

T.R.
GEBZE TECHNICAL UNIVERSITY
INSTITUTE OF BIOTECHNOLOGY

**INVESTIGATION OF THE EFFECTS OF
BENEFICIAL SOIL FUNGI ON THE BORON TOXICITY
TOLERANCE AND ROOT TRAITS OF WHEAT GROWN IN
SOILLESS CULTURE**

ELIF OZLEM GUNCALDI
A THESIS SUBMITTED FOR THE DEGREE OF
MASTER OF SCIENCE
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THESIS SUPERVISOR
ASST. PROF. UMIT BARIS KUTMAN

GEBZE
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T.C.
GEBZE TEKNİK ÜNİVERSİTESİ
BİYOTEKNOLOJİ ENSTİTÜSÜ

YARARLI TOPRAK MANTARLARININ
TOPRAKSIZ KÜLTÜRDE YETİŞTİRİLEN
BUĞDAYLARIN BOR TOKSİSİTE
TOLERANSI VE KÖK ÖZELLİKLERİ
ÜZERİNE ETKİLERİNİN İNCELENMESİ

ELİF ÖZLEM GÜNÇALDI
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BİYOTEKNOLOJİ ANABİLİM DALI

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

SUMMARY

Boron (B) is an essential micronutrient which is required in certain amounts for a healthy plant growth. The range between deficiency and toxicity of the element for plants is extremely narrow and both inadequate and excessive B supply limit yield and diminish quality.

Recently, beneficial soil microorganisms have gotten a lot of attention as environmentally sustainable ways to boost soil fertility and crop productivity. Studies have shown that microorganisms such as rhizobacteria, arbuscular mycorrhizal fungi, other symbiotic fungi such as *Piriformospora indica* can provide benefits to their host plants such as increased growth and yield, improved pest and disease resistance and increased tolerance to abiotic stresses.

The aim of this study was to observe the effects of colonization by arbuscular mycorrhiza (AM) or *Piriformospora indica* on the root traits and stress tolerance of wheat plants that were exposed to Boron (B) toxicity stress, grown in pseudo-hydroponic system.

Results showed that the effect on growth, responses related to abiotic stress tolerance, and alterations in root morphology differed between AM and *P. indica* colonization. *P. indica* performed better in respect to growth promotion, compared to AM. Colonization with AM caused considerable changes in root architecture by reducing root length, surface area and tip number; *P. indica* colonization showed no effect on root length or surface area but average root diameter and number of tips were higher in colonized plants. Major differences between effects on nutritional status and activities of antioxidative enzymes were also detected.

Key words: Boron toxicity, arbuscular mycorrhiza, *Piriformospora indica*, phosphorus deficiency, root morphology, antioxidative activity, soilless culture.

ÖZET

Bor (B), sağlıklı bir büyüme için bitkilerin belirli bir miktarda ihtiyaç duyduğu bir esansiyel mikrobeseindir. Bitkiler için elementin eksikliği ve toksisitesi arasındaki aralık son derece dardır ve hem yetersiz hem de gereğinden fazla B tedariği verimi sınırlar ve kaliteyi düşürür.

Faydalı toprak mikroorganizmalarının tarımda kullanımı, toprak ve mahsul verimliliğini artırmanın çevresel açıdan sürdürülebilir yolları olarak son zamanlarda oldukça dikkat çekmiştir. Çalışmalar, rizobakteriler, arbusküler mikorizal mantarlar ve *Piriformospora indica* gibi simbiyotik mantarlar ve diğer faydalı mikroorganizmaların konak bitkilere artan büyüme ve verim, gelişmiş hasıere ve hastalık direnci ve abiyotik streslere karşı artan tolerans gibi faydalar sağlayabileceğini göstermiştir.

Bu çalışma, arbusküler mikoriza (AM) veya *Piriformospora indica* ile kolonizasyonunun Bor (B) toksisite stresine maruz kalan ve sözde-hidroponik sistemde büyütülen buğday bitkilerinin kök özellikleri ve stres toleransı üzerindeki etkilerini gözlemlemek amacıyla gerçekleştirilmiştir.

