial treatment groups as mock treated pots *P. indica* treated pots, AMF treated pots and *P. indica* (+) AMF treated pots for low and adequate P supply in this experiment. For this experiment 71 days-old re-isolated *P. indica* culture was used. 353 g *P. indica* pellet formed by mycelium and chlamydospores was divided into two group and one of the groups were autoclaved at 121 °C for 30 minutes, two times.

Approximately 176 g non-autoclaved and autoclaved pellet were dissolved in 2.5 L dH₂O. Autoclaved *P. indica* was applied to mock and AMF treated pots. Non-autoclaved *P. indica* was used to inoculate plantlets in *P. indica* treatment and *P. indica* (+) AMF treatment groups (Table 2.2).

Table 2. 2: Inoculum application method that was followed in the first and second experiments.

Inoculation Procedure	
Name of Inoculation	Applied Inoculum
Mock	Autoclaved <i>P. indica</i> (+) Autoclaved AMF
<i>P. indica</i>	<i>P. indica</i> (+) Autoclaved AMF
AMF	AMF (+) Autoclaved <i>P. indica</i>
<i>P. indica</i> (+) AMF	<i>P. indica</i> (+) AMF

Shubhodaya brand *Glomus intradices* and *Glomus proliferum* containing 25 gr powder was divided into two. 12.5 g powder was autoclaved in 100 ml dH₂O to applied for mock and *P. indica* treatment pots. Both 12.5 g non-autoclaved and autoclaved powder were dissolved in 2.5 L dH₂O, separately. Each pot in the experiments had 8 *Triticum durum* cv. Saricanak-98- plantlets. The plantlets were 2-days-old during plantation. 5 pots were taking place in each treatment group as independent replicate. Each pot was treated with total 100 ml respective liquid microbial inoculant. 0.25 g mycorrhizal propagules were applied 1 kg experimental soil based on following protocol. Therefore, 500 mycorrhiza propagules/kg dry soil were applied. Moreover, 12.17×10^5 chlamydospores/ml was given respective pots.

Experiment was started in growth chamber by seed sowing in 04.08.2021. Two days after sowing (DAS), plantlets were transferred to greenhouse for transplantation. 50-days-old wheat plants were harvested in the twenty-second of September 2021. After harvest of above part of wheat, roots sample were taken for microscopic observation.

2.3.2. Second Experiment

P. indica and AMF were used as an inoculant in this experiment. Mineral fertilizers were given with the doses 250 mg/kg N and 100 mg/kg P, 1 mg/kg B and 10 mg/kg CI in all pots before transplantation. 50 DAS, second N fertilizer were applied in the form of $\text{Ca}(\text{NO}_3)_2$ with level of 150 mg/kg N. In this experiment soil were autoclaved at 121 °C for 30 minutes. There were just two factors as cultivar and inoculation in this experiment. Cultivars were *Triticum durum* cv. Saricanak-98 and *Triticum aestivum* cv. Nusrat. Every treatment had four independent pots with 5 plants in each pot. The experiment was started in growth chamber by seed sowing in 22nd of February 2022 and ended 120 DAS in 22nd of June 2022. 3 DAS, plantlets were transferred to greenhouse for plantation. Total number of pots in the experiment was 96. Mock, *P. indica*, AMF and *P. indica* (+) AMF were the treatment groups for inoculation and the inoculation methods was the same with the first experiment (Table 2.2).

Pellet of *P. indica* which contain mycelium and chlamydospores was collected from 26 Erlenmeyer flasks by centrifugate at 7500 rpm for 15 min. Yield was approximately 456 g. Obtained pellet was divided into two and approximately 228 g was autoclaved at 121 °C for 30 min in 100 ml dH₂O. In this experiment 48 pots were inoculated with non-autoclaved *P. indica*. 228 g non-autoclaved pellet was suspended in 5 L dH₂O. 100 ml inoculum was applied to each of 48 pots as inoculum (*P. indica* treated pots and *P. indica* (+) AMF treated pots).

In this experiment, 0.69×10^6 chlamydospores/ml was given each of respective pots. For preparing AMF inoculum, 60 g mycorrhizal powder was taken from commercial Shubhodaya product. 30 g of this powder was dissolved in 100 ml dH₂O and autoclaved at 121 °C for 30 min. Autoclaved and non-autoclaved 30 gr of AMF inoculant was dissolved in 6 L dH₂O, separately. Therefore, each pot was treated with total 100 ml microbial suspension. 0.25 g mycorrhizal propagules were applied 1 kg experimental soil based on following protocol. Therefore, 500 mycorrhizal propagules/kg dry soil were applied.

2.3.3. Third Experiment

The third experiment was conducted to illustrate interactive effects of salinity stress and B toxicity on *Triticum durum* cv. Saricanak-98 and *Triticum aestivum* cv. Nusrat. Applied basal fertilizer was the same with the ones in the second experiment. In this experiment, three levels of salinity as control salinity (no salt treatment), moderate salinity (3 dS/m) and high salinity (6 dS/m) were applied to pots according to experimental design. To increase soil electrical conductivity of saturation, paste extract (EC_e) for 1 dS/m, 300 mg NaCl applied to 1 kg experimental soil. Amount of NaCl must be applied to increase soil EC_e as 1 dS/m was determined by 1:5 soil water extract according to Sonmez et al., [2008].

Toxic or adequate B were the B levels. For causing B toxicity and supplying adequate B on plant, 20 mg/kg B and 1 mg/kg B were applied to pots, respectively according to experimental plan. H_3BO_3 was applied to pots to provide adequate B and toxic B levels based on experimental design. 48 pots were used in this experiment and 12 seed were sowed and 7 of them removed 12 DAS. 4 independent replicates were represented each treatment group. The experiment was started and ended in 5.4.2021 and 7.5.2021, respectively. 32-days-old wheat plants were harvested to estimate effects of the treatments on shoot dry weight and mineral concentrations in shoot. Plants were exposed to the stresses from 2 DAS to ending date of experiment.

2.3.4. Fourth Experiments

Fourth experiment was conducted to investigate physiological and morphological effects of the *P. indica* inoculation. *Triticum durum* cv. Saricanak-98 and *Triticum aestivum* cv. Nusrat cultivars were exposed to salinity stress and B toxicity. The doses of salt and B treatment were the with the third experiment. In contrast to the third experiment, in this experiment salinity stress was applied started from 2 dS/m (moderate salinity) and 4 dS/m (high salinity). 15 DAS, salt addition to respective pots were executed to increase the salt levels from 2 dS/m and 4 dS/m to 3 dS/m and 6 dS/m for moderate and high salinity, respectively.

The same procedure that followed for basal fertilizer treatment in the second and third experiments was also followed in the fourth experiment. Seeds of both cultivars were sowed on 24 April 2021 into perlite and located in growth chamber. Two days later, plantlets were transferred to greenhouse for transplantation. 32 DAS, on 26 May 2021 plants were harvested for spectrophotometric analysis, determination of mineral concentration and shoot dry weight etc. 0.5 g fresh leaves sample were taken and immediately submerged into liquid N to freeze sample rapidly for ensuring that sample did not degrade. Further, samples were stored in refrigerator (-80 °C) until analysis. The experiment had 96 independent pots and there were 4 plants in each pot. To be ensured that obtained results were valid, each treatment had 4 independent pots as replicate. To apply *P. indica* as microbial inoculant, 22 Erlenmeyer flask with 200 ml volume that contained *P. indica* culture was centrifugated at 7500 rpm for 10 min. The culture was started on 9 April 2021 and centrifugated on the day when plantlets were transferred to greenhouse for microbial inoculant applications. Approximately 756 gr pellet that contain mycelium and chlamydospores of *P. indica* was obtained. The pellet was mixed with 6 L dH₂O, and 100 ml pellet-water suspension was applied to *P. indica* (+) pots. The number of *P. indica* (+) pots were 48. Remaining 48 pots were exposed to 100 ml dH₂O. Applied concentration of *P. indica* spores per ml was identified as 1.39×10^6 .

2.3.5. Fifth Experiments

Mineral fertilizer and stress treatments of the fifth experiment were the same with the fourth experiment. Salinity levels of moderate and high salinity were upgraded to 3 dS/m and 6 dS/m from 2 dS/m and 4 dS/m, respectively 25 DAS. 41 DAS, 150 mg/kg N was given as a second N fertilization in the form of Ca (NO₃)₂·4H₂O. Two different B levels as adequate (1 mg/kg) and toxic (20 mg/kg) were initiated. The fifth experiment was initiated and ended on 14 May 2021 and 98 DAS on 20 August 2021, respectively. Plants were harvested when they become completely mature. Straw and spike of the plants were counted and harvested separately. Total number of pots in this experiment was 96. Each pots contain 5 plants, and 4 independent pot replicate was established for each treatment to increase the accuracy of the obtained results.

P. indica inoculant was collected from 26 Erlenmeyer flask by centrifugation at 7500 rpm for 10 min. at room temperature on the starting day of experiment. Until the day that pellet was collected, *P. indica* culture was growth in liquid media by shaking in incubator for 19 days. Approximately 977 g pellet was obtained from 26 Erlenmeyer flask. The collected pellet was dissolved in 5 L dH₂O, and 100 ml suspension was taken and applied to 48 *P. indica* (+) pots. The amount of *P. indica* spores in applied 1 ml suspension was determined as 1.64×10^6 by spore counting. *P. indica* (-) pots was treated the same with *P. indica* (+) pots by 100 ml *P. indica*-free dH₂O. Straw and spike of wheats were dried at 50 °C for 1 days before further analysis.

2.4. Analysis

To investigate effects of the treatment's mineral composition, physiological, morphological and biomass changes were analysis. The analysis was performed by using spectrophotometer, ICP-OES, microscopes etc.

2.4.1. Antioxidative Enzyme Activity and Total Protein Assays

Fresh plant sample were stored in -80 °C was used for these assays. Fully expanded first youngest leaves of two wheat plantlets were harvested and mixed thoroughly. Later, 0.5 g fresh leaves samples from the mixture were taken, weighted, and put in liquid nitrogen until stored in -80 °C for long preservation.

0.5 g fresh leaves sample were extracted by using 50 mM KP buffer which contain 0.1 mM Titriplex III (prepared in K-P buffer). K-P buffer was prepared by addition of KH₂PO₄ to K₂HPO₄ for adjustment of pH to 7.6. 5 ml buffer solution was added plant sample to start extraction process. After homogenization, obtained solution was centrifuged at 4600 rpm for 15 min at 4°C. Supernatant of the centrifuged solution were transferred into microcentrifuge tubes for second centrifuge at 15000 rpm for 15 min at 4°C. Obtained solution was divided into six aliquot and stored in -80 °C for using enzymes activity and total protein assays.

2.4.2. Catalase Assay

Catalase assay was performed by using spectrophotometer. The assay was designed to follow the reduction of H_2O_2 due to the activity of catalase. To measure the catalase activity 0.8 ml 50 mM K-P buffer, 0.1 ml 0.1 mM H_2O_2 and 0.1 ml sample (1:8 diluted with K-P buffer) were used to detect the activity of catalase at 240 nm during 2 min.

2.4.3. Superoxide Dismutase Assay

Superoxide dismutase assay rely on dismutation of superoxide (O_2^-) to H_2O_2 and O_2 . The dismutation causes reduction of absorbance at 560 nm. Reduction was recorded for 2 min after mixture was kept under light during 8 min. Assay mixture was prepared by addition of 0.5 ml from L-methionine, Na_2CO_3 , NBT, Riboflavin with molarity 13 mM, 50 mM, 63 μM and 1.3 μM , respectively. 0.1 ml sample was used for the assay. Reaction was started with the addition of Riboflavin.

2.4.4. Glutathione Reductase Assay

Glutathione reductase assay was performed by the following of reduction of NADPH which is the cofactor of GR that convert oxidized glutathione (GSSG) to reduced glutathione (GSH) at 340 nm by using spectrophotometer during the 2 mi. Total 1 ml assay mixture volume was formed by addition 0.1 ml 0.45 mM from NADPH- Na_4 , and 0.1 ml sample. In addition, 0.1 ml 0.5 mM GSSG and 0.7 ml 50 mM K-P were added to assay mix.

2.4.5. Ascorbate Peroxidase Assay

Activities of ascorbate peroxidase was determined by addition of 0.1 ml 2.55 mM substrate of ascorbate peroxidase namely, L-ascorbic acid and 0.1 ml 12 mM H_2O_2 which contain 10 mM Titriplex III. 0.7 ml 50 mM K-P buffer and 0.1 m sample (1:4 diluted with K-P buffer) were added to reaction mixture.

In this assay, reduction of L-ascorbic acid due to utilization by ascorbate peroxidase for breaking down H_2O_2 into H_2O and O_2 were examined at 290 nm during 2 min.

2.4.6. Peroxidase Assay

Peroxidase assay was carried out based on the increasement of abs at 470 nm because of oxidation of Guaiacol due to reaction with O_2 which was produced by degradation of H_2O_2 into O_2 during 2 min. Reaction mix contain 0.75 ml 10 mM K-P buffer, 0.1 ml 0.25% Guaiacol, 0.1 ml 10 mM H_2O_2 and 0.05 ml sample (1:8 diluted with K-P buffer).

2.4.7. Estimation of Malondialdehyde

For estimating Malondialdehyde level in plants, 0.5 g wheat leaves which were obtained by harvesting fully expanded youngest fourth leaves of two wheat leaves were used. 0.5 g fresh leaves samples were homogenised by homogenizers with a frequency of at 30 Hz for 30 second. 80% v/v ethanol was prepared with deionized water. 12.5 ml ethanol was used for extraction. The extract was centrifuged at 3000 g for 10 min at 4°C. 2 ml supernatants were stored in -80 °C.

For spectrophotometrically measurement of MDA leaves in wheat leaves, three different solution was used namely BHT, (-) TBA and (+) TBA. 10 mM BHT solution was prepared by dissolving 0.02 g BHT in 10 ml absolute ethanol. Both of the (-) TBA and (+) TBA solutions were contain 20 g TCA but in addition to TCA, 0.65 g TBA was also dissolved in deionized water and final volume was adjusted to 95 ml by addition of deionized water.

0.95 ml from (-) TBA and (+) TBA solutions were taken and put into two separate microcentrifuge tubes. 0.05 μl BHT and 1 ml sample were added to microcentrifuge tubes. Microcentrifuge tubes were kept in water bath which has adjusted temperature to 95 °C for 25 min. After, microcentrifuges tubes were centrifuged at 3000 rpm for 10 min at 4 °C. MDA levels were determined by using supernatant at 440, 532 and 600 nm.

2.4.8. Determination of Glycine Betaine

Glycine betaine concentration of plant samples were determined by using 0.5 g dried and grounded. Approximately 0.5 g plant sample were extracted with 20 ml deionized water in 50 ml falcons by shaking at 300 rpm for 24 hours at room temperature. Falcons were centrifugated at 7500 rpm for 20 min. 1 ml supernatant which were taken from centrifugated falcons mixed with 1 ml 2 N H_2SO_4 . 0.5 ml was taken from the 2 ml sample- H_2SO_4 and cooled it ice water for 1 hours. After 1 hours, 0.2 ml KI-I_2 which were prepared by dissolving 15.7 g of iodine (I_2) and 20.0 g of Potassium iodide (KI) in 100 ml deionized water was added to 0.5 ml cooled sample- H_2SO_4 mixture. It was stored at 4 °C for 16 hours.

After 16 hours, stored mixture was centrifuged at 7500 rpm for 20 minutes at 0 °C. Pellets were dissolved by the help of 9 ml 1,2-dichloroethane and 1 ml were taken from the solution to measure glycine betaine concentration at 360 nm. For preparing standards, betaine was dissolved in deionized water and 1 ml of standards were mixed with 1 ml 1 N H_2SO_4 . 2 ml standard- H_2SO_4 mix treated with the same way sample were treated after 1 ml 2 N H_2SO_4 addition. 1 N H_2SO_4 was used as blank.

2.4.9. Estimation of Proline

Proline concentration of wheat leaves were measured by using 10 % sulfosalicylic acid as a buffer to obtain plant extract. ninhydrin, glacial acetic acid, orthophosphoric acid, toluene, proline, 3% (w/v) sulfosalicylic acid, Plant extract (sample). For preparation of samples for spectrophotometric measurement, 2 ml of ninhydrin, glacial acetic acid and sample or standard added centrifuge tubes. Mixture was reacted for 1 hour at 100 °C. After 1 hour, 4ml toluene was added to the mixture and chloroform phase was used to estimate the proline concentration at 520 nm. Proline was used as standards.

2.4.10. Phenylalanine Ammonia Lyase Activity

Determination of phenylalanine ammonia lyase (PAL) activity was performed by using leaves extract which was extracted by acetone and borate buffer respectively. After extraction, borate buffer contained 2 mM EDTA, 1mM DDT, 1% PVP. For standards trans cinnamic acid was used. Sample was measured at 290 nm spectrophotometrically. Total and specific enzyme activities were calculated using Bradford proteins.

2.4.11. Relative Electron Leakage Assay

Fully expanded second youngest leaves of single plants were used for the assay. Approximately 9 ml of leaves from tip margin was measured and cut. Then, it was immersed into 50 ml deionized and wait at room temperature for 6 hours. After 6 hours, first measurement was taken by electrical conductivity (EC) meter. The second measurement was performed when the temperature of 50 ml falcon which contain cut leaves decrease room temperature after keeping plant sample at 95 °C for 1 hours by using water bath.

2.4.12. Root Staining and Microscopic Observation

3 cm of root tips of inoculated and non-inoculated plant were cleaned and then cut and put into 80% v/v ethanol solution after sampling and stored in - 4 °C for until root staining. Root tip sample were waited in 10% HCl solution for 15 minutes, after 15 min root tips sample were washed and put into 1 M HCl solution. After this step, root sample were washed again and stained with 0.05% trypan blue for overnight. To prepare for observation under microscope, root tips were exposed to 50% lactic acid solution for 2 hours prior to observation. Light microscope which equipped by KameraM Microstructure Imaging Software was used.

2.4.13. Polymerase Chain Reaction

In the fourth experiment, root samples from 24 treatment groups were taken for DNA extraction. DNA of wheat cultivars were isolated from the roots by following the protocol designed for “DNeasy Plant Mini Kit” in DNeasy Plant Handbook. 0.2 g root sample were used for extraction. Yield and purity of extracted genomic DNA determined by spectrophotometer, buffer AE was used as a blank. Average concentrations of extracted DNA were between 5 and 10 ng/μl.

To validate root colonization with *P. indica*, roots of wheat were scanned with the primers designed to amplified target region of *EF-1-alpha (tef)* genes (Genbank accession no: AJ249912.1). The product of the amplification was identified by the produced band at 200 bp in agarose gel electrophoresis. PCR conditions and ingredients of PCR reaction were given in Table 2.3.

Table 2. 3: Amplification conditions and components of of specific segment of *EF-1-alpha (tef)* gene by PCR.

PCR Conditions			
PCR Steps	Temperature (°C)	Duration (m:s)	Cycles
Initial Denaturation	94	05:00	1
Deneraturation	94	00:40	35
Annealing	59	00:40	
Extension	72	00:30	
Final Extension	72	10:00	1
	4	30:00	∞
PCR Reaction Set-Up			
Ingredients		Concentration	Volume in Reaction
DNA		5 ng/ μ l	5 μ l
MyTaq DNA Polymerase		5 U/ μ l	1 μ l
5x MyTaq Reaction Buffer	dNTPs	5 mM	10 μ l
	MgCl ₂	15 mM	
Primers	Forward Primer	20 μ M/ μ l	0.5 μ l
	Reverse Primer	20 μ M/ μ l	0.5 μ l
ddH ₂ O		-	33 μ l
Species Specific Primer Pair			
Target Gene	Primer Name	Primer Sequence 5' to 3'	PCR Product Size
<i>EF-1-alpha (tef)</i>	Pitef_F	TCGTCGCTGTCAACAAGATG	220 bp
	Pitef_F	GAGGGCTCGAGCATGTTGT	

2.4.14. Dry Weight, Tiller Number and Yield Parameters

Dry weight was determined by harvesting and drying 4 and 8 plants of first and fourth experiments by using laboratory oven at 60 °C for 2 days, respectively. When the temperature of dried plants reached room temperature after 2 days drying process, they were weighted by using analytical balance. Tiller numbers were determined by counting stems which has three or more than three leaves. Later, tiller number per plant were defined by dividing total tiller number to plant number.

Straw weight and spike weight were calculated by considering sum of 5 plants in the second and fifth experiments. Harvest index was calculated by dividing grain yield to sum of the grain yield and straw weight.

2.4.15. Element Analysis

Shoot and straw samples were dried and powdered but grain was used as intact for element analysis. Approximately 0.2 dried and powdered plant sample were used for the shoot of first, second, third experiment and 0.2 g dried and powdered straw in the second and third experiment was also used for element analysis. Element concentration of grain was detected by using 0.4 g grain.

For preparation of plant sample to element analysis, after weighting required amount of sample, 2 ml 30% H₂O₂ and 5 ml 65% HNO₃ were added into vessels which contain plant sample. Digestion was performed in a closed-vessel microwave system (MarsExpress; CEM Corp., Matthews, NC, USA). After digestion, final volume of solution was adjusted to 20 ml with double deionized water. Solution was filtered after completing to final volume by analytical filter paper (Macherey-Nagel, Ø125 mm, blue band). Mineral concentrations were estimated by inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista-Pro Axial, Varian, Australia).

3. RESULTS

3.1. Results of First Experiment

First experiment was conducted to estimate interaction between P treatments and inoculation of wheat with different microbial inoculant on shoot biomass and shoot mineral concentration. Roots of durum wheats (*Triticum durum* cv. Sarıçanak) were stained by using trypan blue to estimate colonization of the *P. indica* and AMF. As show in Figure 3.1, vesicles and chlamydospores were shown with arrow. Root staining was performed to verify colonization of *P. indica* and AMF in roots of durum wheat.



Figure 3.1: Observation of root colonization of *P. indica* and arbuscular mycorrhizal fungi.

Figure 3.2 shows the combined effects of two different fungi on the shoot dry weight of durum wheat under adequate and low P supply. Based on the Figure, adequate P supply with combination of the two inoculants provide significantly different result in terms of increment of the shoot dry weight when compared to controls. Furthermore, significant increase in shoot dry weight of Sarıçanak-98 cultivar was observed when plant was inoculated and supplied adequate P when compared to low P supply with inoculation. In addition, adequate P supply causes significantly higher shoot dry weight when compared to low ones.

Furthermore, the differences between shoot dry weight of Saricanak-98 were grown with AMF and mock treatment under adequate P supply is higher than differences between mock and AMF inoculation under low P supply. This indicated that effects of AMF treatment supplied with adequate P caused more shoot biomass enhancement.

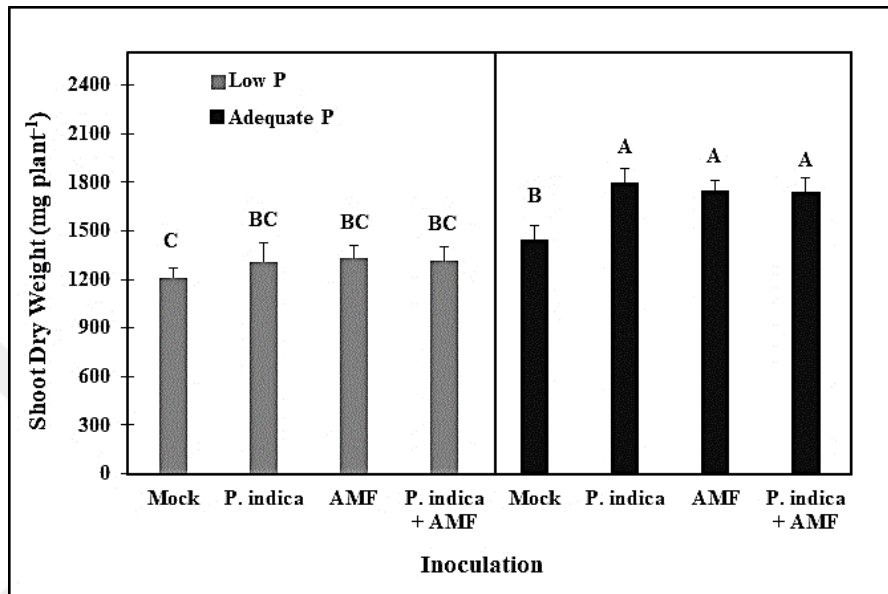


Figure 3. 2: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation with low (20 mg P/kg soil) or adequate (100 mg P/kg of soil) P treatments on shoot biomass of 50-day-olds durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Different letters indicate significant differences according to the Tukey's protected HSD test at 95% confidence intervals. Bars represent means of five independent pot replicates each containing eight individual plants. Error bars represent standard deviation.

Table 3.1 shows that *P. indica* inoculation significantly increased B concentration when compared to mycorrhiza treatment while P supply did not cause any significant differentiation. Interaction between inoculation and P supply revealed highest shoot B concentration was recorded when wheat plant grown with *P. indica* inoculation and treated with adequate P. Moreover, shoot Cu and Fe concentration were only significant altered due to P supply. Adequate P supply significantly increased Cu and Fe concentrations. Furthermore, shoot Zn concentration affected by all the factors, significantly.

The highest Zn concentration was recorded in plant that inoculated with *P. indica* and supplied with low P. Zn concentration of shoot was significantly reduced due to high P supply when compared to low ones. This may be originated from antagonistic relationship between the P and Zn due to formation of insoluble Zn-phosphate complexes.

Table 3.1: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation with low (20 mg P/kg soil) or adequate (100 mg P/kg of soil) P treatments on shoot concentration of selected micronutrients of 50-day-olds durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of five independent replicates each containing eight individual plants.

P	Inoculation	B (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Low	Mock	18 ± 2	10.3 ± 0.4	50 ± 3	150 ± 18	29 ± 3
	<i>P. indica</i>	21 ± 4	9.6 ± 0.5	49 ± 2	197 ± 23	28 ± 3
	AMF*	22 ± 3	10.6 ± 0.4	48 ± 3	194 ± 7	35 ± 2
	<i>P. indica</i> + AMF	20 ± 2	10.0 ± 0.2	46 ± 4	154 ± 13	32 ± 0
Adequate	Mock	31 ± 14	10.2 ± 1.0	52 ± 3	142 ± 16	23 ± 3
	<i>P. indica</i>	31 ± 2	10.7 ± 0.8	54 ± 3	176 ± 16	24 ± 1
	AMF	16 ± 3	10.8 ± 0.4	54 ± 3	181 ± 14	24 ± 2
	<i>P. indica</i> + AMF	16 ± 3	10.3 ± 0.4	51 ± 5	181 ± 14	22 ± 2

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$). AMF*: A commercial product containing *Glomus intraradices* and *Glomus proliferum* were used for inoculation.

HSD_{0.05} values: Inoculation (I); Phosphorus (P); I×P

Shoot B Concentration: 7; *n.s.*; 11

Shoot Cu Concentration: *n.s.*; 0.4; *n.s.*

Shoot Fe Concentration: *n.s.*; 2; *n.s.*

Shoot Mn Concentration: 19; *n.s.*; 32

Shoot Zn Concentration: 3; 1; 5

Table 3.2: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation with low (20 mg P/kg soil) or adequate (100 mg P/kg of soil) P treatments on shoot concentration of selected macronutrients of 50-day-olds durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of five independent replicates each containing eight individual plants.