Sonuçlar, büyüme üzerindeki etkinin, abiyotik stres toleransına bağlı tepkilerin ve kök morfolojisindeki değişikliklerin AM ve *P. indica* kolonizasyonu arasında farklılık gösterdiğini belirtmiştir. *P. indica*, AM'ye kıyasla büyümeyi destekleme açısından daha iyi performans göstermiştir. AM ile kolonizasyon, kök uzunluğunu, yüzey alanını ve uç sayısını azaltarak kök mimarisinde önemli değişikliklere neden olmuştur; bu sırada *P. indica* kolonizasyonu, kök uzunluğu veya yüzey alanı üzerinde hiçbir etki göstermemiştir, ancak kolonize bitkilerde ortalama kök çapı ve uç sayısının daha yüksek olduğu gözlemlenmiştir. Bitki beslenmesi üzerinde etkiler ve antioksidan enzimlerin aktiviteleri arasında da büyük farklılıklar tespit edilmiştir.

Anahtar Kelimeler: Bor toksisitesi, arbusküler mikoriza, *Piriformospora indica*, fosfor eksikliği, kök morfolojisi, antioksidan aktivitesi, topraksız kültür.

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TABLE OF CONTENTS

SUMMARY	i
ÖZET	ii
ACKNOWLEDGEMENTS	iii
LIST of ABBREVIATIONS and ACRONYMS	vi
LIST of FIGURES	viii
LIST of TABLES	ix
1. INTRODUCTION	1
1.1. Climate Change and Abiotic Stress	1
1.2. Plant Mineral Nutrition	2
1.3. Boron	3
1.4. Boron in Plants	4
1.5. Boron Toxicity	5
1.6. Boron Toxicity Tolerance	6
1.7. Beneficial Soil Fungi	7
1.8. Effects of Abiotic Stress and Endophytes on Root System Architecture (RSA)	10
1.9. Wheat	11
2. MATERIAL AND METHODS	12
2.1. Materials and General Information	12
2.2. Plant Growth Facilities and Growing Medium	13
2.3. Experimental Designs and Applied Methods	13
2.3.1 First Experiment with Arbuscular Mycorrhiza Colonization	13
2.3.2 Second Experiment with Arbuscular Mycorrhiza Colonization	14
2.3.3 First Experiment with <i>P. indica</i> Colonization	15
2.3.4 Second Experiment with <i>P. indica</i> Colonization	16
2.4. Relative Electron Leakage (REL) Analysis	17
2.5. Root Morphology Analysis	17
2.6. Element Analysis	17
2.7. Antioxidative Enzymes and Total Protein Analysis	18
2.7.1. Superoxide Dismutase (SOD) Analysis	18
2.7.2. Glutathione Reductase (GR) Analysis	18
2.7.3. Ascorbate Peroxidase (AP) Analysis	19

2.7.4. Catalase (CAT) Analysis	19
2.8. Root Staining	20
2.9. Statistical Analysis	20
3. RESULTS	21
3.1. First Experiment with Arbuscular Mycorrhiza Colonization	21
3.2. Second Experiment with Arbuscular Mycorrhiza Colonization	37
3.3. First Experiment with <i>P. indica</i> Colonization	42
3.4. Second Experiment with <i>P. indica</i> Colonization	53
4. DISCUSSION	57
5. CONCLUSION	62
REFERENCES	63
BIOGRAPHY	75

LIST of ABBREVIATIONS and ACRONYMS

Abbreviations and Acronyms

Explanations

μM	Micromolar
Al	Aluminum
AM	Arbuscular Mycorrhiza
ANOVA	Analysis of variance
AP	Ascorbate peroxidase
B	Boron
Ca	Calcium
$\text{Ca}(\text{NO}_3)_2$	Calcium nitrate
CAT	Catalase
cm^2	Centimeter square
Conc.	Concentration
Cont.	Content
Cu	Copper
cv.	Cultivar
DAS	Days After Sowing
DW	Dry Weight
EC	Electrical Conductivity
Fe	Iron
GR	Glutathione reductase
H_2O_2	Hydrogen peroxide
H_3BO_3	Boric acid
HCl	Hydrochloric acid
K	Potassium
LSD	Fisher's least significant difference
mg	Milligram
Mg	Magnesium
ml	Milliliter

mm	Millimeter
mM	Millimolar
Mn	Manganese
Mo	Molybdenum
N	Nitrogen
<i>n.s.</i>	not significant
Ni	Nickel
O ₂ ⁻	Superoxide radical
OH ⁻	Hydroxyl radical
P	Phosphorus
<i>P. indica</i>	<i>Piriformospora indica</i>
REL	Relative Electron Leakage
RG-II	Rhamnogalacturonan II
ROS	Reactive Oxygen Species
S	Sulphur
SOD	Superoxide dismutase
Zn	Zinc