P	Inoculation	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Low	Mock	0.14 ± 0.01	3.72 ± 0.21	1.02 ± 0.04	0.13 ± 0.01	0.26 ± 0.01
	<i>P. indica</i>	0.10 ± 0.00	3.73 ± 0.39	1.18 ± 0.10	0.13 ± 0.01	0.26 ± 0.01
	AMF*	0.13 ± 0.00	4.25 ± 0.49	1.18 ± 0.09	0.13 ± 0.01	0.28 ± 0.02
	<i>P. indica</i> + AMF	0.12 ± 0.01	3.79 ± 0.15	1.10 ± 0.05	0.12 ± 0.00	0.25 ± 0.01
Adequate	Mock	0.22 ± 0.02	3.84 ± 0.15	1.29 ± 0.08	0.15 ± 0.01	0.31 ± 0.03
	<i>P. indica</i>	0.22 ± 0.03	4.79 ± 0.49	1.56 ± 0.22	0.17 ± 0.01	0.35 ± 0.02
	AMF	0.24 ± 0.03	4.50 ± 0.06	1.45 ± 0.18	0.16 ± 0.01	0.34 ± 0.02
	<i>P. indica</i> + AMF	0.22 ± 0.01	4.39 ± 0.22	1.45 ± 0.18	0.15 ± 0.01	0.33 ± 0.02

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$). AMF*: A commercial product containing *Glomus intraradices* and *Glomus proliferum* were used for inoculation.

HSD_{0.05} values: Inoculation (I); Phosphorus (P); I×P

Shoot P Concentration: *n.s.*; 0.01; *n.s.*

Shoot K Concentration: *n.s.*; 0.14; *n.s.*

Shoot Ca Concentration: *n.s.*; *n.s.*; *n.s.*

Shoot Mg Concentration: 0.01; 0.01; *n.s.*

Shoot S Concentration: *n.s.*; 0.01; *n.s.*

As shown in Table 3.2 there were not any significant interactive effects on macro nutrient concentration of shoots of wheat inoculated with microbial inoculant and grown under low or adequate P supply. Additionally, none of the shoot P, shoot K, shoot Ca, and shoot S concentration of wheat were affected by the inoculation significantly. Moreover, except Ca all the listed minerals in Table 3.2 were significantly affected by P treatment. For instance, shoot P, K, Mg and S concentration of Saricanak-98 were significantly under adequate P supply than low P supply.

As a recap, adequate P supply significantly promoted shoot biomass and macronutrient concentration in shoot except Ca. As a result of the first experiment, interaction between microbial inoculation and P supply were investigated. In vegetative stage, adequate P supply increased plant growth by concentrating macronutrients. Furthermore, shoot growth promotion by microbial inoculant application was enhanced by adequate P supply (Figure 3.2, Table 3.1). In the second experiment, we hypothesis that usage of *P. indica* and AMF as a microbial inoculant increases yield and quality parameters of wheat cultivars namely *Triticum durum* cv. Saricanak-98 and *Triticum aestivum* cv. Nusrat when compared to mock treatment. To test the hypothesis, the second experiment was conducted.



3.2. Results of Second Experiment

Apart from the remaining experiments, only in second experiment photos of wheat plants were taken during the vegetative and generative stages (Figure 3.3, Figure 3.4). In both mentioned stages, *P. indica* (+) AMF treatment group were easily recognizable because pots which were inoculated with *P. indica* (+) AMF provided more growth promotion.



Figure 3.3: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation on bread wheat (*Triticum aestivum* cv. Nusrat) plants grown in autoclaved soil under greenhouse conditions.

Figure 3.4 demonstrated that simultaneous inoculation of *P. indica* (+) AMF increased biomass of *Triticum durum* cv. Sarıçanak-98 when compared to remaining treatment groups. However, effects of inoculation on yield of Sarıçanak-98 cultivar were not recognizable because results of all treatments exhibit similar responses on phenotypes (Figure 3.4).

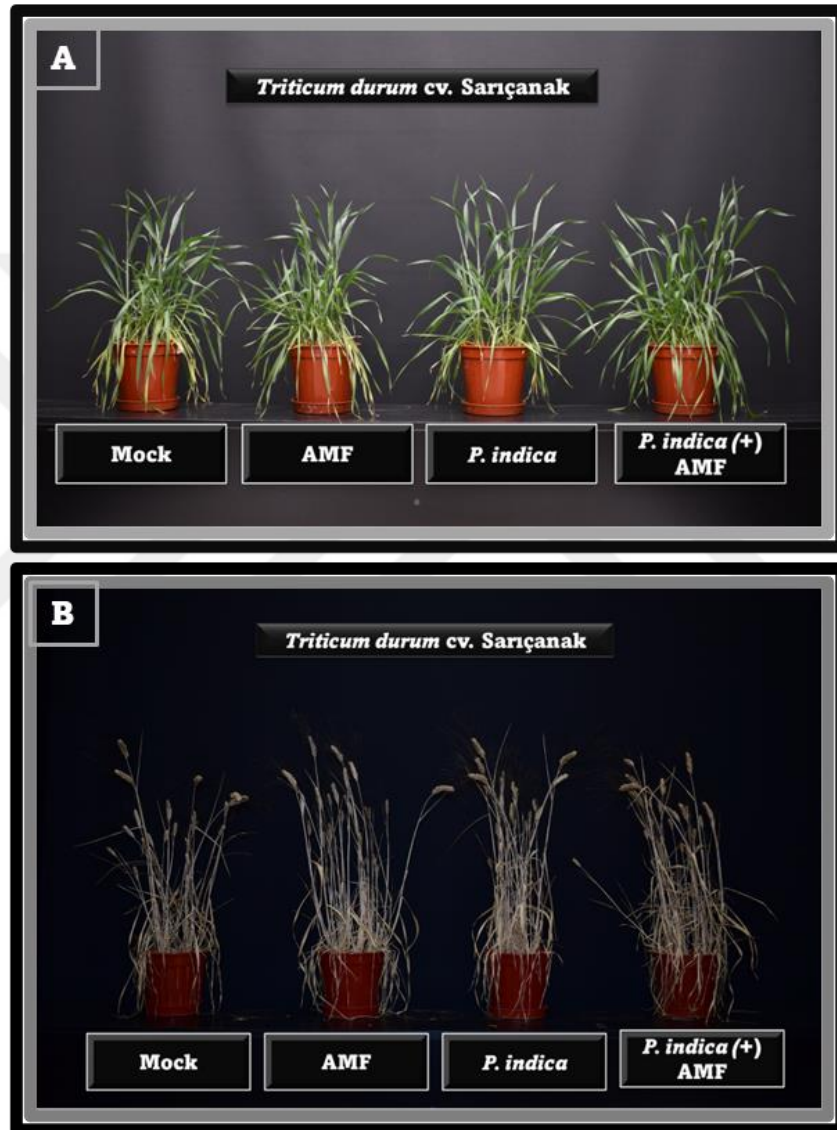


Figure 3.4: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation on durum wheat (*Triticum durum* cv. Sarıçanak-98) plants grown in autoclaved soil under greenhouse conditions.

As shown in Table 3.3, grain yield did not significantly increase with the inoculation when compared to mock treatment. In addition, Harvest index was significantly higher in both cultivar when the cultivars were treated with co-inoculation. Additionally, based on information gathered from Table 3.3, in both cultivars, the maximum straw dry weight was recorded when the cultivars inoculated with both fungi simultaneously.

Table 3.3: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation on grain yield, straw dry weight, and harvest index parameters of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Sarıcanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Cultivar	Inoculation	Grain Yield (g plant ⁻¹)	Straw Dry Weight (g plant ⁻¹)	Harvest Index (%)
Nusrat	Mock	3.7 ± 0.7	4.2 ± 0.2	46 ± 6
	<i>P. indica</i>	5.0 ± 0.1	4.3 ± 0.2	54 ± 1
	AMF*	4.1 ± 0.5	3.9 ± 0.2	51 ± 3
	<i>P. indica</i> + AMF	5.2 ± 0.3	4.7 ± 0.3	52 ± 3
Sarıcanak-98	Mock	3.7 ± 0.6	3.1 ± 0.2	54 ± 6
	<i>P. indica</i>	4.8 ± 0.9	3.3 ± 0.2	59 ± 4
	AMF	4.3 ± 0.3	3.5 ± 0.2	55 ± 3
	<i>P. indica</i> + AMF	5.5 ± 0.4	3.7 ± 0.3	60 ± 1

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$). AMF*: A commercial product containing *Glomus intraradices* and *Glomus proliferum* were used for inoculation.

HSD_{0.05} values: Cultivar (CV); Inoculation (I); CV*I

Grain Yield: *n.s.*; 0.7; *n.s.*

Straw Dry Weight: 0.2; 0.3; 0.5

Harvest Index: 4; 8; *n.s.*

Figure 3.5 shows the thousand grain weight of Nusrat and Saricanak-98 cultivars. The lowest thousand grain weights were recorded in mock treatment in Nusrat and Saricanak-98. In addition, co-inoculation with *P. indica* and AMF provided the highest thousand grain weight in both cultivars. Furthermore, thousand grain weight of Saricanak-98 cultivar was significantly higher than Nusrat cultivar. In both cultivars, the highest thousand grain weight was recorder when cultivars were co-inoculated with fungus.

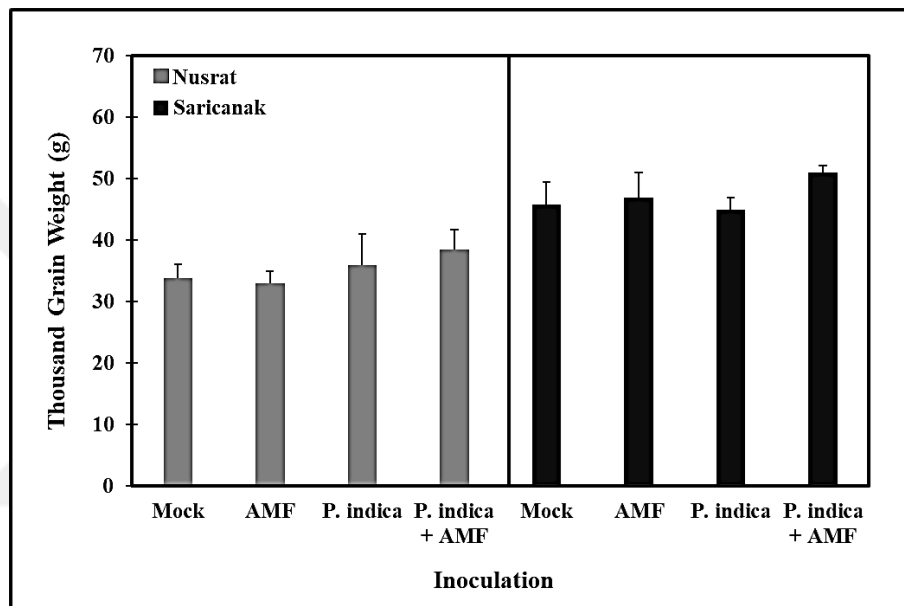


Figure 3.5: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation on thousand grain weight of bread wheat (*Triticum aestivum* Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions.

Changes of concentrations of macronutrients in grain of *Triticum aestivum* cv. Nusrat and *Triticum durum* cv are shown in Table 3.4. According to the Table shown in below, main effects of cultivars and inoculation were found significant on P concentration of grain. Irrespective to cultivars, P concentration was reached the highest value when cultivars were co-inoculated with *P. indica* and AMF. P concentration of *Triticum aestivum* cv. Nusrat significantly higher than *Triticum durum* cv. Saricanak. Although, K concentrations of grains did not significantly affect by any of the factors, main effects of cultivar and inoculation were found significant on P and S concentrations (Table 3.4).

Table 3. 4: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation on grain macronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Cultivar	Inoculation	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Nusrat	Mock	0.32 ± 0.05	0.50 ± 0.07	0.04 ± 0.00	0.13 ± 0.01	0.22 ± 0.03
	<i>P. indica</i>	0.33 ± 0.02	0.46 ± 0.04	0.05 ± 0.00	0.13 ± 0.00	0.21 ± 0.01
	AMF*	0.30 ± 0.00	0.46 ± 0.03	0.05 ± 0.00	0.13 ± 0.01	0.22 ± 0.01
	<i>P. indica</i> + AMF	0.36 ± 0.04	0.47 ± 0.03	0.05 ± 0.00	0.14 ± 0.00	0.21 ± 0.01
Saricanak-98	Mock	0.26 ± 0.00	0.45 ± 0.05	0.06 ± 0.00	0.11 ± 0.00	0.20 ± 0.00
	<i>P. indica</i>	0.28 ± 0.02	0.45 ± 0.03	0.06 ± 0.00	0.11 ± 0.01	0.19 ± 0.00
	AMF	0.29 ± 0.04	0.44 ± 0.03	0.05 ± 0.00	0.11 ± 0.01	0.21 ± 0.01
	<i>P. indica</i> + AMF	0.31 ± 0.02	0.46 ± 0.03	0.06 ± 0.00	0.11 ± 0.00	0.19 ± 0.01

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$). AMF*: A commercial product containing *Glomus intraradices* and *Glomus proliferum* were used for inoculation.

HSD0.05 values: Inoculation (I); Cultivar (CV); I × CV

Grain P Concentration: 0.04; 0.02; *n.s.*

Grain K Concentration: *n.s.*; *n.s.*; *n.s.*

Grain Ca Concentration: 0.004; 0.002; *n.s.*

Grain Mg Concentration: *n.s.*; 0.01; *n.s.*

Grain S Concentration: 0.02; 0.01; *n.s.*

Table 3. 5 shows how micronutrient concentrations of grain of *Triticum aestivum* cv. Nusrat and *Triticum durum* cv. Sarıcanak-98 were modified due to treatments. Grain concentration of B was only significantly changed by main effects of cultivar and inoculation. Grain B concentration of Nusrat cultivar was significantly higher than Sarıcanak-98. Additionally, inoculation with mycorrhizae significantly increased concentration of B in grain of wheat cultivars when compared to remaining inoculant exposures. Furthermore, Cu and Fe concentration only exhibited significant changes because of main effects of cultivars.

Interestingly, while Fe and Zn concentrations of grain of *Triticum aestivum* cv. Nusrat significantly higher than *Triticum durum* cv. Sarıcanak, the result was vice versa for grain Cu concentration. Generally, bread wheat tends to concentrate higher Zn in their tissues than durum wheat. As shown in Table 3.1 and 3.5, bread wheat cultivar Nusrat concentrated higher Zn in shoot and grain than Sarıcanak-98 cultivar, respectively. Only grain Mn concentration was significantly affected by the interaction and main effects of inoculation and cultivar. The significantly greater Mn concentration was observed when *Triticum aestivum* cv. Nusrat was grown under simultaneous inoculation of *P. indica* with AMF (Table 3.5).

Table 3.5: Effects of single *P. indica*, single AMF, combined *P. indica* (+) AMF inoculation on grain micronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Cultivar	Inoculation	B (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Nusrat	Mock	1.1 ± 0.1	7.2 ± 0.8	40 ± 8	37 ± 3	28 ± 6
	<i>P. indica</i>	1.1 ± 0.1	6.8 ± 0.2	37 ± 1	43 ± 2	26 ± 1
	AMF*	1.2 ± 0.1	7.0 ± 0.3	40 ± 3	51 ± 1	28 ± 2
	<i>P. indica</i> + AMF	1.0 ± 0.1	7.0 ± 0.4	37 ± 3	60 ± 11	30 ± 2
Saricanak-98	Mock	0.8 ± 0.0	8.1 ± 0.3	34 ± 4	34 ± 5	22 ± 1
	<i>P. indica</i>	0.8 ± 0.1	8.2 ± 0.2	33 ± 2	33 ± 3	23 ± 1
	AMF	0.9 ± 0.1	8.0 ± 0.3	35 ± 4	34 ± 8	24 ± 4
	<i>P. indica</i> + AMF	0.7 ± 0.0	8.1 ± 0.2	34 ± 1	38 ± 4	27 ± 2

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$). AMF*: A commercial product containing *Glomus intraradices* and *Glomus proliferum* were used for inoculation.

HSD_{0.05} values: Inoculation (I); Cultivar(C); I×C

Grain B Concentration: 01; 0.1; *n.s.*

Grain Cu Concentration: *n.s.*; 0.3; *n.s.*

Grain Fe Concentration: *n.s.*; 3; *n.s.*

Grain Mn Concentration: 8; 4; 13

Grain Zn Concentration: *n.s.*; 3; *n.s.*

Straw P concentration did not significantly affect by any of the source of variability according to Table 3.6. Straw K concentration of cultivars were significantly different from each other. For example, straw K concentrations of *Triticum aestivum* cv. Nusrat significantly higher than *Triticum durum* cv. Saricanak. Nusrat cultivar concentrate 10% higher K in its straw than Saricanak-98 cultivar. In contrast to K straw concentration, Ca concentration was recorded significantly higher in straw of *Triticum durum* cv. Saricanak-98 than *Triticum aestivum* cv. Nusrat. Mg concentration was only significantly affected by cultivars and *Triticum durum* cv. Saricanak-98 was the one which accumulated significantly higher Mg in their straw than *Triticum aestivum* cv. Nusrat.

The last minerals which were examined to illustrate the changes of its concentration in straw tissues was S and it was the only minerals that significantly affected by not only cultivars but also inoculation status. While the highest straw S concentration was recorded when wheat plant inoculated with *P. indica*, the lowest concentration was found in straw tissues of co-inoculated wheat plants. Like straw K and Mg concentration, straw S concentration was higher in Nusrat cultivar than Sarıcanak-98 cultivar (Table 3.6).

Table 3. 6: Effects of single and co-inoculation of *P. indica*, single AMF on straw macronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Sarıcanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Cultivar	Inoculation	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Nusrat	Mock	0.03 ± 0.01	2.0 ± 0.1	0.85 ± 0.05	0.27 ± 0.02	0.31 ± 0.04
	<i>P. indica</i>	0.03 ± 0.00	2.1 ± 0.1	0.92 ± 0.04	0.26 ± 0.02	0.30 ± 0.02
	AMF*	0.03 ± 0.00	2.1 ± 0.1	0.91 ± 0.08	0.26 ± 0.03	0.30 ± 0.03
	<i>P. indica</i> + AMF	0.03 ± 0.01	2.1 ± 0.1	0.84 ± 0.03	0.25 ± 0.01	0.27 ± 0.03
Sarıcanak-98	Mock	0.03 ± 0.00	2.0 ± 0.3	1.14 ± 0.10	0.15 ± 0.01	0.27 ± 0.02
	<i>P. indica</i>	0.03 ± 0.01	1.9 ± 0.4	1.01 ± 0.16	0.17 ± 0.02	0.28 ± 0.04
	AMF	0.03 ± 0.01	1.8 ± 0.2	1.05 ± 0.03	0.15 ± 0.01	0.25 ± 0.03
	<i>P. indica</i> + AMF	0.03 ± 0.00	1.7 ± 0.1	0.98 ± 0.03	0.15 ± 0.01	0.22 ± 0.01

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$). AMF*: A commercial product containing *Glomus intraradices* and *Glomus proliferum* were used for inoculation.

HSD_{0.05} values: Inoculation (I); Cultivar (CV); CV × I

Straw P Concentration: *n.s.*; *n.s.*; *n.s.*

Straw K Concentration: *n.s.*; 0.1; *n.s.*

Straw Ca Concentration: *n.s.*; 0.06; *n.s.*

Straw Mg Concentration: *n.s.*; 0.01; *n.s.*

Straw S Concentration: 0.04; 0.02; *n.s.*

Mn and B concentration were significantly affected by main effects of all factors. Inoculation of *Triticum aestivum* cv. Nusrat with *P. indica* caused more than 2-fold increase in B concentration of straw comparing to mock treatment. Because of low re-translocation ability of B, acquisition of B which promoted by *P. indica* inoculation caused high straw B concentrations (Table 3.7) while grain B concentrations did not significantly differentiate from mock treatment (Table 3.5).

Table 3. 7: Effects of single and co-inoculation of *P. indica*, single AMF on straw micronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Cultivar	Inoculation	B (mg.kg ⁻¹)	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Nusrat	Mock	22 ± 3	5 ± 1	75 ± 15	78 ± 5	6 ± 1
	<i>P. indica</i>	74 ± 2	5 ± 1	75 ± 11	88 ± 8	5 ± 0
	AMF*	19 ± 2	6 ± 1	69 ± 10	158 ± 24	6 ± 1
	<i>P. indica</i> + AMF	35 ± 4	4 ± 1	52 ± 9	160 ± 46	7 ± 1
Saricanak-98	Mock	23 ± 2	6 ± 1	98 ± 14	56 ± 8	7 ± 1
	<i>P. indica</i>	35 ± 2	5 ± 1	70 ± 13	62 ± 13	5 ± 1
	AMF	22 ± 3	6 ± 1	71 ± 7	55 ± 21	6 ± 1
	<i>P. indica</i> + AMF	21 ± 2	5 ± 0	61 ± 8	87 ± 7	8 ± 1

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$). AMF*: A commercial product containing *Glomus intraradices* and *Glomus proliferum* were used for inoculation.

HSD_{0.05} values: Inoculation (I); Cultivar (CV); CV × I

Straw B Concentration: 4; 2; 6

Straw Cu Concentration: 1; *n.s.*; *n.s.*

Straw Fe Concentration: 15; *n.s.*; *n.s.*

Straw Mn Concentration: 29; 15; 49

Straw Zn Concentration: 1; *n.s.*; *n.s.*

Additionally, root colonization of single *P. indica* and combination of *P. indica* with AMF provided 102% and 105% increases in straw Mn concentration of *Triticum aestivum* cv. Nusrat comparing to mock treatment, respectively. Microbial inoculation was also enhanced shoot Mn concentration in the first experiment (Table 3.1). Astonishingly, straw Fe concentration in mock-treated wheat plants were higher than the plants which were inoculated with single or combined fungus. (Table 3.7). Because microbial inoculation did not increase grain Fe concentration while decreasing straw concentration when compared to mock treatment, uptake and/or translocation of Fe may be restricted in wheat plants which grown in autoclaved soil. The restriction of Fe uptake from the roots of wheat plants by microbial inoculation may prevent possible Fe toxicity because soil that used in this thesis had high Fe concentration (Table 2.1).

The following experiment, namely, the third experiment was conducted to investigate relationship between salinity and B toxicity in wheat plants by examination of mineral concentration of shoot and changes of shoot dry weight due to stress treatments.

3.3. Results of Third Experiment

Figure 3.6 shows that combined effects of the salinity and B did not show any significant effects on shoot dry weight, but their interaction had significantly affected on the shoot dry weight. For instance, even there were no significant differences between the control and moderate salinity, high salinity treatment significantly reduces shoot dry weight. Furthermore, B toxicity exhibit significant reduction in shoot dry weight when compared to adequate B supply. Additionally, shoot dry weight of *Triticum durum* cv. Sarıçanak is significantly higher than *Triticum aestivum* cv. Nusrat (Figure 3.6). The lowest shoot dry weight of both cultivars was recorded when cultivars were treated with high salinity and toxic B. Moreover, there was a steady decrease in the shoot dry weight of both cultivars with increasing salt levels when salt and B toxicity applied together. On the other hand, adequate B supply with moderate salinity was resulted with the highest shoot dry weight in Sarıçanak-98.

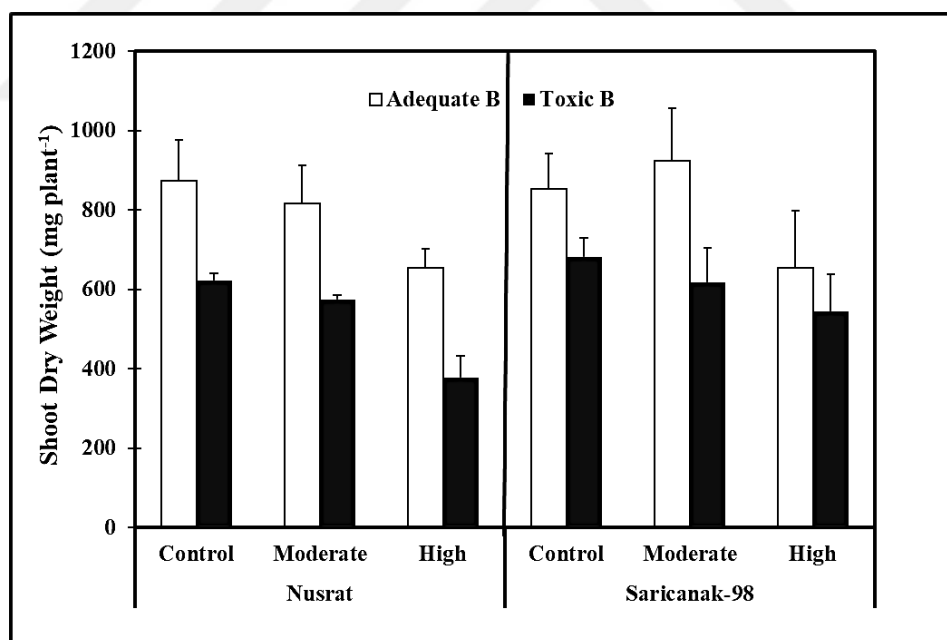


Figure 3.6: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on shoot dry weight of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Sarıçanak-98) plants grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation.

As shown in Figure 3.7A, shoot B concentration significantly affected from the interaction of cultivar, B supply and salinity treatment. However, irrespective to salinity treatment and cultivar, adequate B supply did not exhibit any variation on shoot B concentration.

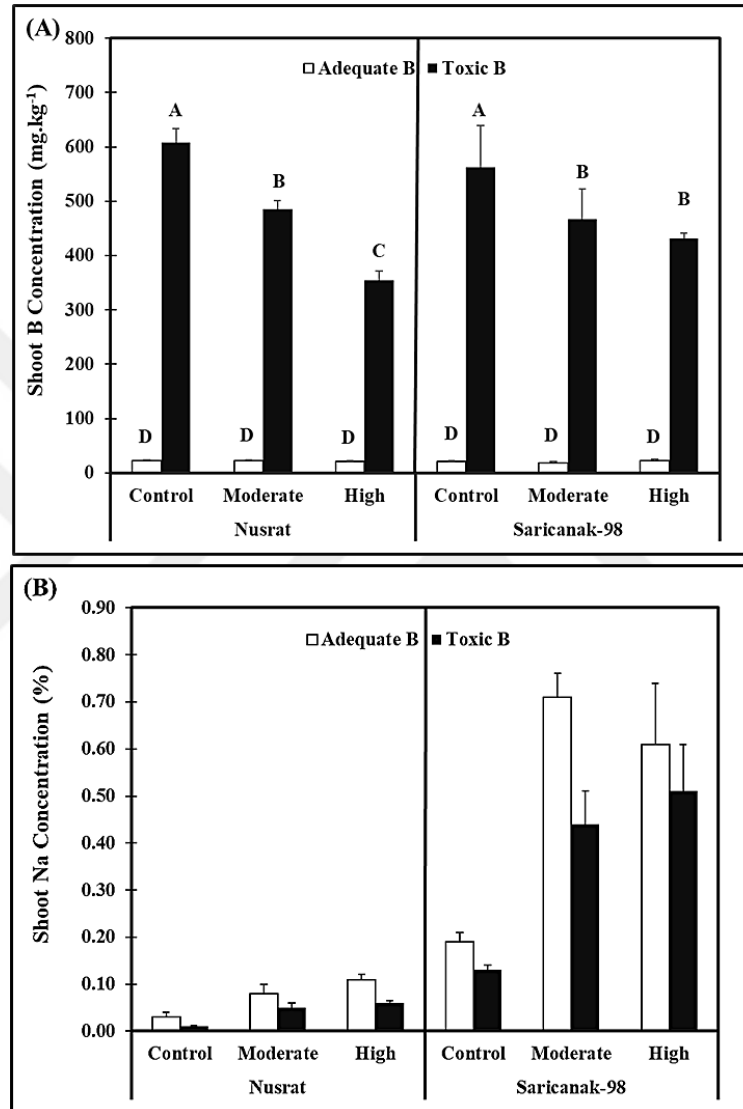


Figure 3.7: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on B (A) and Na (B) concentration of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test.

On the other hand, increasing salinity levels significantly decreased B concentrations in Nusrat which was treated with toxic B and the lowest B concentration was found in wheat plant which exposed toxic B and high salinity (Figure 3.7A). Interaction of factors that employed in the third experiment did not significantly affect Na shoot concentration as shown in Figure 3.7B. Nusrat and Saricanak-98 cultivar behaved differently in terms of concentrating Na in their shoot. Nusrat restricted concentration of Na while Saricanak-98 tend to accumulate significantly higher Na in its shoot tissues (3.7B).

Interactive effects of B supply and salinity treatment on shoot Na concentration did not significant. However, in al treatment's groups increasing salt levels decreased B concentration. Interaction of cultivar and salinity was found significant, and the significantly lowest Na concentration was recorded in Nusrat which did not expose to salinity and grown under adequate B, while the highest shoot Na concentration observed in Saricanak-98 which was treated with toxic B with high salinity. To recap, while toxic B supply reduced shoot Na concentration in both cultivars, increasing Na concentration due to increasing salinity treatment in shoot restricted accumulation of B (Figure 3.1A; Figure 3.1B).

Antagonistic relationship between the salinity and boron is well studied in literature. The reason behind reduction of B concentration due to Na and vice versa may be originate from the reduction of expression of certain genes that encode B and Na transporter. Additionally, because uptake of minerals is restricted by osmotic stress triggered by salinity this may partially inhibited B uptake.

Table 3.8 shows the shoot macronutrient concentration of shoot of Nusrat and Saricanak-98 cultivars. P concentration affected by B supply, significantly. Shoot K concentration significantly affected by cultivar, B supply and interaction of B with salinity and B with cultivar. While toxic B supply significantly increased K concentration comparing to adequate B supply, shoot K concentration of Nusrat was significantly greater than Saricanak-98 (Table 3.8).

Table 3. 8: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on macronutrient concentrations of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Cultivar	Boron	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Control	Nusrat	Adequate	0.28 ± 0.02	3.7 ± 0.2	7.7 ± 0.4	0.25 ± 0.01	0.36 ± 0.01
		Toxic	0.36 ± 0.03	4.8 ± 0.1	7.1 ± 0.8	0.21 ± 0.01	0.41 ± 0.03
	Saricanak-98	Adequate	0.33 ± 0.05	3.5 ± 0.1	10.5 ± 0.4	0.18 ± 0.01	0.38 ± 0.04
		Toxic	0.39 ± 0.04	4.2 ± 0.1	9.9 ± 0.6	0.18 ± 0.01	0.36 ± 0.02
Moderate	Nusrat	Adequate	0.32 ± 0.02	4.0 ± 0.2	7.3 ± 0.3	0.25 ± 0.01	0.35 ± 0.01
		Toxic	0.36 ± 0.03	4.7 ± 0.3	7.0 ± 0.2	0.21 ± 0.01	0.39 ± 0.02
	Saricanak-98	Adequate	0.33 ± 0.03	3.4 ± 0.2	8.5 ± 0.6	0.17 ± 0.00	0.38 ± 0.02
		Toxic	0.40 ± 0.03	4.1 ± 0.1	8.3 ± 0.7	0.17 ± 0.01	0.38 ± 0.02
High	Nusrat	Adequate	0.32 ± 0.05	4.1 ± 0.2	6.8 ± 0.3	0.22 ± 0.01	0.34 ± 0.01
		Toxic	0.38 ± 0.06	4.7 ± 0.2	6.1 ± 0.6	0.19 ± 0.02	0.32 ± 0.01
	Saricanak-98	Adequate	0.31 ± 0.05	3.4 ± 0.3	8.7 ± 0.9	0.16 ± 0.01	0.41 ± 0.04
		Toxic	0.37 ± 0.02	3.7 ± 0.1	8.8 ± 0.7	0.16 ± 0.01	0.34 ± 0.04

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05}: Cultivar (CV); Salinity (S); Boron (B); CV×S; CV×B; S × B; CV× S × B

Shoot P Concentration: *n.s.*; *n.s.*; 0.02; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot K Concentration: 0.1; *n.s.*; 0.1; *n.s.*; 0.3; 0.4; *n.s.*

Shoot Ca Concentration: 0.3; 0.5; 0.3; 0.9; *n.s.*; *n.s.*; *n.s.*

Shoot S Concentration: *n.s.*; 0.02; *n.s.*; 0.04; 0.03; 0.04; *n.s.*

Shoot Mg Concentration: 0.01; 0.01; 0.01; *n.s.*; *n.s.*; 0.02; *n.s.*

Answer of the question why Nusrat concentrates higher K in shoot tissues than Saricanak-98 may be the greater ability of Nusrat to tolerate salinity by excluding Na from roots than Saricanak-98. The significant interaction between cultivar and B supply showed that the significantly highest and lowest K concentration were observed when Nusrat was treated with toxic B and Saricanak-98 was grown under adequate B, respectively.

Therefore, based on the antagonistic interaction between the Na and K, assuming that B toxicity enhanced K uptake indirectly by decreasing Na acquisition may be logical (Table 3.8; Figure 3.7). Shoot Ca concentration reduced by increasing salt treatment and there was a significant difference between control salinity with moderate salinity and control salinity with high salinity. Ca concentration of wheat plants were significantly reduced by B toxicity. Because Saricanak-98 is more susceptible against salinity than Nusrat and Ca-mediated salinity tolerance is one of natural phenomena that is commonly observed in plants that deal with salt stress, Saricanak-98 needed to accumulate significantly higher Ca in its shoot tissues than Nusrat (Table 3.8). Furthermore, significant interaction between the salinity and B affected Mg and S concentration.

Cu, Mn, Zn and Fe shoot concentrations of Nusrat and Saricanak-98 cultivar is shown in Table 3.9. While shoot Fe and Zn concentration significantly reduced by Toxic B supply, Mn concentration was increased significantly due B toxicity. Even though, interaction of salinity and B did not significantly affect Mn concentration, their main effects were found significant. Shoot Mn concentration was higher in wheat plants which grown under B toxicity than the plants were exposed to adequate B. In literature, studies shown that NaCl + B treatment increased Mn concentrations. Furthermore, shoot Mn concentration was increased by increasing salinity. Shoot Fe concentration significantly reduced by toxic B supply and effect of triple interaction on shoot Fe concentration was found significant.

The highest and the lowest shoot Mn concentration were observed in Nusrat which grown under simultaneous high salinity with toxic B and in Saricanak-98 which was treated with control salinity with toxic B supply, respectively. Additionally, shoot Zn concentration was affected by triple interaction of source of variability, significantly. The highest Zn concentration was recorded when Saricanak-98 grown under moderate salinity with adequate B supply. Furthermore, Toxic B supply significantly reduced Zn concentration in shoot when compared to adequate B supply.

Table 3. 9: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on micronutrient concentrations of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Cultivar	Boron	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Control	Nusrat	Adequate	11.6 ± 0.3	80 ± 2	59 ± 1	20 ± 2
		Toxic	13.8 ± 0.9	72 ± 4	75 ± 2	15 ± 1
	Saricanak-98	Adequate	12.2 ± 1.1	80 ± 5	38 ± 1	25 ± 3
		Toxic	13.4 ± 0.1	63 ± 6	40 ± 2	13 ± 1
Moderate	Nusrat	Adequate	12.8 ± 0.3	82 ± 6	73 ± 5	21 ± 2
		Toxic	14.8 ± 0.2	74 ± 2	84 ± 3	16 ± 1
	Saricanak-98	Adequate	12.1 ± 0.5	70 ± 5	44 ± 2	25 ± 3
		Toxic	13.8 ± 1.0	76 ± 6	49 ± 1	7 ± 1
High	Nusrat	Adequate	12.7 ± 0.6	80 ± 8	71 ± 2	18 ± 2
		Toxic	13.0 ± 0.9	87 ± 11	83 ± 7	8 ± 1
	Saricanak-98	Adequate	12.9 ± 0.8	75 ± 8	51 ± 4	17 ± 3
		Toxic	12.8 ± 1.1	71 ± 4	58 ± 4	12 ± 3

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05}: Cultivar (CV); Salinity (S); Boron (B); CV×S; CV× B; S × B; CV× S × B

Shoot Cu Concentration: *n.s.*; 0.6; 0.4; *n.s.*; *n.s.*; 1.1; *n.s.*

Shoot Fe Concentration: 4; *n.s.*; 4; *n.s.*; *n.s.*; 9; 15

Shoot Mn Concentration: 2; 3; 2; 5; 4 *n.s.*; *n.s.*

Shoot Zn Concentration: 1; *n.s.*; 1; 3; 2; *n.s.*; 5

The third experiment was conducted to reveal interaction between salinity and B toxicity in durum and bread wheat cultivars. The following experiment namely fourth experiment was aimed to demonstrate the interplay among salinity, B toxicity and *P. indica* colonization on wheat roots in vegetative stages by analysing morphological, physiological, and molecular changes.

3.4. Results of Fourth Experiment

Figure 3.8 shows that salinity treatment reduced growth of the *Triticum aestivum* cv. Nusrat under absence of B toxicity. However, interaction of B toxicity and salinity treatment caused more detrimentally on plant growth. In addition, salinity treatment and B toxicity lead to formation of necrosis and chlorosis in margins of leaves. Toxic B supply adversely affected vegetative and impaired development of wheat. Toxic B supply without salt stress decreased thickness of stem of wheat. Alone and combined damages of B toxicity and salinity stress were ameliorated by *P. indica*.

The difference between adequate B supply and B toxicity was not easily recognizable when compared to mock treatment. Especially, in plant which were grown under toxic B and high salinity treatment with *P. indica* inoculation were healthier and vigoured when compared to mock treatments. Figure 3.4 showed the effects of the stress and *P. indica* treatments on growth of bread wheat namely *Triticum aestivum* cv. Nusrat.

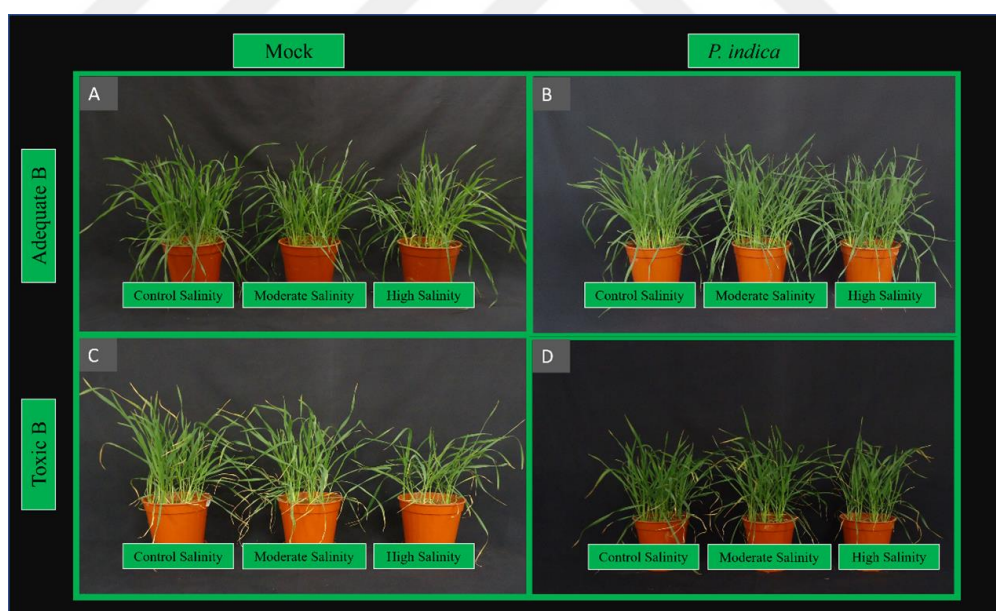


Figure 3.8: Observation of effects of root colonization of *P. indica* on 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) plants grown under different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies in non-autoclaved soil under greenhouse conditions.

In addition to *Triticum aestivum* cv. Nusrat, *Triticum durum* cv. Sarıçanak was also benefit from *in* terms of minimizing their injury which caused by stress treatments. Especially, high salt treatments were adversely affected plants when compared to control salinity in both under adequate and toxic B conditions. Like *Triticum aestivum* cv. Nusrat, *Triticum durum* cv. Sarıçanak was also severely affected from combination of Toxic B and salinity treatments under mock treatments. In contrast to mock treatment, *P. indica* protected plants from stress factors. Under adequate B supply, reduction in growth due to salt stress was nearly eliminated by *P. indica* inoculation (Figure 3.9).

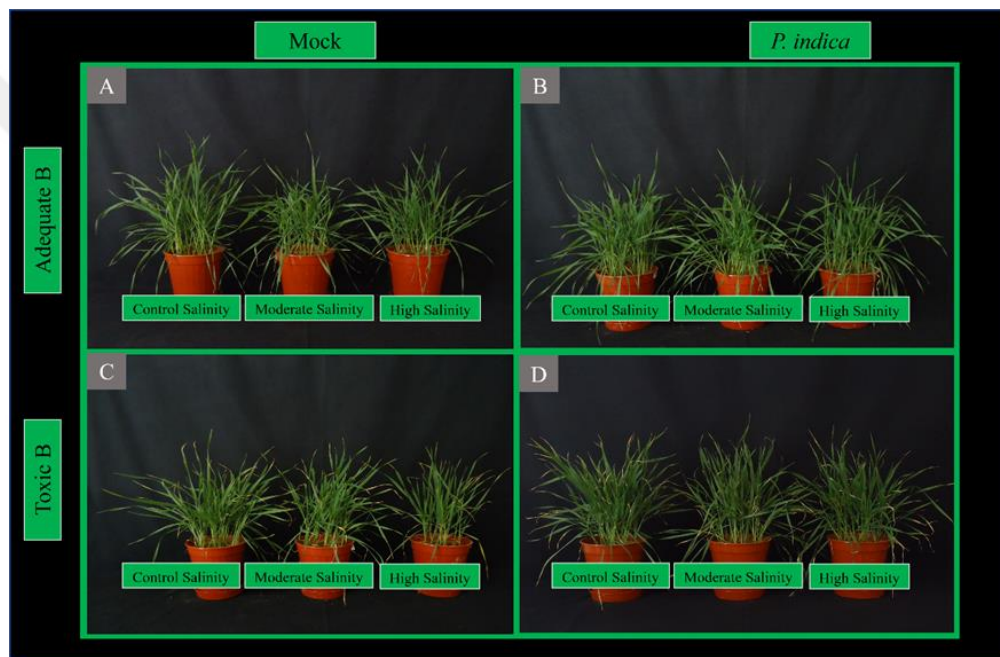


Figure 3. 9: Observation of effects of root colonization of *P. indica* on 32-day-olds bread wheat (*Triticum durum* cv. Sarıçanak-98) plants grown under different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies in non-autoclaved soil under greenhouse conditions.

Figure 3.10 shows the impact of *P. indica* on tiller number and shoot dry weight of Nusrat and Sarıçanak-98 cultivars. Significant interaction among source of variability affected shoot dry weight and tiller number of both cultivars (Figure 3.10).

The lowest shoot dry weight and tiller number were recorded when Nusrat cultivar was grown under high salinity and toxic B supply without *P. indica* inoculation, while the highest shoot dry weight was observed when Nusrat cultivars were inoculated with *P. indica* and grown under adequate B supply with control salinity conditions (Figure 3.10A, B).

Additionally, in Nusrat, root colonization of *P. indica* significantly increased shoot dry weight and tiller number when compared to non-inoculated plants. Furthermore, shoot dry weight and tiller number of wheat plant grown under adequate B supply were greater than toxic B treated plants. In addition, shoot dry weight and tiller number significantly reduced by increasing salinity. %21 and %33 reductions on tiller number and shoot dry weight were recorded when the Nusrat cultivar was exposed to high salinity when compared to the control. (Figure 3.10A, B)

Similar to Nusrat, shoot dry weight and tiller number of Saricanak-98 were also significantly affected from interaction among salinity, B and *P. indica*. For example, the highest shoot dry weight and tiller number were recorded when adequate B applied Saricanak-98 cultivar was inoculated with *P. indica* and grown under with control or moderate salinity, respectively. On the other hand, the lowest shoot dry weight and tiller number were identified in non-inoculated and high salinity with toxic B applied treatment groups (Figure 3.10A; B). Furthermore, *P. indica* inoculation increased shoot dry weight and tiller number. Shoot dry weight and tiller number of *P. indica* treated plants were 22% and 11% greater than non-inoculated. Toxic B supply significantly reduced both parameters when comparing to adequate ones and increasing salinity decreased the parameters, as well (Figure 3.10C; D)

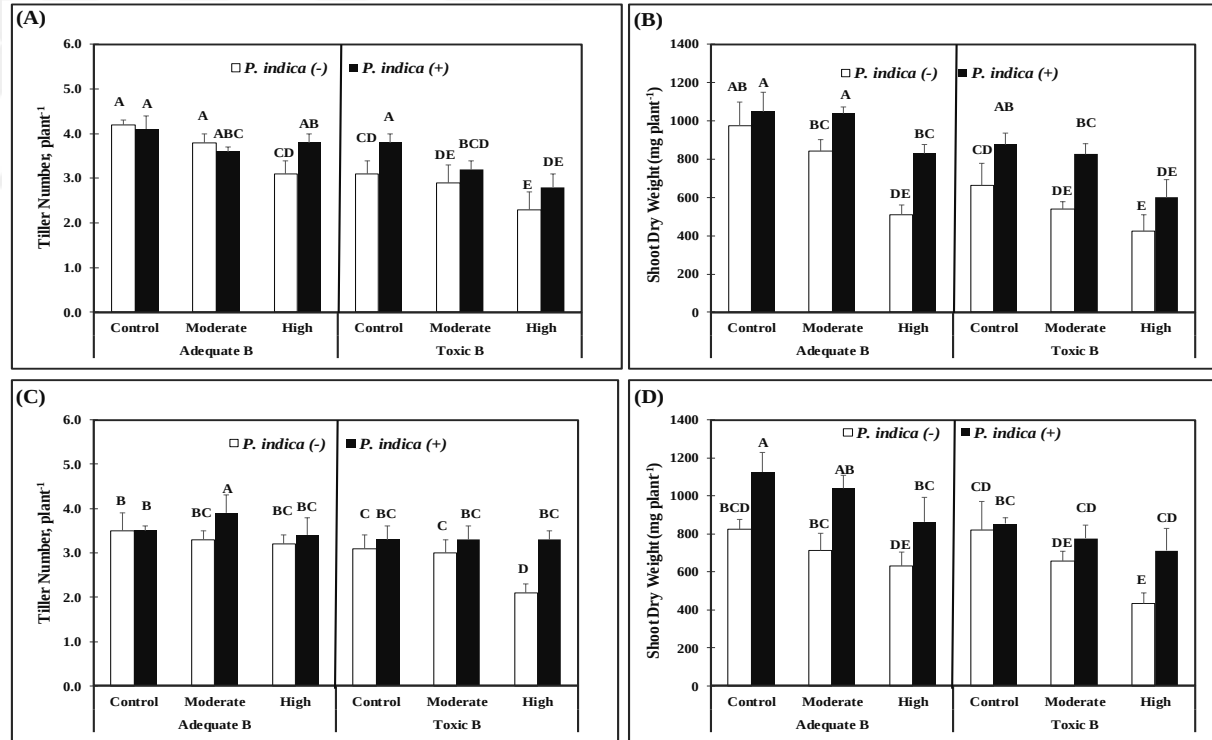


Figure 3.10: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on tiller number and shoot dry weight of 32-day-olds bread wheat (A; B) (*Triticum aestivum* cv. Nusrat) and durum wheat (C;D) (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing four individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test.

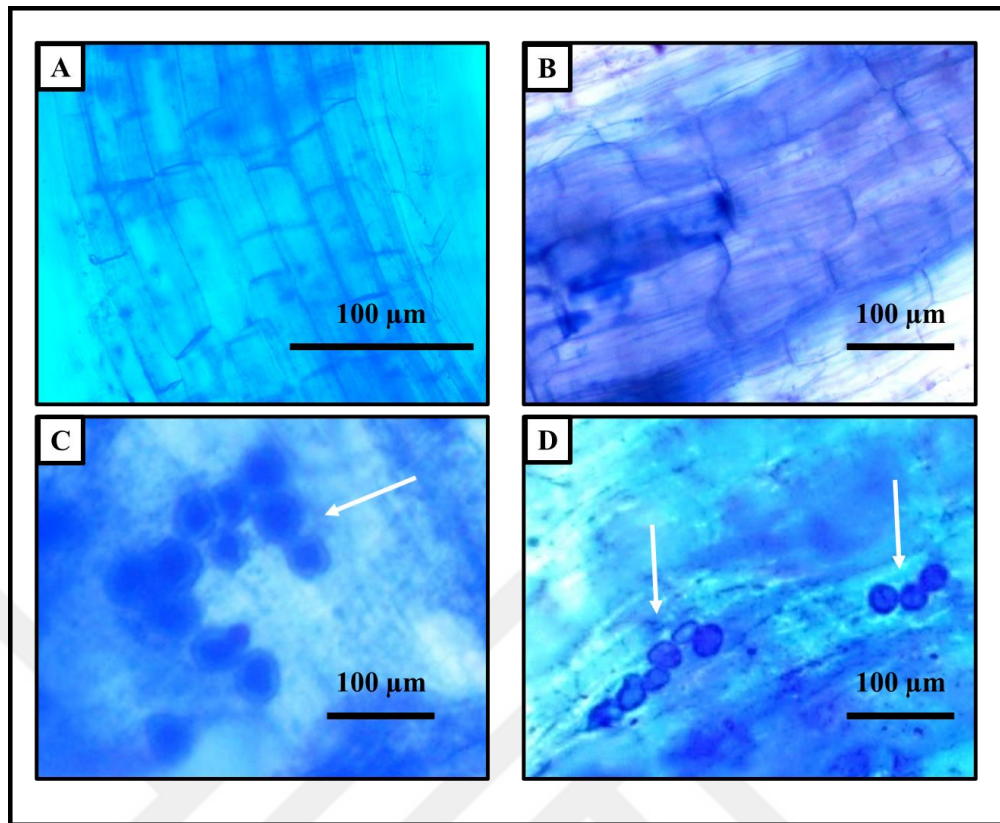


Figure 3. 11: Microscopic observation of roots of 32-days-old wheat plants grown in absence (A and B) and presence of *P. indica* (C and D) inoculation. Chlamydospores of *P. indica* are showed by white arrows. Roots of wheats were stained by trypan blue. Scale bars (horizontal black lines) indicates magnification.

Figure 3.11 shows the observation of wheat roots. Figure 3.11A and B indicate that non-inoculated wheat plants did not contaminate. Figure 3.11C and D demonstrated that roots of wheat were successfully colonized by *P. indica*. By microscopic examination of roots of wheat, we ensure that the effects that shown on wheat physiology was originated from *P. indica* inoculation. Colonization of spores of *P. indica* observed under different scanning objective lens as 10x, 20x and 40x.

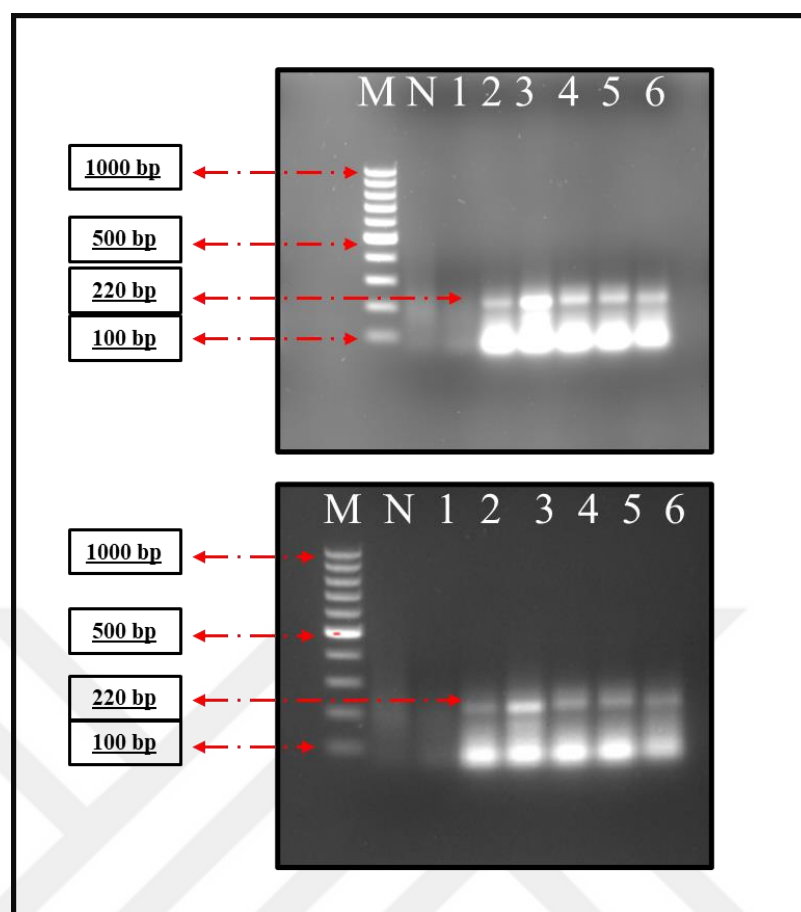


Figure 3.12: PCR amplification result of total DNA samples extracted from roots of 32-days-old-wheat plants which were inoculated with or without *P. indica*. PCR products were loaded into wells of 1.5 % agarose gel and run at 90 V for 2 hours. Except ladder, the remaining well was loaded with 25 μ l PCR product. DNA ladder was loaded with 5 μ l. M: 100bp DNA Ladder; N: Negative control; 1: non-inoculated wheat sample; 2-3-4-5-6: *P. indica* inoculated wheat sample. Amplification products of *EF-1 alpha (tef)* gene produced bands at 220 bp.

Figure 3.12 shows the results of the agarose gel electrophoresis which was used to identify the marker gene of *P. indica* namely *EF-1 alpha (tef)*. The gene produced homozygous band at 220 bp. 100 bp DNA ladder was used and the ladder had two reference band at 500 and 1000 bp. Moreover, non-inoculated plants and negative control did not produce any band. 6 different wheat plants were selected to be ensure that fungi treated experimental groups were inoculated successfully.

Figure 3.13 shows the MDA concentration of Nusrat and Saricanak-98 cultivars. Shoot MDA concentrations of Nusrat significantly reduced by *P. indica* inoculation. Moreover, when severity of salt treatment increased, MDA concentration increased, and the highest and lowest MDA concentration were found under high salinity and control salinity conditions. Furthermore, the third independent variable of this experiment namely B had significant impact on the shoot MDA concentration. Toxic B caused higher accumulation of MDA than adequate B (Figure 3.13A).