LIST of FIGURES

<u>Figure No:</u>	<u>Page</u>
3.1: Effects of P deficiency, B toxicity and inoculation with AM fungi on 5-week-old bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak) plants supplied with two levels of P (Low: 0.02 mM KH ₂ PO ₄ ; Control: 0.2 mM KH ₂ PO ₄) and high level of B (1000 µM H ₃ BO ₃).	22
3.2: Effects of inoculation with AM fungi on 5-week-old bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak) supplied with an inadequate level of P (0.02 mM KH ₂ PO ₄) and two levels of B (Control: 2 µM H ₃ BO ₃ ; High: 1000 µM H ₃ BO ₃).	23
3.3: Mycorrhizal colonization in root samples of wheat plants, stained with trypan blue and observed under light microscope: A, B, non-inoculated (10x resolution); C, D, inoculated with AM (10x resolution).	36
3.4: Effect of colonization with AM fungi (A: non-inoculated; B: inoculated) on total root length of 3-week-old wheat plants supplied with two different levels of Phosphorus (Deficient: 0.02 mM; Adequate: 0.2 mM) and Boron (Control: 2 µM H ₃ BO ₃ ; High 1000 µM H ₃ BO ₃) grown in pseudo-hydroponic system under greenhouse conditions. Different letters indicate significant differences between means according to two-way ANOVA and Fisher's protected LSD test ($p \leq 0.05$).	41
3.5: Effects of inoculation with <i>P. indica</i> on 5-week-old wheat plants supplied with two levels of B (control: 2 µM H ₃ BO ₃ ; high: 1000 µM H ₃ BO ₃).	43
3.6: Effect of colonization with <i>P. indica</i> (A: non-inoculated; B: inoculated) on 5-week-old wheat plants supplied with two different levels of Boron (Control: 2 µM H ₃ BO ₃ ; High 1000 µM H ₃ BO ₃) grown in pseudo-hydroponic system under greenhouse conditions. Different letters indicate significant differences between means according to two-way ANOVA and Fisher's protected LSD test ($p \leq 0.05$).	44
3.7: <i>P. indica</i> colonization in root samples of wheat plants, stained with trypan blue and observed under light microscope: A, non-inoculated (10x resolution); B, C, D, inoculated with <i>P. indica</i> (4x, 10x, 20x resolution, respectively).	52

LIST of TABLES

<u>Table No:</u>	<u>Page</u>
3.1: Shoot dry weight data of 5-week-old non-inoculated (Control) or inoculated (AM) bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak) plants supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H ₃ BO ₃ ; High: 1000 μ M H ₃ BO ₃) grown in pseudo-hydroponic system under greenhouse conditions.	24
3.2: Relative electron leakage (%) from first (REL-1) and second (REL-2) youngest leaves of 5-week-old non-inoculated (Control) or inoculated (AM) bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak) plants supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H ₃ BO ₃ ; High: 1000 μ M H ₃ BO ₃) grown in pseudo-hydroponic system under greenhouse conditions.	25
3.3: Analysis of variance of the main effects of AM inoculation, P deficiency, B toxicity and their interaction on dry weight (DW), relative electron leakage from youngest leaves (REL-1) and second youngest leaves (REL-2) and mineral status of 5-week-old bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and (<i>Triticum durum</i> cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.	26
3.4: Concentrations of selected macronutrients (K, P, Mg, S) of 5-week-old bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak) plants with two inoculation status (Control; Arbuscular Mycorrhiza); supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H ₃ BO ₃ ; High: 1000 μ M H ₃ BO ₃) grown in pseudo-hydroponic system under greenhouse conditions.	28
3.5: Concentrations of selected micronutrients (B, Fe, Zn) of 5-week-old bread wheat (<i>Triticum aestivum</i> cv. Nusrat) and durum wheat (<i>Triticum durum</i> cv. Saricanak) plants with two inoculation status (Control; Arbuscular Mycorrhiza); supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H ₃ BO ₃ ; High: 1000 μ M H ₃ BO ₃) grown in pseudo-hydroponic system under greenhouse conditions.	30
3.6: Analysis of variance of the main effects of AM inoculation, P deficiency, B toxicity and their interaction on total and specific enzymatic activities of ascorbate peroxidase (AP), glutathione reductase (GR) and catalase (CAT) of 5-week-old bread wheat	32