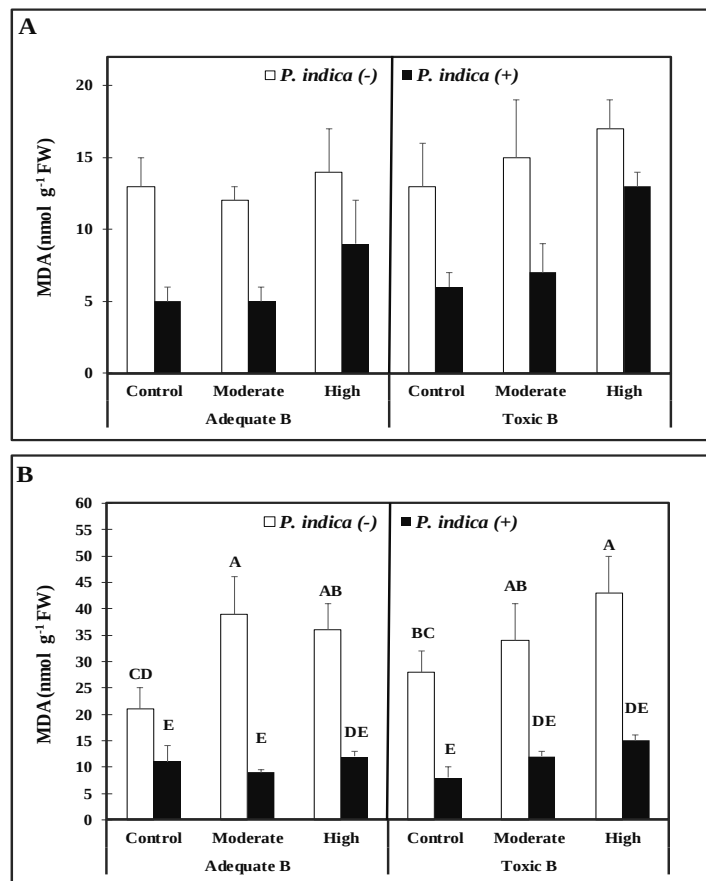


Figure 3.13: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on Malondialdehyde (MDA) concentration of 32-day-olds leaves of bread wheat (A) (*Triticum aestivum* cv. Nusrat) and durum wheat (B) (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing four individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.

Shoot MDA concentration of Saricanak-98 was determined by significant influences of all the source of variabilities. The highest and lowest shoot MDA concentrations were found when non-inoculated wheat plants grown under high salinity with toxic B supply and *P. indica*-inoculated wheat plants grown under the control salinity with toxic B supply, respectively (Figure 3.13B).

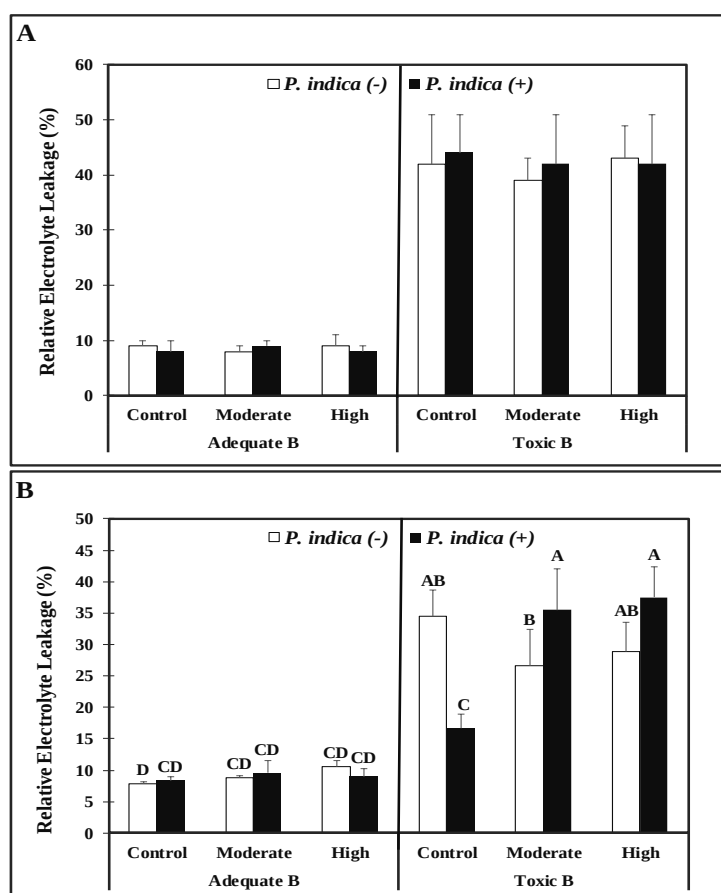


Figure 3.14: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on Relative Electrolyte Leakage of 32-day-olds bread wheat (A) (*Triticum aestivum* cv. Nusrat) and durum wheat (B) (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing four individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.

Figure 3.14 was prepared to illustrate modification of relative electron leakage (REL) levels of leaves of *Triticum durum* Sarıcanak-98 and *Triticum aestivum* cv. Nusrat. Inoculation levels did not significantly affect to REL. In both cultivars, significant increases were observed when cultivars were exposed to toxic B. Additionally, interaction among inoculation, salinity and B treatment was found significant in Sarıcanak-98 cultivar (Figure 3.14B). The lowest REL was recorded when non-inoculated *Triticum durum* cv. Sarıcanak cultivars treated with control salinity and adequate B supply.

Figure 3.15 shows shoot Na and B concentration of Nusrat and Sarıcanak-98 cultivars. Only shoot Na concentration of Sarıcanak-98 significantly affected by interaction of salinity, B, and inoculation. Na concentration of high salinity and toxic B treated non-inoculated wheat plants significantly higher than remaining treatment groups in Sarıcanak-98. The lowest Na concentration was found when wheat plants did not inoculate with *P. indica* and grown under control salinity with toxic B supply (Figure 3.15D). Additionally, Na concentration significantly reduced by 39% and 10 % *P. indica* inoculation in Nusrat and Sarıcanak-98, respectively. Especially in treatment group which were exposed to high salinity, Na reducing effects of *P. indica* inoculation were more significant. Furthermore, shoot Na concentration in both cultivars significantly increased with increasing salinity.

Shoot B concentration of Nusrat and Sarıcanak-98 cultivar did not significantly affect by interaction of salinity, B supply and *P. indica*. However, interaction of salinity and B supply was found significant. In detail, shoot B concentration of Nusrat and Sarıcanak-98 were reached highest levels when the cultivars were exposed to toxic B supply and grown under control salinity conditions. Result of shoot B concentration measurement was revealed 25-fold increase in Nusrat and Sarıcanak-98 due to toxic B supply when compared to adequate supply (Figure 3.15A; C).

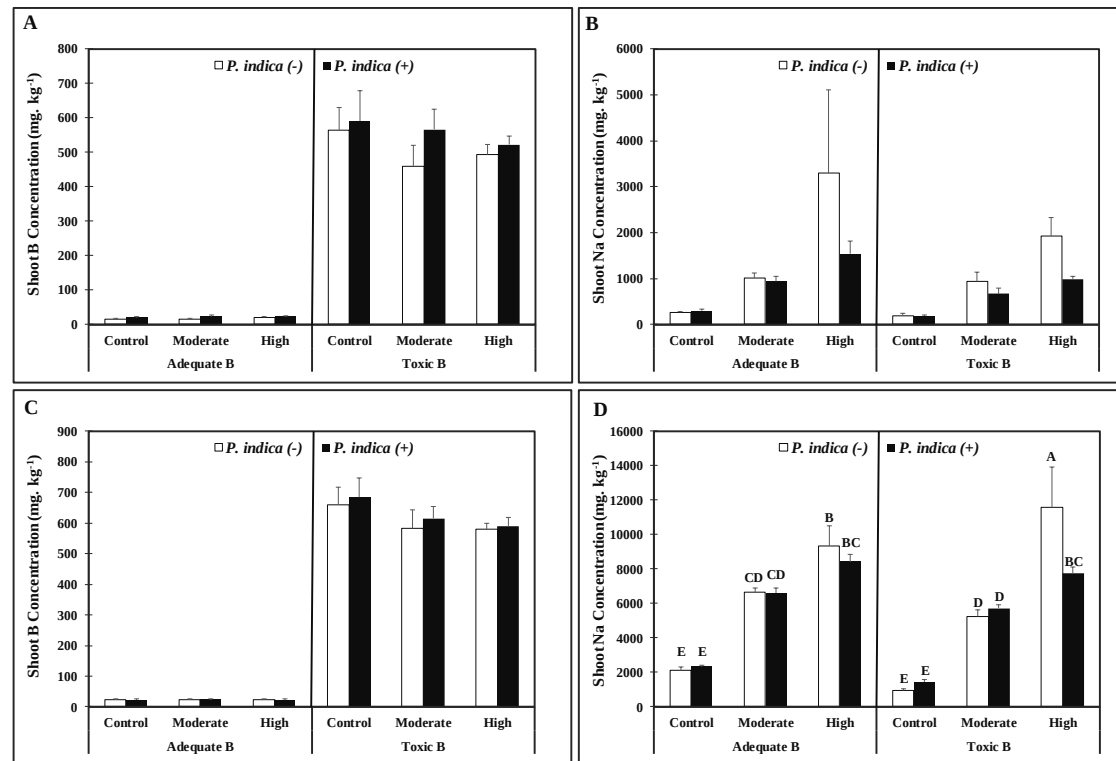


Figure 3.15: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on shoot B (A;C) and Na (B;D) concentration of 32-day-olds bread wheat (A;B) (*Triticum aestivum* cv. Nusrat) and durum wheat (C;D) (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing four individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.

As shown in Table 3.10, shoot S, Mg, Ca, P concentration of *Triticum aestivum* cv. Nusrat cultivar were significantly affected by inoculation except K. While shoot P, K and S concentration were increased by *P. indica* inoculation, Ca concentration was decreased. Moreover, K concentration of shoots of *Triticum aestivum* cv. Nusrat cultivar significantly higher when Nusrat grown under moderate and high salinity when compared to control salinity.

Table 3. 10: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on macronutrient concentration of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Control	Adequate	(-)	0.27 ± 0.01	3.9 ± 0.2	0.78 ± 0.02	0.25 ± 0.01	0.38 ± 0.02
		(+)	0.32 ± 0.03	4.0 ± 0.2	0.80 ± 0.06	0.26 ± 0.01	0.40 ± 0.02
	Toxic	(-)	0.30 ± 0.02	4.5 ± 0.1	0.87 ± 0.13	0.22 ± 0.01	0.44 ± 0.01
		(+)	0.37 ± 0.02	4.5 ± 0.2	0.76 ± 0.05	0.23 ± 0.01	0.45 ± 0.01
Moderate	Adequate	(-)	0.28 ± 0.02	4.4 ± 0.2	0.75 ± 0.05	0.22 ± 0.01	0.37 ± 0.02
		(+)	0.31 ± 0.03	4.3 ± 0.2	0.75 ± 0.07	0.24 ± 0.01	0.39 ± 0.02
	Toxic	(-)	0.34 ± 0.03	5.1 ± 0.6	0.79 ± 0.11	0.20 ± 0.03	0.44 ± 0.01
		(+)	0.34 ± 0.03	4.7 ± 0.2	0.73 ± 0.03	0.21 ± 0.01	0.44 ± 0.01
High	Adequate	(-)	0.30 ± 0.02	4.3 ± 0.2	0.88 ± 0.13	0.20 ± 0.01	0.36 ± 0.01
		(+)	0.30 ± 0.03	4.3 ± 0.1	0.71 ± 0.04	0.21 ± 0.01	0.38 ± 0.01
	Toxic	(-)	0.36 ± 0.03	4.8 ± 0.2	0.80 ± 0.04	0.18 ± 0.01	0.36 ± 0.01
		(+)	0.36 ± 0.04	4.9 ± 0.1	0.70 ± 0.02	0.20 ± 0.01	0.38 ± 0.03

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I × S; I × B; S × B; I × S × B

Shoot P Concentration: 0.02; *n.s.*; 0.02; 0.04; *n.s.*; *n.s.*; *n.s.*

Shoot K Concentration: *n.s.*; 0.2; 0.1; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot Ca Concentration: 0.04; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot Mg Concentration: 0.01; 0.01; 0.01; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot S Concentration: 0.01; 0.01; 0.01; *n.s.*; *n.s.*; 0.02; *n.s.*

Significant main effect of B supply on shoot Mg, K, P and S were found. Similar to findings of third experiment which showed that toxic B supply significantly increased shoot K and P concentration of Nusrat cultivar (Table 3.8), in fourth experiment was also revealed that toxic B supply significantly increased shoot P and K concentration in Nusrat as well. However, Mg concentration in shoot was reduced by toxic B supply when compared to adequate one (Table 3.10).

Table 3. 11: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on micronutrient concentration of 32-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Control	Adequate	(-)	12.3 ± 0.4	83 ± 3	54 ± 4	27 ± 3
		(+)	12.8 ± 0.5	89 ± 6	53 ± 3	31 ± 6
	Toxic	(-)	14.7 ± 0.4	96 ± 21	65 ± 3	25 ± 2
		(+)	15.0 ± 0.6	78 ± 7	70 ± 3	28 ± 2
Moderate	Adequate	(-)	13.0 ± 0.8	77 ± 2	60 ± 6	23 ± 2
		(+)	13.1 ± 0.5	77 ± 3	61 ± 3	28 ± 4
	Toxic	(-)	15.5 ± 0.0	82 ± 15	72 ± 3	28 ± 1
		(+)	15.6 ± 0.5	80 ± 6	73 ± 5	29 ± 5
High	Adequate	(-)	13.1 ± 0.7	76 ± 7	68 ± 2	25 ± 2
		(+)	13.6 ± 0.8	77 ± 3	69 ± 5	25 ± 2
	Toxic	(-)	14.9 ± 1.1	74 ± 7	81 ± 3	14 ± 3
		(+)	15.1 ± 0.3	76 ± 6	81 ± 6	19 ± 3

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Shoot Cu Concentration: *n.s.*; 1; 0.4; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot Fe Concentration: *n.s.*; 8; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot Mn Concentration: *n.s.*; 3; 2; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot Zn Concentration: 2; 3; 2; *n.s.*; *n.s.*; 5; *n.s.*

Shoot Cu, Fe, Mn, and Zn concentration of bread wheat cultivar Nusrat was analysed. The analysis demonstrated that only shoot Zn concentration significantly affected by microbial treatment. Zn concentration of *P. indica* colonized plants were significantly higher than non-inoculated plants (Table 3.11). Zn concentration reduced effects of *P. indica* inoculation was not only seen in shoot (Table 3.11) but also shown in straw of Nusrat and Saricanak-98 in the second experiment (Table 3.7). Additionally, impact of B supply was found significant on shoot Mn, Zn, and Cu concentration in Nusrat. For instance, while B toxicity significantly increased Mn and Cu concentration, it significantly decreased Zn concentration of Nusrat when compared to adequate B supply (Table 3.11). This result was previously found in the third experiment (Table 3.9).



Table 3. 12: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on macronutrient concentration of 32-day-olds durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Control	Adequate	(-)	0.25 ± 0.01	3.6 ± 0.1	0.96 ± 0.04	0.16 ± 0.01	0.36 ± 0.01
		(+)	0.26 ± 0.03	3.5 ± 0.2	0.95 ± 0.07	0.17 ± 0.01	0.39 ± 0.02
	Toxic	(-)	0.28 ± 0.02	4.3 ± 0.2	0.88 ± 0.07	0.17 ± 0.01	0.33 ± 0.01
		(+)	0.27 ± 0.01	3.8 ± 0.2	0.87 ± 0.04	0.17 ± 0.01	0.34 ± 0.00
Moderate	Adequate	(-)	0.23 ± 0.01	3.9 ± 0.1	0.85 ± 0.06	0.15 ± 0.01	0.39 ± 0.02
		(+)	0.25 ± 0.02	3.6 ± 0.1	0.80 ± 0.03	0.15 ± 0.00	0.38 ± 0.02
	Toxic	(-)	0.27 ± 0.02	4.3 ± 0.2	0.79 ± 0.06	0.15 ± 0.01	0.37 ± 0.03
		(+)	0.30 ± 0.03	4.1 ± 0.0	0.76 ± 0.05	0.16 ± 0.00	0.38 ± 0.01
High	Adequate	(-)	0.23 ± 0.01	3.6 ± 0.2	0.99 ± 0.12	0.15 ± 0.01	0.38 ± 0.02
		(+)	0.26 ± 0.02	3.6 ± 0.2	0.82 ± 0.10	0.14 ± 0.01	0.40 ± 0.03
	Toxic	(-)	0.24 ± 0.02	3.6 ± 0.6	1.37 ± 0.41	0.17 ± 0.02	0.36 ± 0.01
		(+)	0.28 ± 0.02	3.9 ± 0.2	0.70 ± 0.03	0.15 ± 0.01	0.36 ± 0.01

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Shoot P Concentration: 0.01; *n.s.*; 0.01; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot K Concentration: *n.s.*; 0.21; 0.14; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Shoot Ca Concentration: 0.08; 0.12; *n.s.*; 0.20; 0.15; *n.s.*; 0.33.

Shoot Mg Concentration: *n.s.*; 0.01; 0.01; 0.01; *n.s.*; *n.s.*; *n.s.*

Shoot S Concentration: *n.s.*; 0.02; 0.01; *n.s.*; *n.s.*; 0.03; *n.s.*

Table 3.12 shows the shoot macronutrient concentrations of Saricanak-98 cultivar. Only shoot Ca concentration of Saricanak-98 was significantly affected from interaction of all sources of variability. The highest shoot Ca concentration of Saricanak-98 was recorded when toxic B-treated and high salinity exposed treatment groups were grown without inoculation. While shoot P concentration significantly increased by *P. indica* inoculation, Ca concentration was reduced due to root colonization of *P. indica* in Saricanak-98. Effect of B toxicity on shoot P, K, Mg and S concentration were significant. There was consistency in terms of effects of B toxicity on shoot P and K concentration between the third and fourth experiment. B toxicity significantly increased shoot P and K concentration comparing to adequate B supply (Table 3.12; 3.10; 3.8). However, effects of B toxicity on shoot S concentration were found insignificant (Table 3.8) or significant (Table 3.10; 3.12). Additionally, even significant effects of B toxicity on shoot S concentration were not consistent with each other because it varied according to cultivar.

As shown in Table 3.13, only shoot Fe and Zn concentration significantly affected by *P. indica* inoculation. *P. indica* inoculation significantly increased shoot Zn concentration while shoot Fe concentration was significantly reduced by inoculation (Table 3.13). Effects of *P. indica* inoculation on shoot Zn concentration may change according to soil status and development period of wheat. In autoclaved soil, inoculation decreased Zn concentration of wheat in grain and straw (Table 3.5; 3.7). However, during vegetative period, shoot Zn concentration of wheat grown in non-autoclaved soil significantly increased by *P. indica* inoculation (Table 3.11; 3.13).

Furthermore, the significant interaction among all source of variability revealed that the highest and lowest shoot Zn concentration in Saricanak-98 cultivar were found when *P. indica* inoculated Saricanak-98 cultivar grown under high salinity with toxic B treatment and control salinity with adequate B supply. It is obvious that salinity and B supply had significant effects of Zn concentration. Additionally, shoot Zn, Mn, Fe and Cu concentration significantly affected by B supply. Shoot Cu and Mn concentration of Saricanak-98 were significantly higher than adequate B treated one (Table 3.13). The enhancement of Mn and Cu concentration mediated by B toxicity was also observed in third experiment for both Nusrat and Saricanak-98 (Table 3.9). Additionally, in the fourth experiment, Nusrat shoot Mn and Cu concentration was also significantly increased due to the toxic B treatment (Table 3.11).

On the other hand, B toxicity applied Saricanak-98 concentrated lower Zn and Fe in shoot than non-treated one (Table 3.13). This result may be the validation of previously obtained findings in the third experiment (Table 3.9).

Table 3. 13: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on selected micronutrient concentration of 32-day-olds durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Control	Adequate	(-)	13 ± 1	64 ± 3	32 ± 2	22 ± 2
		(+)	14 ± 1	78 ± 9	33 ± 2	24 ± 4
	Toxic	(-)	16 ± 2	64 ± 1	39 ± 4	9 ± 1
		(+)	14 ± 1	51 ± 5	38 ± 2	11 ± 1
Moderate	Adequate	(-)	15 ± 1	68 ± 5	39 ± 5	21 ± 3
		(+)	14 ± 0	62 ± 6	40 ± 4	21 ± 1
	Toxic	(-)	18 ± 4	65 ± 17	52 ± 10	9 ± 2
		(+)	16 ± 1	44 ± 6	43 ± 4	8 ± 1
High	Adequate	(-)	13 ± 2	64 ± 5	46 ± 3	15 ± 1
		(+)	14 ± 1	61 ± 2	52 ± 7	22 ± 2
	Toxic	(-)	17 ± 0	54 ± 8	51 ± 4	12 ± 1
		(+)	16 ± 1	40 ± 2	45 ± 4	8 ± 2

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Shoot Cu Concentration: n.s; 1; 1; n.s.; 2; n.s.; n.s.

Shoot Fe Concentration: 4; 6; 4; 11; 8; n.s.; n.s.

Shoot Mn Concentration: n.s.; 42; 3; n.s.; 5; 7; n.s.

Shoot Zn Concentration: 1; 2; 1; n.s.; 2; 3; 5

As shown in Table 3.14, Proline concentration of Saricanak-98 and Nusrat were significantly affected by salinity. The highest Proline concentrations were found in the high salinity treated groups in both cultivars. Additionally, shoot proline concentration of Saricanak-98 which grown under toxic B treatment was recorded as significantly higher than counterparts which were treated with adequate B supply. High salinity with toxic B supply caused significantly highest proline accumulation in shoot of Saricanak-98.

Table 3. 14: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on leaves Glycine Betaine and Proline concentration of 32-day-olds bread (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	Saricanak-98		Nusrat	
			Glycine Betaine (%)	Proline (mg.kg ⁻¹)	Glycine Betaine (%)	Proline (mg.kg ⁻¹)
Control	Adequate	(-)	0.9 ± 0.1	22 ± 0	0.7 ± 0.1	23 ± 1
		(+)	0.7 ± 0.1	25 ± 4	0.7 ± 0.1	24 ± 3
	Toxic	(-)	0.6 ± 0.0	24 ± 2	0.6 ± 0.1	23 ± 1
		(+)	0.6 ± 0.1	23 ± 2	0.6 ± 0.1	25 ± 5
Moderate	Adequate	(-)	0.8 ± 0.1	22 ± 1	0.9 ± 0.1	22 ± 1
		(+)	0.8 ± 0.1	24 ± 1	1.1 ± 0.4	21 ± 2
	Toxic	(-)	0.9 ± 0.3	24 ± 4	0.8 ± 0.2	23 ± 1
		(+)	0.6 ± 0.1	25 ± 3	0.9 ± 0.2	23 ± 2
High	Adequate	(-)	0.9 ± 0.2	25 ± 1	0.8 ± 0.2	24 ± 1
		(+)	0.7 ± 0.1	23 ± 1	0.8 ± 0.2	24 ± 4
	Toxic	(-)	0.9 ± 0.1	30 ± 6	0.9 ± 0.1	26 ± 2
		(+)	0.7 ± 0.0	28 ± 2	0.7 ± 0.0	24 ± 3

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Nusrat Glycine Betaine Concentration: *n.s.*; 0.2: *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Saricanak-98 Glycine Betaine Concentration: 0.08; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*; 0.3

Nusrat Proline Concentration: *n.s.*; 2; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Saricanak-98 Proline Concentration: *n.s.*; 2; 2; *n.s.*; *n.s.*; 4; *n.*

Shoot Glycine Betaine concentration of Nusrat cultivar were significantly influenced by salinity. Accumulation of the Glycine Betaine in shoot was higher moderate and high salinity comparing to control ones. In Saricanak-98 the highest shoot Glycine Betaine concentration was recorded when non-inoculated Saricanak-98 grown under moderate salinity with toxic B supply (Table 3.14).

Table 3.15 designed to explain the how Phenylalanine ammonia-lyase (PAL) and Trolox equivalent antioxidant capacity (TEAC) were changed by source variabilities in Nusrat and Saricanak-98. Specific PAL activity of Saricanak-98 was affected by interaction of all factors. Inoculation affected specific PAL activity of both cultivars significantly. While specific PAL activity of Nusrat significantly increased by *P. indica* inoculation, in Saricanak-98 Pal activity significantly reduced by inoculation. In both cultivar, B toxicity significantly reduced specific PAL activity.

Table 3. 15: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on leaves Phenylalanine ammonia-lyase (PAL) activity and Trolox equivalent antioxidant capacity (TEAC) of 32-day-olds bread (*Triticum aestivum* cv. Nusrat) durum wheat (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	Saricanak-98		Nusrat	
			Sp. PAL ACT. ($\mu\text{mol TCA}$ $\text{mg}^{-1} \text{ prt. hour}^{-1}$)	TEAC ($\mu\text{mol TE}$ $\text{g}^{-1} \text{ TA}$)	Sp. PAL ACT. ($\mu\text{mol TCA}$ $\text{mg}^{-1} \text{ prt. hour}^{-1}$)	TEAC ($\mu\text{mol TE}$ $\text{g}^{-1} \text{ TA}$)
Control	Adequate	(-)	1.5 $\pm$ 0.0	16 $\pm$ 3	1.3 $\pm$ 0.1	13 $\pm$ 1
		(+)	1.4 $\pm$ 0.2	21 $\pm$ 3	1.9 $\pm$ 0.2	16 $\pm$ 1
	Toxic	(-)	1.9 $\pm$ 0.6	15 $\pm$ 2	1.2 $\pm$ 0.2	18 $\pm$ 2
		(+)	1.3 $\pm$ 0.2	21 $\pm$ 2	1.4 $\pm$ 0.2	16 $\pm$ 3
Moderate	Adequate	(-)	2.3 $\pm$ 0.2	16 $\pm$ 1	1.3 $\pm$ 0.2	16 $\pm$ 2
		(+)	1.4 $\pm$ 0.1	17 $\pm$ 4	1.3 $\pm$ 0.2	16 $\pm$ 4
	Toxic	(-)	1.6 $\pm$ 0.2	19 $\pm$ 2	1.2 $\pm$ 0.1	17 $\pm$ 2
		(+)	1.1 $\pm$ 0.1	21 $\pm$ 3	1.4 $\pm$ 0.2	17 $\pm$ 3
High	Adequate	(-)	1.4 $\pm$ 0.2	21 $\pm$ 4	1.2 $\pm$ 0.1	19 $\pm$ 3
		(+)	1.3 $\pm$ 0.1	25 $\pm$ 2	1.3 $\pm$ 0.3	16 $\pm$ 1
	Toxic	(-)	1.3 $\pm$ 0.3	24 $\pm$ 3	1.1 $\pm$ 0.0	20 $\pm$ 3
		(+)	1.2 $\pm$ 0.2	24 $\pm$ 2	1.1 $\pm$ 0.1	18 $\pm$ 1

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I $\times$ S; I $\times$ B; S $\times$ B; I $\times$ S $\times$ B

Saricanak-98 Specific PAL Activity: 0.1; 0.2; 0.1; 0.4; *n.s.*; 0.4; 0.6

Nusrat Specific PAL Activity: 0.1; 0.1; 0.1; 0.3; *n.s.*; 0.3; *n.s.*

Saricanak-98 TEAC: 2; 3; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Nusrat TEAC: *n.s.*; 1.9; 1.3; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Trolox equivalent antioxidant capacity (TEAC) of Saricanak-98 was improved by *P. indica* inoculation by 16% when compared to non-inoculation. In both cultivars, increased of salinity levels caused increment of TEAC. Moreover, significant enhancement of TEAC in Nusrat against B toxicity was recorded (Table 3.15).

Table 3. 16: Effects of inoculation with *P. indica* on specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POX) enzymes of leaves of 32-days-old bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 6 dS/m; high: 9 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	SOD (U mg ⁻¹ prt.)	GR (µmol [NADPH] mg ⁻¹ prt. min ⁻¹)	APX (µmol H ₂ O ₂ mg ⁻¹ prt. min ⁻¹)	CAT (µmol H ₂ O ₂ mg ⁻¹ prt. min ⁻¹)	POX (mmol Tetraguaiaco l mg ⁻¹ prt. min ⁻¹)
Control	Adequate	(-)	4.5 ± 0.2	163 ± 14	3.1 ± 0.2	134 ± 23	1.5 ± 0.2
		(+)	6.3 ± 0.6	219 ± 16	4.4 ± 0.7	265 ± 57	2.0 ± 0.3
	Toxic	(-)	3.9 ± 0.5	174 ± 6	3.0 ± 0.4	182 ± 24	1.6 ± 0.3
		(+)	5.0 ± 0.4	184 ± 11	3.4 ± 0.2	187 ± 44	1.4 ± 0.3
Moderate	Adequate	(-)	3.5 ± 0.5	154 ± 21	2.0 ± 0.4	140 ± 20	0.9 ± 0.1
		(+)	4.8 ± 0.6	151 ± 26	2.4 ± 0.5	214 ± 37	1.3 ± 0.3
	Toxic	(-)	3.7 ± 0.1	163 ± 10	2.2 ± 0.5	148 ± 24	1.3 ± 0.2
		(+)	4.8 ± 0.8	165 ± 18	3.3 ± 0.5	189 ± 27	1.5 ± 0.2
High	Adequate	(-)	3.6 ± 0.4	161 ± 18	1.9 ± 0.3	155 ± 25	1.4 ± 0.3
		(+)	4.2 ± 0.7	144 ± 19	2.1 ± 0.6	174 ± 28	1.3 ± 0.3
	Toxic	(-)	3.4 ± 0.2	125 ± 11	1.9 ± 0.3	154 ± 22	1.1 ± 0.2
		(+)	4.0 ± 0.4	139 ± 9	1.9 ± 0.4	216 ± 13	1.1 ± 0.1

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

SOD: 0.3; 0.4; 0.3; *n.s.*; *n.s.*; 0.7; *n.s.*

GR: 9; 14; *n.s.*; 24; *n.s.*; 24; 40

APX: 0.3; 0.4; *n.s.*; 0.6; *n.s.*; *n.s.*; 0.6; 1.1

CAT: 18; *n.s.*; *n.s.*; *n.s.*; 34; *n.s.*; 76

POX: 0.1; 0.2; *n.s.*; *n.s.*; *n.s.*; 0.4; *n.s.*

Specific activities of major enzymes namely superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POX) were determined in 32-days-old leaves of Nusrat cultivar. The result of enzymes assays showed that except SOD and POX, remaining above mentioned enzymes were significantly affected by interaction of all sources of variabilities. For instance, *P. indica* inoculated Nusrat cultivar grown under control salinity with adequate B supply resulted with the highest specific CAT and GR activity. Additionally, the lowest specific activities of CAT and GR were detected in non-inoculated Nusrat grown under control salinity with adequate B supply and high salinity with toxic B treatment, respectively. The third enzymes which its specific activities were significantly modified due to interaction of all independent variables was APX. The highest and lowest specific activities of APX enzymes were determined when inoculated and non-inoculated Nusrat cultivar were exposed to control salinity with adequate B and high salinity with toxic B, respectively. *P. indica* inoculation had significant effects on all of the specific enzyme activities which are listed in Table 3.16. As expected, all the specific enzymes activities were significantly enhanced by *P. indica* inoculation. 28%, 24%, 36%, 7% and 11% increases in specific enzyme activities of SOD, APX, CAT, GR, and POX by *P. indica* inoculation were determined compared to non-inoculated cases (Table 3.16).

Only specific activities of SOD were affected by B treatment and B toxicity decreased the activity by 7% compared to adequate B supply. Certain enzymes are seemed to more sensitive against salinity stress such as SOD, APX, GR and POX. Reduction of all the enzymes activities against increasing salinity were observed. The highest enzymes activities were recorded under control salinity but increasing salinity significantly reduced activities (Table 3.16).

Table 3.17 showed specific enzymes activities of 32-days-old Saricanak-98 leaves. Except POX, all the remaining specific enzyme activities were significantly affected by interaction of salinity, B and *P. indica*. Specific activities of CAT, APX and SOD were recorded as the lowest when non-inoculated Saricanak-98 were grown under high salinity and toxic B. Only SOD, GR and POX were significantly affected by inoculation and *P. indica* inoculation decreased their specific activities, significantly. Main effect of salinity was the reason of significant changes in the specific activities of all the enzymes that are listed in Table 3.17.

For all the specific enzymes activities, activity-reducing effects of high salinity was common. The lowest specific activities of all the enzymes were measured under high salinity. Furthermore, specific enzyme activities of SOD, GR and CAT were decreased by 12%, 17% and 14% due to toxic B supply compared to adequate B treatment (Table 3.17).

Table 3. 17: Effects of inoculation with *P. indica* on specific activities of superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), catalase (CAT) and peroxidase (POX) enzymes of leaves of 32-days-old durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	SOD (U mg ⁻¹ prt.)	GR (μ mol [NADPH] mg ⁻¹ prt. min ⁻¹)	APX (μ mol H ₂ O ₂ mg ⁻¹ prt. min ⁻¹)	CAT (μ mol H ₂ O ₂ mg ⁻¹ prt. min ⁻¹)	POX (mmol Tetraguaiacol mg ⁻¹ prt. min ⁻¹)
Control	Adequate	(-)	4.1 $\pm$ 0.1	220 $\pm$ 28	3.0 $\pm$ 0.1	205 $\pm$ 17	1.3 $\pm$ 0.1
		(+)	4.2 $\pm$ 0.4	183 $\pm$ 14	3.6 $\pm$ 0.6	264 $\pm$ 52	1.3 $\pm$ 0.2
	Toxic	(-)	4.8 $\pm$ 0.1	185 $\pm$ 13	4.1 $\pm$ 0.6	221 $\pm$ 39	1.6 $\pm$ 0.0
		(+)	3.7 $\pm$ 0.3	143 $\pm$ 12	2.7 $\pm$ 0.8	239 $\pm$ 13	1.1 $\pm$ 0.0
Moderate	Adequate	(-)	6.1 $\pm$ 1.0	274 $\pm$ 22	4.0 $\pm$ 0.7	387 $\pm$ 92	1.9 $\pm$ 0.4
		(+)	4.2 $\pm$ 0.3	154 $\pm$ 13	2.8 $\pm$ 0.2	241 $\pm$ 9	1.2 $\pm$ 0.2
	Toxic	(-)	5.0 $\pm$ 0.2	187 $\pm$ 24	3.2 $\pm$ 0.2	226 $\pm$ 37	1.7 $\pm$ 0.1
		(+)	3.7 $\pm$ 0.4	130 $\pm$ 12	3.0 $\pm$ 0.8	226 $\pm$ 20	1.0 $\pm$ 0.1
High	Adequate	(-)	4.2 $\pm$ 0.6	175 $\pm$ 16	2.2 $\pm$ 0.1	209 $\pm$ 42	1.3 $\pm$ 0.3
		(+)	4.1 $\pm$ 0.2	128 $\pm$ 11	2.9 $\pm$ 0.3	234 $\pm$ 32	1.3 $\pm$ 0.0
	Toxic	(-)	2.9 $\pm$ 0.3	159 $\pm$ 19	1.7 $\pm$ 0.5	200 $\pm$ 23	1.4 $\pm$ 0.4
		(+)	3.4 $\pm$ 0.4	132 $\pm$ 20	2.8 $\pm$ 0.8	214 $\pm$ 15	1.0 $\pm$ 0.1

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

SOD: 0.3; 0.4; 0.3; 0.6; *n.s.*; 0.6; 1.0

GR: 10; 15; 10; 27; 19.5; 27; 44

APX: *n.s.*; 0.5; *n.s.*; 0.8; *n.s.*; *n.s.* 1.4

CAT: *n.s.*; 34; 23; 59; *n.s.*; 59; 97

POX: 0.1; 0.2; *n.s.*; 0.3; 0.2; *n.s.*; *n.s.*

3.5. Results of Fifth Experiment

The fifth experiment was conducted to investigate effects of *P. indica* inoculation on yield and mineral homeostasis of durum and bread wheat plants grown under salinity treatments and B supply.

As shown in Figure 3.16, bread wheat cultivar *Triticum aestivum* cv. Nusrat was grown under in the absence or presence of *P. indica* inoculation. *P. indica* inoculation increased number of tillers per plant but significant differences between the *P. indica* inoculated plants and non-inoculated ones could not be observed visually. However, effects of toxic B to general appearance of *Triticum aestivum* cv. Nusrat plant may identify with naked eyes.

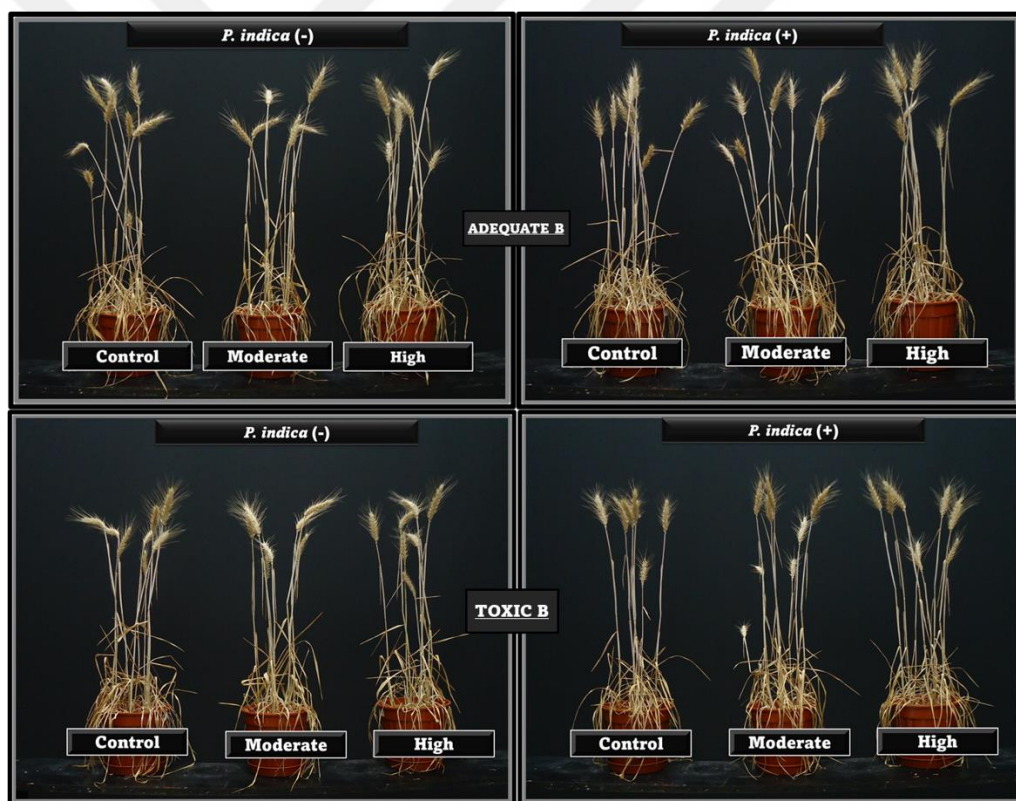


Figure 3.16: Observation of effects of *P. indica* colonization on morphology of 98-day-olds bread wheat (*Triticum aestivum* cv. Nusrat) plants grown under different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies in non-autoclaved soil under greenhouse conditions.

Durum wheat cultivar *Triticum durum* cv. Sarıçanak was exhibit similar physiological responses that Nusrat cultivar did against inoculation status and stress conditions. Figure 3.17 demonstrated that stem thickness of *Triticum durum* cv. Sarıçanak cultivars with *P. indica* inoculation seem to be greater than non-inoculated counterparts. However, it is not possible to accurately assess the effects of the *P. indica* on yield just examining Figure 3.17.



Figure 3.17: Observation of effects of *P. indica* colonization on morphology of 98-day-olds durum wheat (*Triticum durum* cv. Sarıçanak-98) plants grown under different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies in non-autoclaved soil under greenhouse conditions.

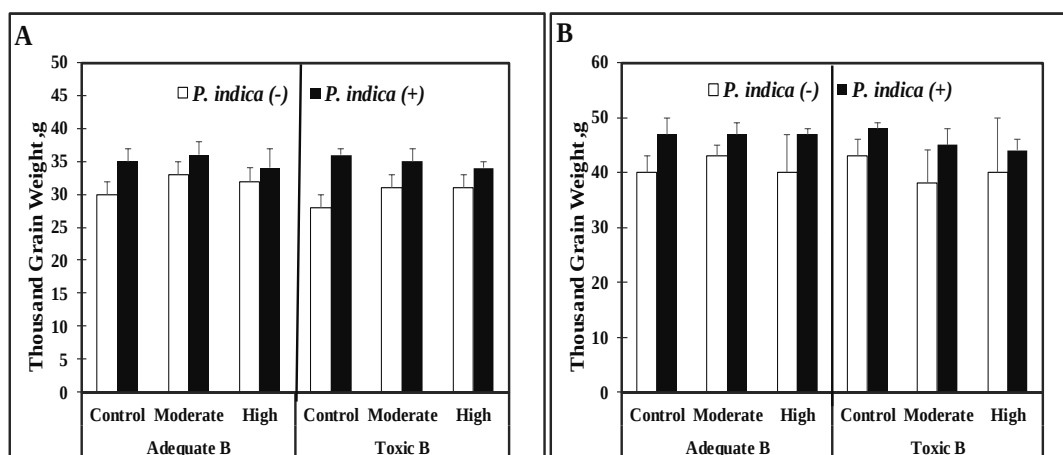


Figure 3.18: : Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on thousand grain weight of 32-day-olds bread wheat (A) (*Triticum aestivum* cv. Nusrat) and durum wheat (B) (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.

Figure 3.18 shows the thousand grain weight of Nusrat and Saricanak-98 cultivar. *P. indica* inoculation significantly increased thousand grain weight by 16% and 15% in Nusrat and Saricanak-98 compared to non-inoculated conditions, respectively. The main effects of the remaining factors did not exhibit any significant effects on thousand grain weight.

Triticum aestivum cv. Nusrat was harvested to estimate changes in grain yield, straw dry weight, and harvest index (Table 3.18). Although, grain yield of *Triticum aestivum* cv. Nusrat cultivar did not significantly affected by inoculation status, main effects of salinity and B supply showed significant influence on grain yield. Grain yield and harvest index of *Triticum aestivum* cv. Nusrat was significantly increased by moderate and high salinity treatment when compared to control ones. Adequate B supply was also significantly enhanced grain yield comparing to toxic B supply (Table 3.18).

Table 3. 18: Effects of inoculation with *P. indica* on grain yield, straw dry weight and harvest index of bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	Grain Yield (g plant ⁻¹)	Straw Dry Weight (g plant ⁻¹)	Harvest Index (%)
Control	Adequate	(-)	1.8 ± 0.1	2.7 ± 0.2	40 ± 1
		(+)	1.8 ± 0.2	2.6 ± 0.1	41 ± 3
	Toxic	(-)	1.6 ± 0.2	2.5 ± 0.1	40 ± 3
		(+)	1.5 ± 0.1	2.4 ± 0.2	40 ± 3
Moderate	Adequate	(-)	2.1 ± 0.1	2.5 ± 0.2	46 ± 2
		(+)	2.0 ± 0.3	2.8 ± 0.2	42 ± 2
	Toxic	(-)	2.0 ± 0.2	2.4 ± 0.3	45 ± 1
		(+)	1.9 ± 0.0	2.3 ± 0.2	45 ± 2
High	Adequate	(-)	2.2 ± 0.2	2.3 ± 0.2	49 ± 3
		(+)	1.9 ± 0.2	2.2 ± 0.2	46 ± 2
	Toxic	(-)	1.7 ± 0.3	2.3 ± 0.3	43 ± 6
		(+)	1.7 ± 0.2	2.1 ± 0.1	45 ± 4

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

Grain Yield: *n.s.*; 0.2; 0.1; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Straw Dry Weight: *n.s.*; 0.18; 0.12; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Harvest Index: *n.s.*; 2; *n.s.*; *n.s.*; *n.s.*; 4; *n.s.*

Table 3.19 shows that main effect of the salinity, B supply and inoculation had significant effects on the straw dry weight of *Triticum durum* cv. Sarıçanak. Adequate B supply significantly increased grain yield and straw dry weight, when compared to B toxicity. Thousand seed weight and harvest index were recorded significantly higher under inoculation with *P. indica* than non-inoculated conditions. Moreover, main effects of salinity revealed that moderate salinity treatment provide significantly higher grain yield when compared to control and high salinity.

Furthermore, there were significant interactions between inoculation with B supply and salinity with B supply in grain yield of *Triticum durum* cv. Sarıçanak. The lowest grain yield was obtained when non-inoculated *Triticum durum* cv. Sarıçanak grown under B toxicity with high salinity.

Table 3. 19: Effects of inoculation with *P. indica* on grain yield, straw dry weight, and harvest index of durum wheat (*Triticum aestivum* cv. Sarıçanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	Grain Yield (g plant ⁻¹)	Straw Dry Weight (g plant ⁻¹)	Harvest Index (%)
Control	Adequate	(-)	1.8 ± 0.2	2.7 ± 0.2	39 ± 4
		(+)	1.7 ± 0.2	2.5 ± 0.2	41 ± 4
	Toxic	(-)	1.5 ± 0.1	2.1 ± 0.0	42 ± 2
		(+)	1.7 ± 0.2	2.0 ± 0.1	46 ± 2
Moderate	Adequate	(-)	2.3 ± 0.2	2.7 ± 0.2	46 ± 1
		(+)	1.9 ± 0.2	2.3 ± 0.1	45 ± 3
	Toxic	(-)	1.5 ± 0.4	1.9 ± 0.3	44 ± 4
		(+)	1.6 ± 0.1	1.7 ± 0.2	49 ± 3
High	Adequate	(-)	1.7 ± 0.1	2.4 ± 0.3	42 ± 2
		(+)	1.7 ± 0.1	2.1 ± 0.1	45 ± 2
	Toxic	(-)	1.2 ± 0.3	1.6 ± 0.2	43 ± 3
		(+)	1.6 ± 0.1	1.7 ± 0.1	48 ± 0

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

Grain Yield: *n.s.*; 0.2; 0.1; *n.s.*; 0.2; 0.3; *n.s.*

Straw Dry Weight: 0.1; 0.2; 0.1; *n.s.*; 0.2; *n.s.*; *n.s.*

Harvest Index: 2; 2; 2; *n.s.*; 3; *n.s.*; *n.s.*

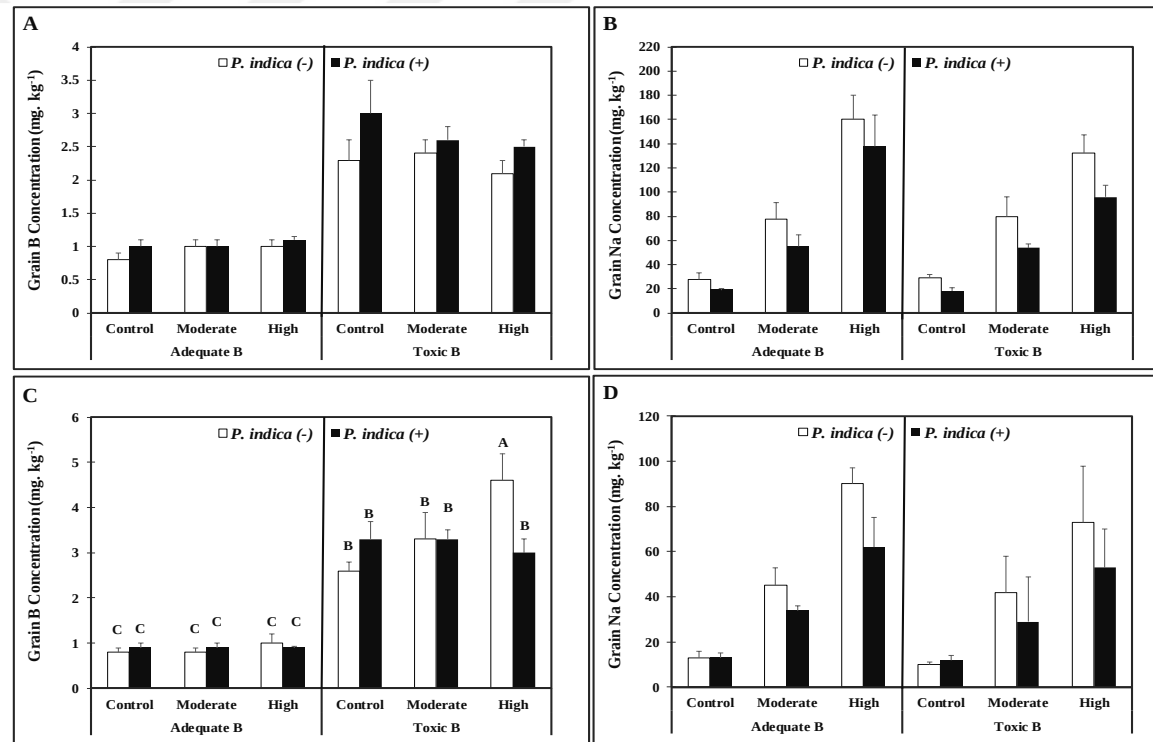


Figure 3.19: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on grain B (A;C) and Na (B;D) concentration of bread wheat (A;B) (*Triticum aestivum* cv. Nusrat) and durum wheat (C;D) (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation. Different letters indicate significant differences between means of treatments group according to Tukey's protected HSD test. Letters appear above the bar if the interaction of the three variables (Salinity levels $\times$ B supply $\times$ Inoculation) is significant.

Figure 3.19 shows the grain B and Na concentration of Nusrat and Saricanak-98 cultivar. 1.5-fold and 2.8-fold increase in grain B concentration of Nusrat and Saricanak-98 were caused by B toxicity (Figure 3.19A; C). The increases in shoot B concentration because of toxic B supply was 25-fold in Nusrat and Saricanak-98 in the fourth experiment (Figure 3.15A; C). This result is particularly important as a validation of low phloem mobility of B in wheat. In Nusrat, grain B concentration was significantly reduced by *P. indica* inoculation. Furthermore, interaction between the inoculation and B supply was found significant in Nusrat. The interaction indicates that *P. indica* inoculation with B toxicity resulted with the highest B concentration. Furthermore, antagonistic relationship between B and salinity was observed in both cultivars. Increases of severity of salinity decreases B concentration significantly. Therefore, the highest grain B concentration of Nusrat and Saricanak-98 were determined under control salinity with toxic B application. Grain B concentration of Saricanak-98 was influenced by interaction of all factors. The highest and lowest grain B concentrations were found non-inoculated Saricanak-98 was exposed to high salinity with toxic B and moderate salinity with adequate B (Figure 3.19).

Grain Na concentration of Nusrat and Saricanak-98 were significantly affected by salinity and inoculation. *P. indica* inoculation significantly decreased grain Na concentration in Nusrat and Saricanak-98. Moreover, in both cultivar Na concentration significantly increased when severity of salinity increased. Significant interaction between salinity and B supply in Saricanak-98 showed that in the absence of *P. indica* inoculation with high salinity Na concentrations reached the highest level. Significant interaction between salinity and B supply revealed that high salinity with adequate B supply resulted with the highest grain Na concentration in Nusrat, while the lowest one was recorded under control salinity with adequate B application (Figure 3.19).

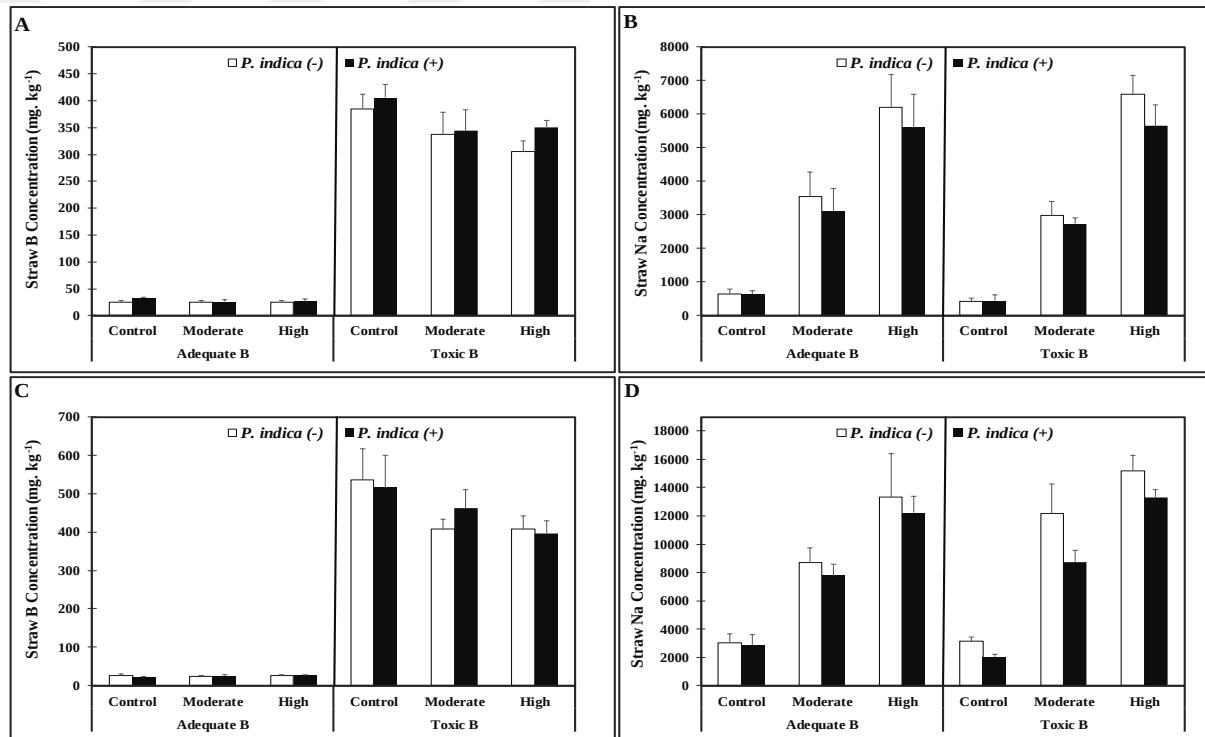


Figure 3.20: Effects of different salinity treatments (control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) and adequate (1 mg/kg B) or toxic (20 mg/kg B) B supplies on grain B (A;C) and Na (B;D) concentration of bread wheat (A;B) (*Triticum aestivum* cv. Nusrat) and durum wheat (C;D) (*Triticum durum* cv. Saricanak-98) plants which were inoculated with or without *P. indica* and grown in non-autoclaved soil under greenhouse conditions. Bars represent means of four independent pot replicates each containing five individual plants. Error bars represent standard deviation.

Figure 3.20 illustrates the changes of straw B and Na concentrations of Nusrat and Saricanak-98 cultivar. Interaction among salinity, inoculation and B did not exhibit any significant effect on straw B and Na concentrations of both cultivars (Figure 3.20A; B; C; D). Straw B concentration of both cultivars increased by *P. indica* inoculation especially under moderate and high salinity with toxic B supply (Figure 3.20A; C;). However, only straw B concentration of Nusrat significantly increased by in the presence of *P. indica* inoculation (Figure 3.20A).