(*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

- 3.7: Total antioxidative enzyme; ascorbate peroxidase (AP), 33
glutathione reductase (GR) and catalase (CAT) activities of 5-
week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum
wheat (*Triticum durum* cv. Saricanak) plants with two inoculation
status (Control; Arbuscular Mycorrhiza); supplied with two
different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and
B (Control: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃) grown in
pseudo-hydroponic system under greenhouse conditions.
- 3.8: Specific antioxidative enzyme; ascorbate peroxidase (AP), 35
glutathione reductase (GR) and catalase (CAT) activities of 5-
week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum
wheat (*Triticum durum* cv. Saricanak) plants with two inoculation
status (Control; Arbuscular Mycorrhiza); supplied with two
different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and
B (Control: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃) grown in
pseudo-hydroponic system under greenhouse conditions.
- 3.9: Analysis of variance of the main effects of AM inoculation, P 37
deficiency, B toxicity and their interaction on shoot dry weight
(DW) and root/shoot ratio of 3-week-old bread wheat (*Triticum
aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown
in pseudo-hydroponic system under greenhouse conditions.
- 3.10: Effect of colonization with AM on shoot dry weight, root dry 38
weight and root/shoot ratio of 3-week-old bread wheat (*Triticum
aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv.
Saricanak) plants supplied with two different levels of P
(Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Adequate: 2 μ M
H₃BO₃; High 1000 μ M H₃BO₃) grown in pseudo-hydroponic
system under greenhouse conditions.
- 3.11: Analysis of variance of the main effects of AM inoculation, P 39
deficiency, B toxicity and their interaction on root length and root
surface area, root volume, average diameter of roots and number
of root tips of 3-week-old bread wheat (*Triticum aestivum* cv.
Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-
hydroponic system under greenhouse conditions.
- 3.12: Root morphological traits of 3-week-old bread wheat (*Triticum* 40
aestivum cv. Nusrat) and (*Triticum durum* cv. Saricanak) plants
with two inoculation status (Control; Arbuscular Mycorrhiza)
supplied with two different levels of P (Deficient: 0.02 mM;
Adequate: 0.2 mM) and B (Control: 2 μ M H₃BO₃; High 1000
 μ M H₃BO₃).