Salinity significantly affected straw B concentration of both cultivars. The highest B concentration were observed when Saricanak-98 and Nusrat did not exposed to salinity. The lowest B concentrations were recorded in straw of high salinity treated Nusrat and Saricanak-98 cultivars (Figure 3.20A; C). Furthermore, interaction between the salinity and B was found significant. The highest straw B concentration were found when toxic B applied Nusrat and Saricanak-98 cultivars grown under control salinity. Furthermore, 12-fold and 18-fold increases in straw B concentrations of Nusrat and Saricanak-98 were caused by toxic B supply compared to adequate B supply, respectively (Figure 3.20A; C).

Straw Na concentration significantly reduced by 10% and 15% with *P. indica* inoculation in Nusrat and Saricanak-98, respectively. In both cultivars, increases in salinity levels significantly increased straw Na concentrations (Figure 3.20B; D). Moreover, straw Na concentration of Saricanak-98 was modified significantly due to B supply. Astonishingly, B toxicity significantly enhanced Na concentration compared adequate one. Moreover, the lowest Na concentration was found in straw of toxic B supplied Saricanak-98 grown under control salinity while the highest one was recorded when B toxicity applied Saricanak-98 cultivars grown under high salinity (Figure 3.20D).

Table 3. 20: Effects of inoculation with *P. indica* on selected grain macronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Boron	<i>P. indica</i>	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Control	Adequate	(-)	0.49 ± 0.03	0.55 ± 0.04	0.06 ± 0.01	0.16 ± 0.01	0.23 ± 0.01
		(+)	0.43 ± 0.06	0.47 ± 0.04	0.05 ± 0.00	0.16 ± 0.01	0.24 ± 0.01
	Toxic	(-)	0.45 ± 0.03	0.56 ± 0.02	0.07 ± 0.00	0.15 ± 0.00	0.23 ± 0.01
		(+)	0.43 ± 0.01	0.49 ± 0.03	0.05 ± 0.01	0.16 ± 0.01	0.25 ± 0.02
Moderate	Adequate	(-)	0.47 ± 0.04	0.55 ± 0.02	0.05 ± 0.00	0.16 ± 0.01	0.22 ± 0.01
		(+)	0.43 ± 0.07	0.51 ± 0.02	0.05 ± 0.01	0.16 ± 0.01	0.23 ± 0.00
	Toxic	(-)	0.47 ± 0.03	0.57 ± 0.03	0.06 ± 0.00	0.16 ± 0.00	0.23 ± 0.02
		(+)	0.39 ± 0.04	0.49 ± 0.03	0.05 ± 0.00	0.15 ± 0.01	0.23 ± 0.01
High	Adequate	(-)	0.50 ± 0.05	0.58 ± 0.04	0.06 ± 0.00	0.17 ± 0.01	0.22 ± 0.00
		(+)	0.43 ± 0.06	0.51 ± 0.03	0.05 ± 0.00	0.16 ± 0.01	0.22 ± 0.01
	Toxic	(-)	0.49 ± 0.04	0.57 ± 0.02	0.06 ± 0.01	0.16 ± 0.01	0.22 ± 0.01
		(+)	0.42 ± 0.02	0.51 ± 0.01	0.06 ± 0.00	0.16 ± 0.00	0.23 ± 0.01

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Grain P Concentration: 0.03; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain K Concentration: 0.02; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain Ca Concentration: 0.003; 0.003; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain Mg Concentration: *n.s.*; *n.s.*; 0.01; *n.s.*; *n.s.*; *n.s.*

Grain S Concentration: 0.01; 0.01; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Table 3.20 shows the changes of selected grain macronutrient concentrations of Nusrat. Except grain Mg concentration of *Triticum aestivum* cv. Nusrat, all the remaining minerals which are listed in Table 3.20 significantly affected from the inoculation. *P. indica* inoculation significantly reduced grain concentration of P, K, Ca, while it increased S concentration in grain. Additionally, grain Mg concentration significantly increased under adequate B supply when compared to toxic B supply. Moreover, high, and moderate salinity decreased grain Ca and S concentration compared to control salinity (Table 3.20).

Table 3. 21: Effects of inoculation with *P. indica* on selected grain micronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Boron	<i>P. indica</i>	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Control	Adequate	(-)	7.3 ± 0.3	42 ± 2	33 ± 2	33 ± 3
		(+)	7.0 ± 0.2	40 ± 4	35 ± 1	27 ± 4
	Toxic	(-)	7.3 ± 0.1	41 ± 3	35 ± 2	31 ± 4
		(+)	7.8 ± 0.3	42 ± 4	40 ± 2	30 ± 3
Moderate	Adequate	(-)	7.6 ± 0.5	41 ± 3	39 ± 3	38 ± 5
		(+)	7.4 ± 0.5	39 ± 1	38 ± 2	34 ± 9
	Toxic	(-)	7.9 ± 0.2	40 ± 3	41 ± 3	34 ± 4
		(+)	7.7 ± 0.3	37 ± 2	40 ± 2	27 ± 3
High	Adequate	(-)	7.6 ± 0.4	41 ± 4	46 ± 3	43 ± 7
		(+)	8.1 ± 0.5	37 ± 5	39 ± 4	33 ± 6
	Toxic	(-)	7.7 ± 0.3	39 ± 6	47 ± 3	45 ± 5
		(+)	8.2 ± 0.2	37 ± 2	44 ± 2	33 ± 5

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Grain Cu Concentration: *n.s.*; 0.3; 0.2; 0.5; *n.s.*; *n.s.*; *n.s.*

Grain Fe Concentration: *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain Mn Concentration: *n.s.*; 2; 2; 4; *n.s.*; *n.s.*; *n.s.*

Grain Zn Concentration: 3; 4; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Table 3.21 shows the grain micronutrient concentration of Nusrat. Only grain Zn concentration was affected from *P. indica* inoculation. *P. indica* inoculation significantly reduced Zn concentration. Moreover, increasing salinity significantly increases grain Mn and Zn concentration. Furthermore, grain Mn and Cu concentration were significantly higher in toxic B supplied plants than adequate B supplied plants (Table 3.21). Cu and Mn concentration improved effects of B toxicity was observed in previous experiment, as well (Table 3.9; 11; 13)

Table 3. 22: Effects of inoculation with *P. indica* on selected grain macronutrient concentration of durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing four individual plants.

Salinity	Boron	<i>P. indica</i>	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Control	Adequate	(-)	0.36 ± 0.02	0.51 ± 0.05	0.05 ± 0.00	0.12 ± 0.01	0.22 ± 0.01
		(+)	0.34 ± 0.05	0.43 ± 0.02	0.05 ± 0.00	0.12 ± 0.01	0.22 ± 0.01
	Toxic	(-)	0.41 ± 0.06	0.53 ± 0.04	0.05 ± 0.01	0.12 ± 0.01	0.21 ± 0.01
		(+)	0.38 ± 0.04	0.46 ± 0.02	0.05 ± 0.00	0.12 ± 0.00	0.21 ± 0.01
Moderate	Adequate	(-)	0.32 ± 0.04	0.46 ± 0.01	0.04 ± 0.00	0.11 ± 0.01	0.21 ± 0.01
		(+)	0.32 ± 0.04	0.42 ± 0.01	0.04 ± 0.00	0.13 ± 0.02	0.21 ± 0.01
	Toxic	(-)	0.36 ± 0.04	0.55 ± 0.09	0.05 ± 0.01	0.12 ± 0.01	0.21 ± 0.01
		(+)	0.40 ± 0.06	0.51 ± 0.01	0.05 ± 0.00	0.13 ± 0.01	0.21 ± 0.01
High	Adequate	(-)	0.36 ± 0.03	0.49 ± 0.04	0.04 ± 0.01	0.14 ± 0.02	0.22 ± 0.01
		(+)	0.36 ± 0.06	0.45 ± 0.02	0.04 ± 0.01	0.13 ± 0.01	0.21 ± 0.00
	Toxic	(-)	0.43 ± 0.06	0.49 ± 0.03	0.05 ± 0.01	0.13 ± 0.01	0.22 ± 0.01
		(+)	0.32 ± 0.01	0.47 ± 0.01	0.04 ± 0.00	0.11 ± 0.00	0.20 ± 0.01

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Grain P Concentration: *n.s.*; *n.s.*; 0.03; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain K Concentration: 0.02; *n.s.*; 0.02; *n.s.*; *n.s.*; 0.06; *n.s.*

Grain Ca Concentration: 0.003; 0.004; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain Mg Concentration: *n.s.*; *n.s.*; *n.s.*; 0.02; *n.s.*; *n.s.*; *n.s.*

Grain S Concentration: *n.s.*; *n.s.*; *n.s.*; 0.01; *n.s.*; *n.s.*; *n.s.*

Tables 3.22 demonstrated that only grain K and Ca concentration were significantly affected by *P. indica* inoculation and inoculation with *P. indica* significantly reduced their concentration. Grain P and K concentration of toxic B supplied Saricanak-98 cultivar were significantly higher than adequate B supplied counterparts (Table 3.22). P and K concentration enhancing activity of B toxicity was observed in previous experiments of this thesis, as well (Table 3.8; 3.10; 3.12).

Table 3. 23: Effects of inoculation with *P. indica* on selected grain micronutrient concentration of durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Boron	<i>P. indica</i>	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Control	Adequate	(-)	7.3 ± 0.7	34 ± 6	25 ± 3	25 ± 2
		(+)	6.6 ± 0.3	31 ± 4	22 ± 5	22 ± 3
	Toxic	(-)	7.0 ± 0.4	27 ± 4	28 ± 4	25 ± 5
		(+)	7.3 ± 0.4	27 ± 2	29 ± 2	22 ± 1
Moderate	Adequate	(-)	7.0 ± 0.5	28 ± 1	28 ± 2	23 ± 3
		(+)	7.2 ± 0.4	26 ± 3	30 ± 6	23 ± 6
	Toxic	(-)	7.4 ± 0.4	28 ± 2	36 ± 6	25 ± 3
		(+)	7.9 ± 0.2	26 ± 1	35 ± 5	24 ± 4
High	Adequate	(-)	7.3 ± 0.3	27 ± 3	39 ± 6	31 ± 8
		(+)	7.7 ± 0.2	26 ± 2	31 ± 5	22 ± 2
	Toxic	(-)	7.8 ± 0.3	31 ± 3	43 ± 7	31 ± 8
		(+)	8.1 ± 0.3	23 ± 2	31 ± 1	20 ± 1

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

Grain Cu Concentration: *n.s.*; 0.3; 0.2; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain Fe Concentration: *n.s.*; 3; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Grain Mn Concentration: 3; 4; 3; 7; *n.s.*; *n.s.*; *n.s.*

Grain Zn Concentration: 3; *n.s.*; *n.s.*; 7; *n.s.*; *n.s.*; *n.s.*

Grain Mn and Zn concentration of Saricanak-98 cultivar were significantly affected by *P. indica* inoculation. Both minerals significantly reduced due to *P. indica* inoculation when compared to non-inoculated ones (Table 3.23). This result was also observed in grain of Nusrat (Table 3.21). Boron toxicity significantly increased grain Mn and Cu concentrations irrespective to cultivar and tissues (Table 3.23; 3.21; 3.11; 3.13; 3.19).

Table 3. 24: Effects of inoculation with *P. indica* on selected straw macronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Boron	<i>P. indica</i>	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Control	Adequate	(-)	0.04 ± 0.01	2.4 ± 0.2	1.09 ± 0.05	0.25 ± 0.03	0.26 ± 0.03
		(+)	0.04 ± 0.01	2.4 ± 0.0	1.18 ± 0.09	0.27 ± 0.01	0.30 ± 0.02
	Toxic	(-)	0.06 ± 0.01	2.8 ± 0.0	1.21 ± 0.06	0.24 ± 0.02	0.27 ± 0.03
		(+)	0.05 ± 0.00	3.2 ± 0.2	1.18 ± 0.10	0.24 ± 0.03	0.29 ± 0.02
Moderate	Adequate	(-)	0.05 ± 0.01	2.8 ± 0.1	1.14 ± 0.09	0.26 ± 0.01	0.31 ± 0.04
		(+)	0.04 ± 0.01	2.7 ± 0.3	1.15 ± 0.04	0.26 ± 0.02	0.28 ± 0.02
	Toxic	(-)	0.06 ± 0.00	2.9 ± 0.1	1.14 ± 0.12	0.23 ± 0.02	0.26 ± 0.01
		(+)	0.04 ± 0.00	3.1 ± 0.3	1.19 ± 0.10	0.22 ± 0.01	0.28 ± 0.02
High	Adequate	(-)	0.05 ± 0.01	3.1 ± 0.3	1.32 ± 0.07	0.24 ± 0.03	0.34 ± 0.06
		(+)	0.03 ± 0.00	3.1 ± 0.4	1.45 ± 0.18	0.25 ± 0.02	0.33 ± 0.01
	Toxic	(-)	0.06 ± 0.01	3.2 ± 0.4	1.20 ± 0.03	0.20 ± 0.02	0.27 ± 0.02
		(+)	0.04 ± 0.00	3.1 ± 0.2	1.26 ± 0.05	0.20 ± 0.02	0.29 ± 0.03

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Straw P Concentration: 0.005; *n.s.*; 0.005; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Straw K Concentration: *n.s.*; 0.2; 0.1; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Straw Ca Concentration: 0.05; 0.07; *n.s.*; *n.s.*; *n.s.*; 0.13; *n.s.*

Straw Mg Concentration: *n.s.*; 0.02; 0.01; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Straw S Concentration: *n.s.*; 0.03; .0.02; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Table 3.24 reveals that only main effects of the experimental factors had significantly affected on straw macronutrients concentrations of *Triticum aestivum* cv. Nusrat. Because of the colonization with *P. indica*, concentrations of P and K significantly changed. While P concentration were reduced significantly when compared to mock treatment, Ca concentrations in straw of Nusrat was significantly enhanced.

Moreover, salinity became decisive on concentration of all the minerals which are listed in Table 3.24 except P. For example, mineral analysis demonstrated that under high salinity, K, Ca, and S straw concentrations of Nusrat significantly increased when compared moderate and control salinity. However, Mg concentration was significantly reduced due to high salinity when compared to moderate and control ones. Additionally, toxic B supply significantly increased straw P and K concentration in Nusrat. This result is consistent the findings of previous experiments (Table 3.12; 3.12; 3.10; 3.8).

Table 3. 25: Effects of inoculation with *P. indica* on selected straw micronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Boron	<i>P. indica</i>	Cu (mg kg ⁻¹)	Fe (mg kg ⁻¹)	Mn (mg kg ⁻¹)	Zn (mg kg ⁻¹)
Control	Adequate	(-)	7.5 ± 0.3	87 ± 6	42 ± 6	7.0 ± 0.5
		(+)	8.0 ± 0.5	95 ± 9	47 ± 3	6.5 ± 0.6
	Toxic	(-)	8.5 ± 0.7	96 ± 9	55 ± 7	7.1 ± 0.8
		(+)	8.7 ± 1.0	70 ± 13	54 ± 4	7.3 ± 1.1
Moderate	Adequate	(-)	8.3 ± 0.7	110 ± 5	70 ± 13	7.0 ± 0.8
		(+)	8.3 ± 0.3	70 ± 11	66 ± 8	7.5 ± 1.8
	Toxic	(-)	8.2 ± 0.5	96 ± 12	68 ± 6	6.4 ± 0.9
		(+)	8.2 ± 0.5	68 ± 3	67 ± 7	6.5 ± 0.4
High	Adequate	(-)	8.2 ± 1.1	104 ± 19	77 ± 5	7.3 ± 1.4
		(+)	8.7 ± 0.6	75 ± 14	66 ± 6	6.6 ± 0.9
	Toxic	(-)	8.0 ± 0.3	84 ± 10	82 ± 6	7.2 ± 0.9
		(+)	8.2 ± 0.4	77 ± 14	73 ± 10	6.5 ± 0.4

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD_{0.05} values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B

Straw Cu Concentration: *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*; 0.9; *n.s.*

Straw Fe Concentration: 7; *n.s.*; 7; 17; *n.s.*; *n.s.*; 28

Straw Mn Concentration: *n.s.*; 6; 4; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Straw Zn Concentration: *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Selected straw micronutrient concentration of Nusrat is shown in Table 3.25. Based on the Table, Fe concentrations of straw significantly reduced by *P. indica* inoculation, and the lowest Fe concentration was identified when *P. indica* inoculated *Triticum aestivum* cv. Nusrat was grown under moderate salinity and toxic B supply. Reduction on Fe concentration did not cause by retranslocation because grain Fe concentration was also reduced by *P. indica* inoculation (Table 2.21). On the other hand, the highest Fe straw concentration was occurred when non-inoculated Nusrat cultivar grown under moderate salinity with adequate B supply (Table 3.25). Toxic B supply significantly enhanced straw Mn concentrations Nusrat but straw Fe concentration significantly reduced by toxic B supply compared adequate B.

Table 3.26 demonstrates that straw P, Ca, and Mg concentration were significantly altered based on inoculation status. *P. indica* inoculation significantly reduced concentrations of P, Ca and Mg in straw of Saricanak-98. Salinity treatment had significant effects on straw P, Ca, and Mg concentration. High salinity significantly increased straw Ca concentration when compared to moderate and control salinity. In contrast to Ca, concentration of Mg and P were found higher under control salinity than moderate and high salinity. Furthermore, Toxic B supply significantly increased straw P, S and K concentrations in straw when compared to adequate B supply (Table 3.26). The effects of B toxicity on P and K concentrations were consisted with previous results. Significant interaction between salinity and B supply showed that the lowest straw K concentration was found when adequate B applied Saricanak-98 cultivar grown under control salinity and the highest one was recorder in moderate salinity exposed Saricanak-98 cultivar grown under toxic B supply (Table 3.26).

Table 3. 26: Effects of inoculation with *P. indica* on selected straw macronutrient concentration of durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Boron	<i>P. indica</i>	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Control	Adequate	(-)	0.04 ± 0.01	2.2 ± 0.1	1.1 ± 0.1	0.15 ± 0.01	0.23 ± 0.01
		(+)	0.03 ± 0.01	2.0 ± 0.3	1.2 ± 0.1	0.15 ± 0.01	0.25 ± 0.03
	Toxic	(-)	0.07 ± 0.01	2.8 ± 0.1	1.0 ± 0.1	0.14 ± 0.00	0.28 ± 0.01
		(+)	0.05 ± 0.01	2.8 ± 0.2	1.1 ± 0.1	0.13 ± 0.01	0.26 ± 0.02
Moderate	Adequate	(-)	0.03 ± 0.00	2.3 ± 0.2	1.1 ± 0.1	0.16 ± 0.00	0.24 ± 0.01
		(+)	0.03 ± 0.01	2.3 ± 0.2	0.9 ± 0.0	0.14 ± 0.02	0.25 ± 0.03
	Toxic	(-)	0.05 ± 0.01	2.8 ± 0.1	1.1 ± 0.0	0.13 ± 0.00	0.26 ± 0.03
		(+)	0.05 ± 0.01	2.9 ± 0.1	1.0 ± 0.1	0.12 ± 0.01	0.26 ± 0.02
High	Adequate	(-)	0.03 ± 0.01	2.4 ± 0.1	1.4 ± 0.2	0.16 ± 0.01	0.27 ± 0.04
		(+)	0.03 ± 0.00	2.4 ± 0.3	1.2 ± 0.1	0.14 ± 0.01	0.25 ± 0.02
	Toxic	(-)	0.05 ± 0.01	2.7 ± 0.2	1.3 ± 0.0	0.14 ± 0.01	0.27 ± 0.02
		(+)	0.04 ± 0.01	2.7 ± 0.1	1.2 ± 0.1	0.12 ± 0.01	0.25 ± 0.02

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD 0.05 values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B
 Straw P Concentration: 0.01; 0.01; 0.01; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Straw K Concentration: *n.s.*; *n.s.*; 0.1; *n.s.*; *n.s.*; 0.3; *n.s.*

Straw Ca Concentration: 0.1; 0.1; *n.s.*; 0.2; *n.s.*; *n.s.*; *n.s.*

Straw Mg Concentration: 0.01; 0.01; *n.s.*; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Straw S Concentration: *n.s.*; *n.s.*; 0.01; *n.s.*; *n.s.*; *n.s.*; *n.s.*

Selected straw micronutrients are listed below (Table 3.27). Main effect of inoculation significantly affected straw Fe and Mn concentrations. 43% and 30% decreases in straw Fe and Mn concentrations were recorded due to *P. indica* inoculation, respectively. Salinity treatment affected Cu, Fe and Mn concentration significantly. Mn and Cu concentration were promoted with high and moderate salinity. In addition, the highest concentration of both minerals was recorded when Saricanak-98 grown under high salinity.

In contrast to Mn and Cu, Fe concentration reduced by increasing salinity and the highest Fe concentration was found when Saricanak-98 grown under high salinity. Furthermore, only straw Cu concentration was significantly altered against B supply. A significant increase in the straw Cu concentration was determined by toxic B supply when compared to adequate B supplied counterparts.

Moreover, significant interactions among salinity, B and inoculation affected straw Zn concentration. Because main effects of high salinity and inoculation with *P. indica* decreased straw Fe concentration, the highest Fe concentration in straw of Saricanak-98 was identified when non-inoculated Saricanak-98 cultivar grown under control salinity with adequate B supply while the lowest Fe concentration was determined in *P. indica* inoculated Saricanak-98 grown under high salinity with toxic B supply. The fifth experiment was conducted to reveals effects of *P. indica* inoculation on durum and bread wheat plants grown under simultaneous salinity and B treatment (Table 3.27).

Table 3. 27: Effects of inoculation with *P. indica* on selected straw micronutrient concentration of durum wheat (*Triticum durum* cv. Saricanak-98) plants grown at adequate (1 mg B kg⁻¹ soil) or toxic (20 mg B kg⁻¹ soil) B supply and different salinity treatment (Control: No salt treatment; moderate: 3 dS/m; high: 6 dS/m) in non-autoclaved soil under greenhouse conditions. Values represent mean and standard deviation of four independent replicates each containing five individual plants.

Salinity	Boron	<i>P. indica</i>	Cu (mg.kg ⁻¹)	Fe (mg.kg ⁻¹)	Mn (mg.kg ⁻¹)	Zn (mg.kg ⁻¹)
Control	Adequate	(-)	6.9 ± 0.6	179 ± 11	31 ± 3	7 ± 2
		(+)	6.2 ± 0.7	78 ± 11	25 ± 2	9 ± 0
	Toxic	(-)	8.5 ± 0.9	134 ± 26	32 ± 3	10 ± 2
		(+)	7.9 ± 0.3	76 ± 5	24 ± 4	8 ± 1
Moderate	Adequate	(-)	7.0 ± 0.3	132 ± 28	35 ± 2	8 ± 2
		(+)	8.1 ± 0.9	84 ± 16	23 ± 1	7 ± 1
	Toxic	(-)	8.1 ± 0.8	131 ± 4	34 ± 4	9 ± 1
		(+)	8.8 ± 0.4	76 ± 14	28 ± 4	7 ± 1
High	Adequate	(-)	8.3 ± 1.3	90 ± 20	45 ± 4	8 ± 1
		(+)	8.6 ± 0.5	70 ± 5	25 ± 1	8 ± 1
	Toxic	(-)	8.4 ± 0.9	131 ± 34	43 ± 5	9 ± 2
		(+)	8.7 ± 0.3	63 ± 6	29 ± 3	7 ± 2

Higher differences between means of treatment groups than their corresponding HSD scores indicate significant differences according to Tukey's protected HSD test ($p \leq 0.05$).

HSD 0.05 values: Inoculation (I); Salinity (S); Boron (B); I×S; I × B; S × B; I × S × B
Straw Cu Concentration: *n.s.*; 0.6; 0.4; 1.1; *n.s.*; 1.1; *n.s.*

Straw Fe Concentration: 11; 16; *n.s.*; 27; *n.s.*; 27; 44

Straw Mn Concentration: 2; 3; *n.s.*; 5; *n.s.*; *n.s.*; *n.s.*

Straw Zn Concentration: *n.s.*; *n.s.*; *n.s.*; *n.s.*; 2; *n.s.*; *n.s.*

The fifth experiment was conducted to investigate effects of interaction of salinity, B toxicity and *P. indica* inoculation on wheat yield. The result of the fifth experiment showed that thousand grain weight significantly increased by *P. indica* inoculation. Furthermore, *P. indica* inoculation was also reduced Na concentration of straw and grain.

4. DISCUSSION

To produce enough food for meeting markedly increasing human population depends on not only increasing yield but also preventing yield losses which are caused by aggravated abiotic factors. Therefore, development of multidimensional approaches is necessary to come up with better and long-term solutions. That's why for better understanding of multicomplexes behind the interaction of salinity and B toxicity prompt us to discuss their interaction firstly.

Although, combination of salinity and B in soil declared as one of the commonly pronounced growth diminishing and yield losing combination, in literature there are numerous studies which shows that B treatment may mitigate adverse effects of salinity [Rahman et al., 2021], [Dong et al., 2021]. However, combination of salinity and B toxicity is accepted as combined stress. Therefore, many studies focus on single and combined effects but obtained the results are controversial and not consistent [Smith et al., 2010]. If we follow the principles of Occam's razor, plausible explanation of obtained controversial result might be originated from the differences among genotypes, soil pH, soil type, experimental design etc.

To explain interaction of salinity and B toxicity, we may firstly mention their main effects. For instance, salinity has two obvious effects on plant as limiting water uptake and causing ionic toxicity. When availability of water is decreased due to salt accumulation in soil, plant closes their stomata for preventing water losses. It is known that uptake of B from soil depends on the passive diffusion. Therefore, thinking that salinity limits B uptake by decreasing transpiration seem to be plausible [Masood et al., 2012]. Furthermore, Ca is an important component of plant tolerance mechanisms against salinity because of its role in the sensing and signalling of salt stress [Seifikalhor et al., 2019], plant tend to accumulate higher Ca to cope with salinity stress for maintaining proper functioning of cell membrane. Restricted transport of Ca by salinity is reversed by B. B and Ca bind each other to form more transferable compound. In this way, B indirectly mitigate harmful effects of salinity [Masood et al., 2012]. Additionally, B reduced not only Na concentration but also Cl concentration in plants [Dong et al., 2021].

Furthermore, the one of the main effects of the salinity on plant mineral homeostasis is reducing the K concentration by effluxion from different tissues. So, decreases in severity of salinity might be originated from promoted uptake of K-mediated by B toxicity [Assaha et al., 2017]. Studies shown that cytosolic K^+/Na^+ ratio has vital importance to maintain ionic homeostasis which may be degraded by high accumulation of Na in plant tissues under saline conditions [Shabala and Cuin, 2008]. So, after this brief introduction, it might be beneficial to discuss how interaction of salinity and B toxicity occurred.

In the third experiment, B concentration was significantly reduced by increased salinity in both cultivars and Na concentration was also reduced due to B supply (Figure 3.7). The reduction of Na concentration might be caused by significant increases of K concentration (Table 3.8). Moreover, similar trend was also observed in the fourth experiment. In Saricanak-98 cultivar shoot B concentration reduced by increasing salinity and B toxicity was also reduced Na concentration (Figure 3.15). Significant increases in the shoot K concentration were found due to toxic B supply (Table 3.12). Similar result was also found in the fifth experiment. Grain Na concentration of both cultivars reduced by B toxicity compared to adequate B supply (Figure 3.19). In addition, straw K concentration significantly increased by toxic B treatment. Therefore, it might be logical to hypothesis that toxic B treatment may be reduced Na concentration by increasing concentration of K. Moreover, B toxicity enhanced concentration of P in addition to K (Table 3.8; 12).

Numerous studies revealed that root colonization of *P. indica* protects plant from many abiotic stresses including salinity [L. Li et al., 2017], [Gill et al., 2016]. The mechanism behind the plant protective abilities of *P. indica* showed in different studies. This beneficial fungi improved antioxidant defences mechanism, enhance uptake of minerals, accumulate osmolyte and exclude toxic ions etc. [Gill et al., 2016]. Abdelaziz et al., [2019] showed that *P. indica* inoculation significantly reduced Na concentration while increasing K concentration.

Mineral analysis results of both experiment which salinity and *P. indica* were integrated as factors showed that irrespective to cultivar and tissues, Na concentration reduced by *P. indica* inoculation when compared to non-inoculated ones (Figure 3.15; 19;20). *P. indica* inoculation increased shoot, grain, and straw B concentration of Nusrat cultivar significantly (Table 3.20A; 3.19A; 3.15).

Moreover, when wheat plants grown under high salinity and toxic B supply grain and straw P and K concentrations were reduced with *P. indica* inoculation in both cultivar (Table 3.21; 22; 24; 26). However, during vegetative period, P and K concentration enhanced by *P. indica* inoculation under high salinity with B toxicity (Table 3.10; 12). Studies showed that *P. indica* inoculation enhanced concentration of K and P in literature [Abdelaziz et al., 2019], [M. Wu et al., 2018]. *P. indica* inoculation mediated promotion of K and P concentration in early developmental stages provided higher shoot biomass than non-inoculated ones in our fourth experiment (Figure 3.10). In addition to the reduction in concentration of P and K by *P. indica* inoculation, and inoculation also did not provide any positive or negative impact on grain yield in both cultivar when wheat plants grown under non-autoclaved soil. Therefore, we might suppose that for prolonged inoculation period, activities of *P. indica* as an alien microorganism was reduced by local microbial community and combined salinity with B toxicity. In the second experiment, 32% grain yield promotion was obtained by *P. indica* inoculation when wheat plants grown under non-stress treated conditions in autoclaved soil. (Table 3.3). Alternatively, we may assume that lower Na accumulation in grain and straw of wheat plants may be achieved by restriction of uptake of Na. That's why, accumulation of high amount of K in tissues may not be required.

In literature, studies shown that inoculation with *P. indica* enhance salt tolerance of barley by triggering antioxidant defence mechanisms and accumulating protein [Becker et al., 2005]. In this study, our findings demonstrate that *P. indica* inoculated plants were more resistant to salt stress and this is coherent to findings of the Becker et. al [2005]. Moreover, Moreira et al., [2015] demonstrated that *P. indica* inoculation promotes plant biomass and nutrient uptake. Beside significant biomass increases were observed in our experimental plant, nutrient uptake promotion changes based on the tissues.

In addition, generally bread wheat cultivars are more tolerant to salinity than durum wheat because they have greater capability to exclude Na when compared to durum wheat [Genc et al., 2019]. So, we may expect that shoot, grain and straw Na concentration of bread wheat could be lower than durum wheat because of exclusion mechanisms.

In our study, based on the mentioned hypothesis, we compared Na concentrations of bread and durum wheat and we found that bread wheat namely *Triticum aestivum* cv. Nusrat accumulate significantly lower Na in their tissues when compared to durum wheat namely *Triticum durum* cv. Sarıçanak-98.

Under abiotic stress conditions plants tend to accumulate certain osmolytes to ameliorate detrimental effects of the abiotic stresses. One of these osmolytes is glycine betaine [Ashraf and Foolad, 2007]. Datir et al., [2020] showed that glycine betaine levels increased with increasing salinity in wheat plants. In our study, glycine betaine levels were also significantly increased in moderate salinity when compared to control.

MDA is produced as a result of the oxidative stress which may be triggered by abiotic stress factors such as salinity and B toxicity. Esim et al., [2013] showed that excess B concentration in maize caused significant increment of MDA levels. In addition, Shafi et al., [2009] figured out that high salinity significantly increase MDA concentrations of wheat. In our study, MDA levels were significantly raised by high salinity, but B toxicity did not significantly affected formation of MDA. Furthermore, previous studies demonstrated that, *P. indica* colonization in roots protects plants from stress factors and alleviate formation of harmful product such as MDA [Hui et al., 2015]. In this thesis, result of MDA analysis showed that *P. indica* inoculation significantly reduced MDA levels in wheats.

In literature, multiple studies revealed that inoculation with endophyte fungus like *P. indica* and other certain beneficial fungus enhanced plant yield and growth and in our studies in especially experiment four we found that there was significant increases in growth because of inoculation with fungus [Khalid et al., 2019], [Rho et al., 2018]. Under B toxicity shoot growth of the wheat plants diminish significantly due to accumulation of excess B in tissues and this accumulation and certain other side effects reduce the shoot growth [Roessner et al., 2006]. In our third experiment, shoot growth of the wheat plants decreases statistically due to B toxicity when compared to adequate B supply. *P. indica* is particularly important to enhance grain yield of the wheat crops because even it can trigger some of the basic plant metabolic processes, yield is the final output.

That's why yield is relatively more important than physiological parameters. *P. indica* was used to assess its performance under two different conditions and the result showed that the fungus did not provide any grain yield promotion in wheat in fifth experiment [Serfling et al., 2007]. However, grain yield of barley crop was significantly increased by *P. indica* inoculation [Achatz et al., 2010]. In contrast to result of fifth experiment, result of grain yield and other yield parameters of the second experiment revealed that *P. indica* inoculation and combination *P. indica* with AMF provided significant increased when compared to mock treatment.

In literature, there are many studies which shows that the enhancer and supporter function of the fungus on stress tolerance. Here, we report that in addition to ability of *P. indica* to support plant growth and development and stress tolerance mechanism, it is also significantly increased wheat yield. Moreover, mycorrhizae provide significant yield enhancement for agricultural production. Numerous studies show that mycorrhizal association between plants and fungi enhance grain yield in many crops [S. Zhang et al., 2019].

Furthermore, in literature a recent study proposed that interaction between *P. indica* and mycorrhizae seem to be beneficial to boost grain yield because of their combined effect on growth [Moreira et al., 2015]. Similar to results of above-mentioned researchers, our result proves that under non-stress condition combination of *P. indica* and mycorrhizae significantly increased grain yield and shoot dry weight in wheat crops. Increment of grain yield reached by up to 45% with co-inoculation when compared non-inoculated conditions.

Colonization of *P. indica* in plant roots were detected by PCR in literature. For this purpose, different genes were amplified by PCR reactions. These genes are of *EF-1 alpha (tef)* and *beta-tubulin (tub2)* gene [Lakshmipriya et al., 2017], [Kumar et al., 2009], *tef* gene produced band at 220 bp [Lakshmipriya et al., 2017]. Results of the DNA extraction and PCR reactions of samples which were taken from roots of wheats was produced band at 220 bp in our study as well. Moreover, the first steps of the detection of *P. indica* inoculation are assessment of spores of *P. indica* by trypan blue methods. Similar to findings of literature we also observe chlamydospores of the fungus in roots of wheat. [Kumar et al., 2009]. Moreover, vesicles of AMF were also detected in colonized roots.

5. CONCLUSION

Agricultural production is accepted as one of the most critical global sectors which has power to manage, design and control future of humanity. People started to inhabited world from millions of years ago and back to the time that we start to settle down we also started to grow, collect, and harvest foods. That's way many peoples think that farmer is one of the most historical occupations. Farming system is changing and because of the transformation of the agriculture from conventional to modern, new problems are arising. Therefore, these problems need to be solved by innovative ideas. For example, climate change, global warming, soil depletion, excess energy usage by agricultural sector, yield reduction and destruction of the arable lands are just the most pronounced modern problems of farming system.

Modern agricultural problems required sustainable, innovative, environmentally friendly, and cost-effective solutions. For example, abiotic stresses which are getting more and more severe day by day because of climate crisis and anthropogenic activities should be solved by immediately. The aim of this thesis is creating a solution to alleviate salinity stress and B toxicity by nature-based solution. Plant-fungus interaction has long history, and both sides have been benefited from this interaction. *P. indica* is partially newly known fungus when compared to arbuscular mycorrhizal fungi.

In this thesis, ameliorative effects of two different beneficial fungi were studied. The result of the study showed that *P. indica* can reduce Na concentration in plant tissues and increased the biomass of the wheat plants. Moreover, co-inoculation with AMF and *P. indica* increased yield of wheat. Therefore, we proposed that both fungi are capable to enhance the wheat yield even under non-optimal growing conditions by maintaining mineral homeostasis and triggering defence mechanism.

Yield enhancement, stress protection, growth of vegetative promotion and many more other benefits of inoculation with *P. indica* and arbuscular mycorrhizal fungi have shown in this thesis. We hope that results of these thesis will be beneficial to contribute studies which are goaling investigating plant-fungus interaction under severe stress conditions. Furthermore, agricultural production needs news perspectives and approach because commonly employed approach are no longer applicable to solve

today's agricultural problems. Therefore, we hope advantageous of beneficial fungi especially *P. indica* and its ability to eliminate harmful effects of the B toxicity will be used to avoid yield losses which are originated from B toxicity together with salinity stress.



REFERENCES

- Abdelaziz M. E., Abdelsattar M., Abdeldaym E. A., Atia M. A. M., Mahmoud A. W. M., Saad M. M., Hirt H., (2019), "Piriformospora indica alters Na^+/K^+ homeostasis, antioxidant enzymes and LeNHX1 expression of greenhouse tomato grown under salt stress," *Scientia Horticulturae*, 256 (June), 108532.
- Achatz B., Kogel K. H., Franken P., Waller F., (2010), "Piriformospora indica mycorrhization increases grain yield by accelerating early development of barley plants," *Plant Signaling and Behavior*, 5 (12), 1685–1687.
- Acosta-Motos J. R., Penella C., Hernández J. A., Díaz-Vivancos P., Sánchez-Blanco M. J., Navarro J. M., Barba-Espín G., (2020), "Towards a sustainable agriculture: Strategies involving phytoprotectants against salt stress," *Agronomy*, 10 (2), 10.3390.
- Agrimonti C., Lauro M., Visioli G., (2021), "Smart agriculture for food quality: facing climate change in the 21st century," *Critical Reviews in Food Science and Nutrition*, 61 (6), 971-981, doi: 10.1080/10408398.2020.1749555.
- Al-Huqail A. A., Khan M. N., Ali H. M., Siddiqui M. H., Al-Huqail A. A., AlZuaibr F. M., Al-Humaid L. A., (2020), "Exogenous melatonin mitigates boron toxicity in wheat," *Ecotoxicology and Environmental Safety*, 201 (May), 110822.
- Anwar A., Kim J.-K., (2020), "Molecular Sciences Transgenic Breeding Approaches for Improving Abiotic Stress Tolerance: Recent Progress and Future Perspectives." *International Journal of Molecular Science*, 21(8), 2695.
- Arzani A., Ashraf M., (2017), "Cultivated Ancient Wheats (*Triticum* spp.): A Potential Source of Health-Beneficial Food Products," *Comprehensive Reviews in Food Science and Food Safety*, 16 (3), 477–488.
- Ashraf M., Foolad M. R., (2007), "Roles of glycine betaine and proline in improving plant abiotic stress resistance," *Environmental and Experimental Botany*, 59 (2), 206–216.
- Assaha D. V. M., Ueda A., Saneoka H., Al-yahyai R., (2017), "The Role of Na^+ and K^+ Transporters in Salt Stress Adaptation in Glycophytes," *Frontiers in Physiology*, 8, 509.
- Ayca M., Baslam M., Asiloglu R., Mitsui T., Yildiz M., (2021), "Development of new high-salt tolerant bread wheat (*Triticum aestivum* L.) genotypes and insight into the tolerance mechanisms," *Plant Physiology and Biochemistry*, 166 (May), 314–327.
- Balakrishnan D., Subrahmanyam D., Badri J., Raju A. K., Rao Y. V., Beerelli K., Neelamraju S., (2016), "Genotype $\times$ environment interactions of yield traits in backcross introgression lines derived from *Oryza sativa* cv. swarna/*oryza nivara*," *Frontiers in Plant Science*, 7 (2016), 1530.

Becker K., Fischer M., Heier T., Waller F., Achatz B., Baltruschat H., Hu R., (2005), "The endophytic fungus *Piriformospora indica* reprograms barley to salt-stress tolerance, disease resistance, and higher yield," *Proceedings of the National Academy of Sciences*, 102 (38), 13386–13391.

Begum N., Qin C., Ahanger M. A., Raza S., Khan M. I., Ashraf M., Zhang L., (2019), "Role of Arbuscular Mycorrhizal Fungi in Plant Growth Regulation: Implications in Abiotic Stress Tolerance," *Frontiers in Plant Science*, 10 (September), 1–15, 1068.

Bhanbhro N., Xiao B., Han L., Lu H., Wang H., Yang C., (2020), "Adaptive strategy of allohexaploid wheat to long-term salinity stress," *BMC Plant Biology*, 20 (1), 1–14.

Biel W., Jaroszewska A., Stankowski S., Sobolewska M., Kępińska-Pacelik J., (2021), "Comparison of yield, chemical composition and farinograph properties of common and ancient wheat grains," *European Food Research and Technology*, 247 (6), 1525–1538.

Bolaños L., Lukaszewski K., Bonilla I., Blevins D., (2004), "Why boron?," *Plant Physiology and Biochemistry*, 42 (11), 907–912.

Bourdenx B., Bernard A., Domergue F., Pascal S., Léger A., Roby D., Joubès J., (2011), "Overexpression of *Arabidopsis* ECERIFERUM1 promotes wax very-long-chain alkane biosynthesis and influences plant response to biotic and abiotic stresses," *Plant Physiology*, 156 (1), 29–45.

Bowles T. M., Barrios-Masias F. H., Carlisle E. A., Cavagnaro T. R., Jackson L. E., (2016), "Effects of arbuscular mycorrhizae on tomato yield, nutrient uptake, water relations, and soil carbon dynamics under deficit irrigation in field conditions," *Science of the Total Environment*, 566–567, 1223–1234.

Brdar-Jokanović M., (2020), "Boron toxicity and deficiency in agricultural plants," *International Journal of Molecular Sciences*, 21 (4), 1424.

Brenchley R., Spannagl M., Pfeifer M., Barker G. L. A., D'Amore R., Allen A. M., Hall N., (2012), "Analysis of the bread wheat genome using whole-genome shotgun sequencing," *Nature*, 491 (7426), 705–710.

Byrt C. S., Xu B., Krishnan M., Lightfoot D. J., Athman A., Jacobs A. K., Gilliam M., (2014), "The Na⁺ transporter, TaHKT1;5-D, limits shoot Na⁺ accumulation in bread wheat," *Plant Journal*, 80 (3), 516–526.

Cakmak I., Pfeiffer W. H., McClafferty B., (2010), "Biofortification of durum wheat with zinc and iron," *Cereal Chemistry*, 87 (1), 10–20.

Camacho-Cristóbal J. J., Navarro-Gochicoa M. T., Rexach J., González-Fontes A., Herrera-Rodríguez M. B., (2018), "Plant Response to Boron Deficiency and Boron Use Efficiency in Crop Plants," *Plant Micronutrient Use Efficiency: Molecular and Genomic Perspectives in Crop Plants*, Jan 1, 109–121.

Camacho-Cristóbal J. J., Rexach J., Herrera-Rodríguez M. B., Navarro-Gochicoa M. T., González-Fontes A., (2011), "Boron deficiency and transcript level changes," *Plant Science*, 181 (2), 85–89.

Chaudhry S., Preet G., Sidhu S., (2022), "Climate change regulated abiotic stress mechanisms in plants: a comprehensive review," *Plant Cell Reports*, 41, 1–31.

Chen J. T., Aroca R., Romano D., (2021), "Molecular aspects of plant salinity stress and tolerance," *International Journal of Molecular Sciences*, 22.9, 4918.

Chitarra W., Hamayun M., Khan A. W., Mardan U., Sandrini M., Ray P., Tomczyk M., (2021), "Fungal Endophyte: An Interactive Endosymbiont With the Capability of Modulating Host Physiology in Myriad Ways," *Frontiers in Plant Science*, 12, 701800.

Chormova D., Fry S. C., (2016), "Boron bridging of rhamnogalacturonan-II is promoted in vitro by cationic chaperones, including polyhistidine and wall glycoproteins," *New Phytologist*, 209 (1), 241–251.

Choudhury F. K., Rivero R. M., Blumwald E., Mittler R., (2016), "Reactive oxygen species, abiotic stress and stress combination," *The Plant Journal* 90.5, 586-867.

Christie P., Li X., Chen B., (2004), "Arbuscular mycorrhiza can depress translocation of zinc to shoots of host plants in soils moderately polluted with zinc," *Plant and Soil*, 261 (1–2), 209–217.

Colmer T. D., Flowers T. J., Munns R., (2006), "Use of wild relatives to improve salt tolerance in wheat," *In Journal of Experimental Botany* (Vol. 57, pp. 1059–1078).

Dahro B., Wang F., Peng T., Liu J. H., (2016), "PtrA/NINV, an Alkaline/neutral invertase gene of *Poncirus trifoliata*, confers enhanced tolerance to multiple abiotic stresses by modulating ROS levels and maintaining photosynthetic efficiency," *BMC Plant Biology*, 16 (1), 1–18.

Datir S., Singh N., Joshi I., (2020), "Effect of NaCl-Induced Salinity Stress on Growth, Osmolytes and Enzyme Activities in Wheat Genotypes," *Bulletin of Environmental Contamination and Toxicology*, 104 (3), 351–357.

Sousa T., Ribeiro M., Sabeñça C., Igrejas G., (2021), "The 10,000-year success story of wheat!," *Foods*, 10 (9), 2124.

Denison R. F., Kiers E. T., (2011), "Life histories of symbiotic rhizobia and mycorrhizal fungi," *Current Biology*, 21 (18), R775–R785.

Deshmukh S., Hückelhoven R., Schäfer P., Imani J., Sharma M., Weiss M., Kogel K. H., (2006), "The root endophytic fungus *Piriformospora indica* requires host cell for proliferation during mutualistic symbiosis with barley," *Proceedings of the National Academy of Sciences of the United States of America*, 103 (49), 18450–18457.

Doering O., Sorensen A., (2018), "The land that shapes and sustains us," *How to Feed the World*, 46–58.

Dong X., Sun L., Guo J., Liu L., Han G., Wang B., (2021), "Exogenous boron alleviates growth inhibition by NaCl stress by reducing Cl⁻ uptake in sugar beet (*Beta vulgaris*)," *Plant and Soil*, 464 (1–2), 423–439.

Dora S., Terrett O. M., Sánchez-Rodríguez C., (2022), "Plant-microbe interactions in the apoplast: Communication at the plant cell wall," *The Plant Cell*, 34 (5), 1532–1550.

Druege U., Baltruschat H., Franken P., (2007), "*Piriformospora indica* promotes adventitious root formation in cuttings," *Scientia Horticulturae*, 112 (4), 422–426.

du Jardin P., (2015), "Plant biostimulants: Definition, concept, main categories and regulation," *Scientia Horticulturae*, 196, 3–14.

Duplessis S., Cuomo C. A., Lin Y. C., Aerts A., Tisserant E., Veneault-Fourrey C., Martin F., (2011), "Obligate biotrophy features unraveled by the genomic analysis of rust fungi," *Proceedings of the National Academy of Sciences of the United States of America*, 108 (22), 9166–9171.

Esim N., Tiryaki D., Karadagoglu O., Atici O., (2013), "Toxic effects of boron on growth and antioxidant system parameters of maize (*Zea mays* L.) roots," *Toxicology and Industrial Health*, 29 (9), 800–805.

Fan M. S., Zhao F. J., Fairweather-Tait S. J., Poulton P. R., Dunham S. J., McGrath S. P., (2008), "Evidence of decreasing mineral density in wheat grain over the last 160 years," *Journal of Trace Elements in Medicine and Biology*, 22 (4), 315–324.

Fraj H., Werbrouck S. P. O., (2023), "Constant and Intermittent Contact with the Volatile Organic Compounds of *Serendipita indica* Alleviate Salt Stress In Vitro *Ocimum basilicum* L.," *Applied Sciences (Switzerland)*, 13 (3), 1776.

Funakawa H., Miwa K., (2015), "Synthesis of borate cross-linked rhamnogalacturonan II," *Frontiers in Plant Science*, 6 (APR), 6- 223.

García-Sánchez F., Simón-Grao S., Martínez-Nicolás J. J., Alfosea-Simón M., Liu C., Chatzissavvidis C., Cámara-Zapata J. M., (2020), "Multiple stresses occurring with boron toxicity and deficiency in plants," *Journal of Hazardous Materials*, 397 (December 2019), 122713.

Genc Y., Taylor J., Lyons G., Li Y., Cheong J., Appelbee M., Sutton T., (2019), "Bread Wheat With High Salinity and Sodicity Tolerance," *Frontiers in Plant Science*, 10, 1280.

Gill S. S., Gill R., Trivedi D. K., Anjum N. A., Sharma K. K., Ansari M. W., Tuteja N., (2016), "*Piriformospora indica*: Potential and Significance in Plant Stress Tolerance," *Frontiers in Microbiology*, 7 (MAR), 1–20.

Godoy F., Olivos-Hernández K., Stange C., Handford M., (2021),“Plants Abiotic Stress in Crop Species: Improving Tolerance by Applying Plant Metabolites,” *Plants*, 2 (2021), 186.

Hänsch R., Mendel R. R., (2009),“Physiological functions of mineral micronutrients (Cu, Zn, Mn, Fe, Ni, Mo, B, Cl),”*Current Opinion in Plant Biology*, 12 (3), 259–266.

Harris J. M., Balint-Kurti P., Bede J. C., Day B., Gold S., Goss E. M., Alvarez M. E., (2020),“What are the Top 10 Unanswered Questions in Molecular Plant-Microbe Interactions?,” *Molecular Plant Microbe Interaction*, 33 (12), 1354-1365.

Harwood J., (2019),“ Was the Green Revolution intended to maximise food production?,” *International Journal of Agricultural Sustainability*, 17 (4), 312-325.

He M., He C. Q., Ding N. Z., (2018),“Abiotic stresses: General defenses of land plants and chances for engineering multistress tolerance,”*Frontiers in Plant Science*, 9 (2018), 1771.

Hill T. W., Kafer E., (2001),“Improved protocols for *Aspergillus* minimal medium: trace element and minimal medium salt stock solutions,”*Fungal Genetics Reports*, 48 (1), 20–21.

Holland C., Ryden P., Edwards C. H., Grundy M. M. L., (2020),“Plant cell walls: Impact on nutrient bioaccessibility and digestibility,”*Foods*, 9 (2), 201.

Hongqiao L., Suyama A., Mitani-Ueno N., Hell R., Maruyama-Nakashita A., (2021),“A low level of NaCl stimulates plant growth by improving carbon and sulfur assimilation in *arabidopsis thaliana*,”*Plants*, 10 (10), 2138.

Horton P., Long S. P., Smith P., Banwart S. A., Beerling D. J., (2021),“Technologies to deliver food and climate security through agriculture,”*Nature Plants*, 7 (3), 250–255.

Hossain A., Skalicky M., Brestic M., Maitra S., Alam M. A., Syed M. A., Islam T., (2021),“Consequences and mitigation strategies of abiotic stresses in wheat (*Triticum aestivum* l.) under the changing climate,”*Agronomy*, 11 (2), 241.

Hosseini F., Mosaddeghi M. R., Dexter A. R., (2017),“Effect of the fungus *Piriformospora indica* on physiological characteristics and root morphology of wheat under combined drought and mechanical stresses,”*Plant Physiology and Biochemistry*, 118, 107–120.

Houston K., Tucker M. R., Chowdhury J., Shirley N., Little A., (2016),“The plant cell wall: A complex and dynamic structure as revealed by the responses of genes under stress conditions,”*Frontiers in Plant Science*, 7, 984.

Hui F., Liu J., Gao Q., Lou B., (2015),“*Piriformospora indica* confers cadmium tolerance in *Nicotiana tabacum*,”*Journal of Environmental Sciences (China)*, 37, 184–191.

Hyde K. D., Xu J., Rapior S., Jeewon R., Lumyong S., Grace Niego A. T., Stadler M., (2019), "The amazing potential of fungi: 50 ways we can exploit fungi industrially," *Fungal Diversity*, 97, 1–136.

Igiehon N. O., Babalola O. O., (2017), "Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi," *Applied Microbiology and Biotechnology*, 101, 4871–4881.

Ishii T., (2018), "Crop Gene-Editing: Should We Bypass or Apply Existing GMO Policy?," *Trends in Plant Science*, 23 (11), 947–950.

Jahandideh Mahjen Abadi V. A., Sepehri M., Khatabi B., Rezaei M., (2021), "Alleviation of zinc deficiency in wheat inoculated with root endophytic fungus *Piriformospora indica* and rhizobacterium *Pseudomonas putida*," *Rhizosphere*, 17 (September 2020), 100311.

James R. A., Blake C., Byrt C. S., Munns R., (2011), "Major genes for Na⁺ exclusion, *Nax1* and *Nax2* (wheat *HKT1;4* and *HKT1;5*), decrease Na⁺ accumulation in bread wheat leaves under saline and waterlogged conditions," *Journal of Experimental Botany*, 62 (8), 2939–2947.

James R. A., Davenport R. J., Munns R., (2006), "Physiological characterization of two genes for Na⁺ exclusion in durum wheat, *Nax1* and *Nax2*," *Plant Physiology*, 142 (4), 1537–1547.

James R. A., Von Caemmerer S., Condon A. G., Zwart A. B., Munns R., (2008), "Genetic variation in tolerance to the osmotic stress component of salinity stress in durum wheat," *Functional Plant Biology*, 35 (2), 111–123.

Jiang M., Jiang J., Li S., Li M., Tan Y., Song S., Huang J., (2020), "Glutamate alleviates cadmium toxicity in rice via suppressing cadmium uptake and translocation," *Journal of Hazardous Materials*, 384 (July 2019), 121319.

Jiang W., Pan R., Buitrago S., Wu C., Abdelaziz M. E., Oelmu R., (2021), "Transcriptome analysis of *Arabidopsis* reveals freezing-tolerance related genes induced by root endophytic fungus *Piriformospora indica*," *Physiological and Molecular Biology of Plants*, 27 (February), 189–201.

Jonsson K., Hamant O., Bhalerao R. P., (2022), "Plant cell walls as mechanical signaling hubs for morphogenesis," *Current Biology*, 32 (7), R334–R340.

Kabu M., Akosman M. S., (2013), "Biological effects of boron," *Reviews of Environmental Contamination and Toxicology*, 225 (March), 57–75.

Khalid M., Rahman S. U., Huang D., (2019), "Molecular mechanism underlying *Piriformospora indica*-mediated plant improvement/protection for sustainable agriculture," *Acta Biochimica et Biophysica Sinica*, 51 (3), 229–242.

Khalvandi M., Amerian M., Pirdashti H., Keramati S., (2021), "Does co-inoculation of mycorrhiza and *Piriformospora indica* fungi enhance the efficiency of chlorophyll fluorescence and essential oil composition in peppermint under irrigation with saline water from the Caspian Sea?," *PLoS ONE*, 16 (7 July), p.e0254076.

Krings M., Taylor T. N., Hass H., Kerp H., Dotzler N., Hermsen E. J., (2007), "Fungal endophytes in a 400-million-yr-old land plant: infection pathways, spatial distribution, and host responses," *New Phytologist*, 174 (3), 648-657.

Kumar M., Yadav V., Tuteja N., Johri A. K., (2009), "Antioxidant enzyme activities in maize plants colonized with *Piriformospora indica*," *Microbiology*, 155 (3), 780-790.

Lakshmipriya P., Nath S V., Veena S. S., Anith K. N., Sreekumar J., Jeeva M. L., (2017), "Piriformospora indica, a Cultivable Endophyte for Growth Promotion and Disease Management in Taro (*Colocasia esculenta* (L.)), " *Journal of Root Crops*, 42 (2), 107-114.

Lawas L. M. F., Erban A., Kopka J., Jagadish S. V. K., Zuther E., Hinch D. K., (2019), "Metabolic responses of rice source and sink organs during recovery from combined drought and heat stress in the field," *GigaScience*, 8 (8), 1-15.

Lawas L. M. F., Li X., Erban A., Kopka J., Jagadish S. V. K., Zuther E., Hinch D. K., (2019), "Metabolic responses of rice cultivars with different tolerance to combined drought and heat stress under field conditions," *GigaScience*, 8 (5), 1-21.

Li L., Li L., Wang X., Zhu P., Wu H., Qi S., (2017), "Plant growth-promoting endophyte *Piriformospora indica* alleviates salinity stress in *Medicago truncatula*," *Plant Physiology and Biochemistry*, 119, 211-223.

Li M., Zhao Z., Zhang Z., Zhang W., Zhou J., Xu F., Liu X., (2017), "Effect of boron deficiency on anatomical structure and chemical composition of petioles and photosynthesis of leaves in cotton (*Gossypium hirsutum* L.), " *Scientific Reports*, 7 (1), 1-9.

Li Q., Kuo Y. W., Lin K. H., Huang W., Deng C., Yeh K. W., Chen S. P., (2021), "Piriformospora indica colonization increases the growth, development, and herbivory resistance of sweet potato (*Ipomoea batatas* L.), " *Plant Cell Reports*, 40 (2), 339-350.

Lohani N., Singh M. B., Bhalla P. L., (2022), "Biological Parts for Engineering Abiotic Stress Tolerance in Plants," *BioDesign Research*, 2022, 1-41.

Lu H., Wei T., Lou H., Shu X., Chen Q., Azcón Aguilar C., (2021), "A Critical Review on Communication Mechanism within Plant-Endophytic Fungi Interactions to Cope with Biotic and Abiotic Stresses," *Journal of Fungi*, 7, 719.

Lücking R., Aime M. C., Robbertse B., Miller A. N., Aoki T., Ariyawansa H. A., "How to publish a new fungal species, or name, version 3.0", (2021), "Nomenclature," 6

(May), 1500-1508.

Ludwiczak A., Osiak M., Cárdenas-Pérez S., Lubińska-Mielińska S., Piernik A., (2021), "Osmotic stress or ionic composition: Which affects the early growth of crop species more?," *Agronomy*, 11 (3), 435.

Maathuis F. J. M., Ahmad I., Patishtan J., (2014), "Regulation of Na⁺ fluxes in plants," *Frontiers in Plant Science*, Frontiers in Plant Science, 5 (2014), 467.

Marchin R. M., Backes D., Ossola A., Leishman M. R., Tjoelker M. G., Ellsworth D. S., (2022), "Extreme heat increases stomatal conductance and drought-induced mortality risk in vulnerable plant species," *Global Change Biology*, 28 (3), 1133–1146.

Marti J., Slafer G. A., (2014), "Bread and durum wheat yields under a wide range of environmental conditions," *Field Crops Research*, 156, 258–271.

Masood S., Saleh L., Witzel K., Plieth C., Mühling K. H., (2012), "Determination of oxidative stress in wheat leaves as influenced by boron toxicity and NaCl stress," *Plant Physiology and Biochemistry*, 56, 56–61.

Mehri M., Ghabooli M., Movahedi Z., (2023), "Contribution of *Serendipita indica* on growth improvement, antioxidative capacity of *Dracocephalum kotschyi*, and its resistance against cadmium stress," *International Microbiology*, 17 (2023), 1-11.

Mittler R., (2006), "Abiotic stress, the field environment and stress combination," *Trends in Plant Science*, 11 (1), 15–19.

Mohd S., Shukla J., Kushwaha A. S., Mandrah K., Shankar J., Arjaria N., ... Kumar M., (2017), "Endophytic fungi *Piriformospora indica* mediated protection of host from arsenic toxicity," *Frontiers in Microbiology*, 8 (May), 1–14.

Moreira B. C., Mendes F. C., Mendes I. R., Paula T. A., Prates Junior P., Salomão L. C. C., ... Kasuya M. C. M., (2015), "The interaction between arbuscular mycorrhizal fungi and *Piriformospora indica* improves the growth and nutrient uptake in micropropagation-derived pineapple plantlets," *Scientia Horticulturae*, 197, 183–192.

Mukhopadhyay R., Sarkar B., Jat H. S., Sharma P. C., Bolan N. S., (2021), "Soil salinity under climate change: Challenges for sustainable agriculture and food security," *Journal of Environmental Management*, 280 (December 2020), 111736.

Munns R., Tester M., (2008), "Mechanisms of salinity tolerance," *Annual Review of Plant Biology*, 59, 651–681.

Nable R. O., Ba G. S., Paull J. G., (1997), "Boron toxicity," *Plant and Soil*, 193, 181–198.

Nadarajah K. K., (2020), "Molecular Sciences ROS Homeostasis in Abiotic Stress Tolerance in Plants," *International Journal of Molecular Sciences*, 21 (15), 5208.

Nanda R., Agrawal V., (2018),“*Piriformospora indica*, an excellent system for heavy metal sequestration and amelioration of oxidative stress and DNA damage in *Cassia angustifolia* Vahl under copper stress.” *Ecotoxicology and Environmental Safety*, 156, 409-419.

Naranjo-Ortiz M. A., Gabaldón T., (2019),“Fungal evolution: diversity, taxonomy and phylogeny of the Fungi,”*Biol. Rev.*, 94, 2101–2137.

Nehe A., Akin B., Sanal T., Evlice A. K., Ünsal R., Dinçer N., Morgounov A., (2019),“Genotype x environment interaction and genetic gain for grain yield and grain quality traits in Turkish spring wheat released between 1964 and 2010,”*PLoS ONE*, 14 (7).

Ngwene B., Boukail S., Söllner L., Franken P., Andrade-Linares D. R., (2016),“Phosphate utilization by the fungal root endophyte *Piriformospora indica*,”*Plant and Soil*, 405 (1–2), 231–241.

Nielsen F. H., (2014),“Update on human health effects of boron,”*Journal of Trace Elements in Medicine and Biology*, 28 (4), 383–387.

Padash A., Shahabivand S., Behtash F., Aghaee A., (2016),“A practicable method for zinc enrichment in lettuce leaves by the endophyte fungus *Piriformospora indica* under increasing zinc supply,”*Scientia Horticulturae*, 213, 367–372.

Pereira G. L., Siqueira J. A., Batista-Silva W., Cardoso F. B., Nunes-Nesi A., Araújo W. L., (2021),“Boron: More Than an Essential Element for Land Plants?,”*Frontiers in Plant Science*, 11 (January), 610307.

Petipas R. H., Geber M. A., Lau J. A., (2021),“Microbe-mediated adaptation in plants,”*Ecology Letters*, 24 (7), 1302–1317.

Poudel R., Bhinderwala F., Morton M., Powers R., Rose D. J., (2021),“Metabolic profiling of historical and modern wheat cultivars using proton nuclear magnetic resonance spectroscopy,”*Scientific Reports*, 11 (1), 3080.

Princi M. P., Lupini A., Araniti F., Longo C., Mauceri A., Sunseri F., Abenavoli M. R., (2015),“Boron Toxicity and Tolerance in Plants: Recent Advances and Future Perspectives,”*Plant Metal Interaction: Emerging*, 115-147.

Qin S., Xu Y., Liu H., Li C., Yang Y., Zhao P., (2021),“Effect of different boron levels on yield and nutrient content of wheat based on grey relational degree analysis,”*Acta Physiologiae Plantarum*, 43 (9), 1–8.

Rahman M., Rahman K., Sathi K. S., Alam M. M., Nahar K., Fujita M., Hasanuzzaman M., (2021),“Supplemental selenium and boron mitigate salt-induced oxidative damages in *glycine max* l.,”*Plants*, 10 (10), 1–16.

Rajendran K., Tester M., Roy S. J., (2009),“Quantifying the three main components of salinity tolerance in cereals,”*Plant, Cell and Environment*, 32 (3), 237–249.

Rakshit A., Singh H. B., Singh A. K., Singh U. S., Fraceto L., (2020),“New frontiers in stress management for durable agriculture,”*New Frontiers in Stress Management for Durable Agriculture*, 1–660.

Rawat N., Neelam K., Tiwari V. K., Dhaliwal H. S., (2013),“Biofortification of cereals to overcome hidden hunger,”*Plant Breeding*.

Ray D. K., Mueller N. D., West P. C., Foley J. A., (2013),“Yield Trends Are Insufficient to Double Global Crop Production by 2050,”*PLoS ONE*, 8 (6).

Raynaud T., Pivato B., Siol M., Spor A., Blouin M., 2021,“Soil microbes drive the effect of plant species and genotypic diversity interaction on productivity.”*Plant and Soil*, 467, 165-180.

Raza A., Razzaq A., Mehmood S. S., Zou X., Zhang X., Lv Y., Xu J., (2019),“Impact of Climate Change on Crops Adaptation and Strategies to Tackle Its Outcome: A Review,” *Plants*, 8 (2), 34.

Rehaman A., Mishra A. K., Ferdose A., Per T. S., Hanief M., Jan A. T., Asgher M., (2021),“Melatonin in plant defense against abiotic stress,”*Forests*, 12 (10).

Reid R., (2007),“Identification of boron transporter genes likely to be responsible for tolerance to boron toxicity in wheat and barley,”*Plant and Cell Physiology*, 48 (12), 1673–1678.

Reid R., (2010),“Can we really increase yields by making crop plants tolerant to boron toxicity?,”*Plant Science*, 178 (1), 9–11.

Reid R. J., Hayes J. E., Post A., Stangoulis J. C. R., Graham R. D., (2004),“A critical analysis of the causes of boron toxicity in plants,”*Plant, Cell and Environment*, 27 (11), 1405–1414.

Reid Robert J., Fitzpatrick K. L., (2009),“Redistribution of boron in leaves reduces boron toxicity,”*Plant Signaling & Behavior*, 4 (11), 1091–1093.

Rerkasem B., Jamjod S., (2004),“Boron deficiency in wheat: A review,”*Field Crops Research*, 89 (2–3), 173–186.

Rho H., Hsieh M., Kandel S. L., Cantillo J., Doty S. L., Kim S. H., (2018),“Do Endophytes Promote Growth of Host Plants Under Stress? A Meta-Analysis on Plant Stress Mitigation by Endophytes,”*Microbial Ecology*, 75 (2), 407–418.

Riaz M., Kamran M., El-Esawi M. A., Hussain S., Wang X., (2021),“Boron-toxicity induced changes in cell wall components, boron forms, and antioxidant defense system in rice seedlings,”*Ecotoxicology and Environmental Safety*, 216, 112192.

Ricci M., Tilbury L., Daridon B., Sukalac K., (2019),“General principles to justify plant biostimulant claims,”*Frontiers in Plant Science*, 10 (March), 1–8.

Roessner U., Patterson J. H., Forbes M. G., Fincher G. B., Langridge P., Bacic A., (2006),“An investigation of boron toxicity in Barley using metabolomics,”*Plant Physiology*, 142 (3), 1087–1101.

Rosenzweig C., Elliott J., Deryng D., Ruane A. C., Müller C., Arneth A., Jones J. W., (2014),“Assessing agricultural risks of climate change in the 21st century in a global gridded crop model intercomparison,”*Proceedings of the National Academy of Sciences of the United States of America*, 111 (9), 3268–3273.

Rouphael Y., Franken P., Schneider C., Schwarz D., Giovannetti M., Agnolucci M., Colla G., (2015),“Arbuscular mycorrhizal fungi act as biostimulants in horticultural crops,”*Scientia Horticulturae*, 196, 91–108.

Rout G. R., Das A. B., (2013),“Molecular stress physiology of plants,”*Molecular Stress Physiology of Plants*, 1–440.

Roy S. J., Negrão S., Tester M., (2014),“Salt resistant crop plants,”*Current Opinion in Biotechnology*, 26, 115–124.

Roylawar P., Khandagale K., Randive P., Shinde B., Murumkar C., Ade A., Morelli M., (2021),“*Piriformospora indica* primes onion response against stemphylium leaf blight disease,”*Pathogens*, 10 (9), 1–29.

Rudgers J. A., Afkhami M. E., Bell-Dereske L., Chung Y. A., Crawford K. M., Kivlin S. N., Nuñez M. A., (2020),“Climate Disruption of Plant-Microbe Interactions,”*Annual Review of Ecology, Evolution, and Systematics*, 51, 561–586.

Rui Y., Dinnyen J. R., (2020),“A wall with integrity: surveillance and maintenance of the plant cell wall under stress,”*New Phytologist*, 225 (4), 1428–1439.

Sahab S., Suhani I., Srivastava V., Chauhan P. S., Singh R. P., Prasad V., (2021),“Potential risk assessment of soil salinity to agroecosystem sustainability: Current status and management strategies,”*Science of the Total Environment*, 764, 144164.

Saman M., Sepehri A., (2021),“Alleviating Effects of *Serendipita indica* and Nitric Oxide in Proso Millet Plants (*Panicum miliaceum* L.) Exposed to Copper Toxicity,”*Journal of Soil Science and Plant Nutrition*, 21 (4), 3065–3075.

Scheublin T. R., Sanders I. R., Keel C., Roelof Van Der Meer J., (2010),“Characterisation of microbial communities colonising the hyphal surfaces of arbuscular mycorrhizal fungi,”*The ISME Journal*, 4, 752–763.

Schikora A., Prakash Verma J., Vishwakarma K., Varma A., Kumar N., Shandilya C., Bhayana S., (2020),“And Varma A (2020) Revisiting Plant-Microbe Interactions and Microbial Consortia Application for Enhancing Sustainable Agriculture: A Review.

Revisiting Plant-Microbe Interactions and Microbial Consortia Application for Enhancing Sustainable Agriculture: ,”*Front. Microbiol.*, 11, 560406.

Schüßler A., Schwarzott D., Walker C., (2001),“A new fungal phylum, the Glomeromycota: phylogeny and evolution,”*Mycological Research*, 105 (12), 1413–1421.

Seifikalhor M., Aliniaieifard S., Shomali A., Azad N., Hassani B., Lastochkina O., Li T., (2019),“Calcium signaling and salt tolerance are diversely entwined in plants,”*Plant Signaling and Behavior*, 14 (11).

Serfling A., Wirsal S. G. R., Lind V., Deising H. B., (2007),“Performance of the biocontrol fungus *Piriformospora indica* on wheat under greenhouse and field conditions,”*Phytopathology*, 97 (4), 523–531.

Shabala S., Cuin T. A., (2008),“Potassium transport and plant salt tolerance,”*Physiologia Plantarum*, 133 (4), 651–669.

Shafi M., Bakht J., Hassan M. J., Raziuddin M., Zhang G., (2009),“Effect of cadmium and salinity stresses on growth and antioxidant enzyme activities of wheat (*Triticum aestivum* L.),”*Bulletin of Environmental Contamination and Toxicology*, 82 (6), 772–776.

Shah A., Wu X., Ullah A., Fahad S., Muhammad R., Yan L., Jiang C., (2017),“Deficiency and toxicity of boron: Alterations in growth, oxidative damage and uptake by citrange orange plants,”*Ecotoxicology and Environmental Safety*, 145 (April), 575–582.

Shaw R. G., Etterson J. R., (2012),“Rapid climate change and the rate of adaptation: insight from experimental quantitative genetics,” *New Phytologist*, 195 (4), 752-765.

Shewry P. R., (2009),“Wheat,”*Journal of Experimental Botany*, 60 (60), 1537-53.

Shewry Peter R., (2018),“Do ancient types of wheat have health benefits compared with modern bread wheat?,”*Journal of Cereal Science*, 79, 469–476.

Shewry Peter R., Hey S., (2015a),“Do ‘ancient’ wheat species differ from modern bread wheat in their contents of bioactive components?,”*Journal of Cereal Science*, 65, 236–243.

Shewry Peter R., Hey S. J., (2015b),“The contribution of wheat to human diet and health,”*Food and Energy Security*.

Shi H., Ishitani M., Kim C., Zhu J. K., (2000),“The *Arabidopsis thaliana* salt tolerance gene *SOS1* encodes a putative Na⁺/H⁺ antiporter,”*Proceedings of the National Academy of Sciences of the United States of America*, 97 (12), 6896–6901.

Shireen F., Nawaz M. A., Chen C., Zhang Q., Zheng Z., Sohail H., Bie Z., (2018),“Boron: Functions and approaches to enhance its availability in plants for

sustainable agriculture,”*International Journal of Molecular Sciences*, 19 (7), 95–98.

Sinha R., Zandalinas S. I., Fichman Y., Sen S., Zeng S., Gómez-Cadenas A., Mittler R., (2022), “Differential regulation of flower transpiration during abiotic stress in annual plants,”*New Phytologist*, 235 (2), 611-629.

Smith T. E., Grattan S. R., Grieve C. M., Poss J. A., Suarez D. L., (2010), “Salinity’s influence on boron toxicity in broccoli: II. Impacts on boron uptake, uptake mechanisms and tissue ion relations,”*Agricultural Water Management*, 97 (6), 783–791.

Sonmez O., Aydemir S., Kaya C., (2009), “Mitigation effects of mycorrhiza on boron toxicity in wheat (*Triticum durum*) plants,”*New Zealand Journal of Crop and Horticultural Science*, 37 (2), 99–104.

Sonmez S., Buyuktas D., Okturen F., Citak S., (2008), “Assessment of different soil to water ratios (1:1, 1:2.5, 1:5) in soil salinity studies,”*Geoderma*, 144 (1–2), 361–369.

Soriano J. M., Colasuonno P., Marcotuli I., Gadaleta A., (2021), “Meta-QTL analysis and identification of candidate genes for quality, abiotic and biotic stress in durum wheat,”*Scientific Reports*, 11 (1), 11877.

Sowell A., Swearingen B., (2022), “Wheat Outlook : May 2022 U . S . Wheat Price Projected at Record High , Supported by Tight Stocks Domestic Changes at a Glance :,”*U.S. Department of Agriculture*, (Vol. Economic r).

Su Z., Zeng Y., Li X., Perumal A. B., Zhu J., Lu X., Lin F., (2021), “The Endophytic Fungus *Piriformospora indica*-Assisted Alleviation of Cadmium in Tobacco,” *Journal of Fungi*, 7 (8), 675.

Subedi K. D., Gregory P. J., Summerfield R. J., Gooding M. J., (1998), “Cold temperatures and boron deficiency caused grain set failure in spring wheat (*Triticum aestivum* L.),”*Field Crops Research*, 57 (3), 277–288.

Suzuki N., Rivero R. M., Shulaev V., Blumwald E., Mittler R., (n.d.), “Abiotic and biotic stress combinations,” *New Phytologist*, 203 (1), 32-43.

Takano J., Miwa K., Fujiwara T., (2008), “Boron transport mechanisms: collaboration of channels and transporters,”*Trends in Plant Science*, 13 (8), 451–457.

Tenhaken R., (2015a), “Cell wall remodeling under abiotic stress,”*Frontiers in Plant Science*, 5 (2015), 771.

Tenhaken R., (2015b), “Cell wall remodeling under abiotic stress,”*Frontiers in Plant Science*, 5 (JAN), 1–9.

Tilman D., Balzer C., Hill J., Befort B. L., (2011), “Global food demand and the sustainable intensification of agriculture,”*Proceedings of the National Academy of Sciences of the United States of America*, 108 (50), 20260–20264.

Timsina J., (2018),“Can organic sources of nutrients increase crop yields to meet global food demand?,”*Agronomy*, 8 (10), 214.

Torun A. A., Yazici A., Erdem H., Çakmak I., (2006),“Genotypic variation in tolerance to boron toxicity in 70 durum wheat genotypes,”*Turkish Journal of Agriculture and Forestry*, 30 (1), 49–58.

Tripathi D. K., Singh S., Singh S., Mishra S., Chauhan D. K., Dubey N. K., (2015),“Micronutrients and their diverse role in agricultural crops: advances and future prospective,”*Acta Physiologiae Plantarum*, 37 (7), 1–14.

Turan M. A., Taban S., Kayin G. B., Taban N., (2018),“Effect of boron application on calcium and boron concentrations in cell wall of durum (*Triticum durum*) and bread (*Triticum aestivum*) wheat,”*Journal of Plant Nutrition*, 41 (11), 1351–1357.

Van Der Heijden M. G. A., Klironomos J. N., Ursic M., Moutoglis P., Streitwolf-Engel R., Boller T., Sanders I. R., (1998),“Mycorrhizal fungal diversity determines plant biodiversity, ecosystem variability and productivity,”*Nature*, 396 (6706), 69–72.

Varma A., Verma S., Sudha, Sahay N., Bütehorn B., Franken P., (1999),“*Piriformospora indica*, a cultivable plant-growth-promoting root endophyte,”*Applied and Environmental Microbiology*, 65 (6), 2741–2744.

Velu G., Ortiz-Monasterio I., Cakmak I., Hao Y., Singh R. P., (2014),“Biofortification strategies to increase grain zinc and iron concentrations in wheat,”*Journal of Cereal Science*, 59 (3), 365–372.

Vera A., Moreno J. L., García C., Morais D., Bastida F., (2019),“Boron in soil: The impacts on the biomass, composition and activity of the soil mi-crobial community,”*Science of the Total Environment*, 685, 564–573.

Verma S., Chakdar H., Kumar M., Varma A., Saxena A. K., (2021),“Microorganisms as a Sustainable Alternative to Traditional Biofortification of Iron and Zinc: Status and Prospect to Combat Hidden Hunger,”*Journal of Soil Science and Plant Nutrition*, 21 (2), 1700–1717.

Vij S., Tyagi A. K., (2007),“Emerging trends in the functional genomics of the abiotic stress response in crop plants,”*Plant Biotechnology Journal*, 5, 361–380.

Walkowiak S., Gao L., Monat C., Haberer G., Kassa M. T., Brinton J., Pozniak C. J., (2020),“Multiple wheat genomes reveal global variation in modern breeding,”*Nature*, 588 (7837), 277–283.

Warrington K., (1923),“The Effect of Boric Acid and Borax on the Broad Bean and certain other Plants,”*Annals of Botany*, 37 (148), 629–672.

White P. J., Brown P. H., (2010),“Plant nutrition for sustainable development and global health,”*Annals of Botany*, 105 (7), 1073–1080.

Wilck N., Balogh A., Markó L., Bartolomaeus H., Müller D. N., (2019), "The role of sodium in modulating immune cell function," *Nature Reviews Nephrology*, 15 (9), 546–558.

Wimmer M. A., Eichert T., (2013), "Review: Mechanisms for boron deficiency-mediated changes in plant water relations," *Plant Science*, 203–204, 25–32.

Wu M., Wei Q., Xu L., Li H., Oelmüller R., Zhang W., (2018), "Piriformospora indica enhances phosphorus absorption by stimulating acid phosphatase activities and organic acid accumulation in *Brassica napus*," *Plant and Soil*, 432 (1–2), 333–344.

Wu X., Lu X., Riaz M., Yan L., Jiang C., (2019), "Boron toxicity induced specific changes of cell ultrastructure and architecture of components in leaf center and tip of trifoliate orange [*Poncirus trifoliata* (L.) Raf.]," *Journal of Environmental Management*, 246 (August 2018), 426–433.

Xynias I. N., Mylonas I., Korpetis E. G., Ninou E., Tsaballa A., Avdikos I. D., Mavromatis A. G., (2020), "Durum wheat breeding in the Mediterranean region: Current status and future prospects," *Agronomy*, 10 (3), 432.

Yang L., Zou Y. N., Tian Z. H., Wu Q. S., Kuča K., (2021), "Effects of beneficial endophytic fungal inoculants on plant growth and nutrient absorption of trifoliate orange seedlings," *Scientia Horticulturae*, 277 (May 2020).

Yang Y., Li T., Wang Y., Cheng H., Chang S. X., Liang C., An S., (2021), "Negative effects of multiple global change factors on soil microbial diversity," *Soil Biology and Biochemistry*, 156 (April), 108229.

Yoshinari A., Takano J., (2017), "Insights into the mechanisms underlying boron homeostasis in plants," *Frontiers in Plant Science*, 8 (November), 1–8.

Yu Z., Duan X., Luo L., Dai S., Ding Z., Xia G., (2020), "Salt Tolerance Mechanisms of Plants," *Trends in Plant Science*, 7 (16), 478–784.

Zabel R. A., Morrell J. J., (2020), "The characteristics and classification of fungi and bacteria," *Wood Microbiology*, 2020-55-98.

Zhang S., Lehmann A., Zheng W., You Z., Rillig M. C., (2019), "Arbuscular mycorrhizal fungi increase grain yields: a meta-analysis," *New Phytologist*, 222 (1), 543–555.

Zhang Y., Lv Y., Jahan N., Chen G., Ren D., Guo L., (2018), "Sensing of abiotic stress and ionic stress responses in plants," *International Journal of Molecular Sciences*, 19 (11), 1–16.

Zhang Z., Penev E. S., Yakobson B. I., (2017), "Two-dimensional boron: Structures, properties and applications," *Chemical Society Reviews*, 46 (22), 6746–6763.

Zhao S., Zhang Q., Liu M., Zhou H., Ma C., Wang P., (2021),“Regulation of plant responses to salt stress,”*International Journal of Molecular Sciences*, 22 (9), 4609.

Zhou R., Yu X., Ottosen C. O., Rosenqvist E., Zhao L., Wang Y., Wu Z., (2017),“Drought stress had a predominant effect over heat stress on three tomato cultivars subjected to combined stress,”*BMC Plant Biology*, 17 (1), 1–13.

Zhou Y., Li X., Gao Y., Gao Y. B., van der Heijden M. G. A., Ren A.-Z., (2018),“Plant endophytes and arbuscular mycorrhizal fungi alter plant competition,” 5 (2018), 1168-1179.

Zhu J. K., (2016),“Abiotic Stress Signaling and Responses in Plants,”*Cell*, 167 (2), 313–324.

Zimny T., Sowa S., Tyczewska A., Twardowski T., (2019),“Certain new plant breeding techniques and their marketability in the context of EU GMO legislation – recent developments,”*New Biotechnology*, 51 (November 2018), 49–56.

Zuccaro A., Lahrmann U., Güldener U., Langen G., Pfiffli S., Biedenkopf D., Howlett B. J., (2011),“Endophytic Life Strategies Decoded by Genome and Transcriptome Analyses of the Mutualistic Root Symbiont *Piriformospora indica*,” *Plos pathogens*, 7 (10), 1002090.

BIOGRAPHY

Özgür ALTUNDAŞ graduated as an Agricultural Genetic Engineer with GPA 3.14 from Nigde Omer Halisdemir University. He attended congresses during his undergraduate education. He received an honourable mention from the Plant Breeders Sub-Union of Turkey (BISAB) because of his essay about plant breeding. He performed his internship at Potato Research Institute for 3 months in 2019. Later, he earned his master's degree in the field of biotechnology with GPA 3.84. He worked as a researcher on TUBITAK-1001 project for 2 years. He made oral presentations during his master's in national and international congress at IV. Agribalkan Congress, 2nd International Graduate Studies Congress, 6th Graduate Research Symposium and 2nd International Molecular Plant Protection Congress.