- 3.13: Relative electron leakage (%) from second youngest leaves of 5- 45
week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum
wheat (*Triticum durum* cv. Saricanak) plants supplied with two
different levels of B (Adequate: 2 μ M H₃BO₃; High 1000 μ M
H₃BO₃) grown in pseudo-hydroponic system under greenhouse
conditions.
- 3.14: Analysis of variance of the main effects of *P. indica* inoculation 46
and B toxicity and their interaction on dry weight (DW), relative
electron leakage (REL) and mineral status of 5-week-old bread
wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv.
Saricanak) grown in pseudo-hydroponic system under greenhouse
conditions.
- 3.15: Concentrations of selected macronutrients (K, P, Mg, S) of 5- 47
week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum
wheat (*Triticum durum* cv. Saricanak) plants supplied with two
different levels of B (Adequate: 2 μ M H₃BO₃; High: 1000 μ M
H₃BO₃) grown in pseudo-hydroponic system under greenhouse
conditions.
- 3.16: Concentrations of selected micronutrients (B, Fe, Zn) of 5-week- 48
old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat
(*Triticum durum* cv. Saricanak) plants supplied with two different
levels of B (Adequate: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃)
grown in pseudo-hydroponic system under greenhouse conditions.
- 3.17: Analysis of variance of the main effects of *P. indica* inoculation, 49
B toxicity and their interaction on total and specific enzymatic
activities of superoxide dismutase (SOD), ascorbate peroxidase
(AP), glutathione reductase (GR) and catalase (CAT) of 5-week-
old wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv.
Saricanak) grown in pseudo-hydroponic system under greenhouse
conditions.
- 3.18: Total antioxidative enzyme; superoxide dismutase (SOD), 50
ascorbate peroxidase (AP), glutathione reductase (GR) and
catalase (CAT) activities of 5-week-old bread wheat (*Triticum*
aestivum cv. Nusrat) and durum wheat (*Triticum durum* cv.
Saricanak) plants with two inoculation status (Control; *P. indica*);
supplied with two different levels of B (Control: 2 μ M H₃BO₃;
High: 1000 μ M H₃BO₃) grown in pseudo-hydroponic system
under greenhouse conditions.
- 3.19: Specific antioxidative enzyme; superoxide dismutase (SOD), 51
ascorbate peroxidase (AP), glutathione reductase (GR) and
catalase (CAT) activities of 5-week-old bread wheat (*Triticum*
aestivum cv. Nusrat) and durum wheat (*Triticum durum* cv.
Saricanak) plants with two inoculation status (Control; *P. indica*);
supplied with two different levels of B (Control: 2 μ M H₃BO₃;

High: 1000 μM H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions.

- 3.20: Analysis of variance of the main effects of *P. indica* inoculation 53 and B toxicity and their interaction on shoot dry weight (DW), root dry weight and root/shoot ratio of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.
- 3.21: Effect of *P. indica* colonization on shoot dry weight, root dry 54 weight and root/shoot ratio of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two different levels of B (Adequate: 2 μM H_3BO_3 ; High 1000 μM H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions.
- 3.22: Analysis of variance of the main effects of *P. indica* inoculation 55 and B toxicity and their interaction on root length, surface area, root volume, average diameter, and number of root tips of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.
- 3.23: Root surface area, average diameter, root volume and number of 56 root tips of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two different levels of B (Adequate: 2 μM H_3BO_3 ; High 1000 μM H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions.

1. INTRODUCTION

1.1. Climate Change and Abiotic Stress

The production of enough food to fulfill population demands despite losses in the quantity and quality of arable land and water, as well as increasingly erratic weather patterns linked with climate change, is a twenty-first-century challenge [Mickelbart et al., 2015].

Crop productivity is being hindered worldwide due to abiotic stress factors such as drought, salinity, ion toxicity, high temperatures, and pollution. Plants' response to abiotic stress is mediated by reactive oxygen species (ROS). They are mostly signal transduction molecules that regulate several pathways during plant stress acclimation, but they are also harmful consequences of stress metabolism causing oxidative stress in addition [Choudhury et al., 2017].

The usual response to abiotic and oxidative stress is growth limitation. Furthermore, severe and/or prolonged stress conditions may end up causing individual cells and possibly the entire plant to die, putting the yield at risk [Gechev and Petrov, 2020].

ROS levels must be strictly restricted to avoid toxicity and to maintain precise execution of their signalling functions. As a detoxification mechanism, plants have developed a complex enzymatic and non-enzymatic antioxidant system that, in combination with ROS-producing enzymes, regulates ROS homeostasis in all cellular compartments and maintains the adjustment of ROS levels based on the cellular requirement at any given moment [Gechev et al., 2006].

1.2. Plant Mineral Nutrition

The elements absorbed in the form of inorganic ions from soil by plant roots for utilization in biological processes and functions are called mineral nutrients. Plants contribute to the element cycles in organisms by acting as miners in the sense of taking up elements and make them enter biosphere through their root system [Epstein, 1999]. Mineral nutrition studies the mechanisms of element absorption by roots, translocation of elements through the plants and their various functions in plant metabolism [Taiz and Zeiger, 1991].

Studies on essentiality of elements revealed that only certain elements are needed in certain amounts for plants to complete their life cycle and to grow healthily. The elements which are:

- required for a plant to complete its life cycle,
- directly involved in plant metabolism,
- not replaceable

are determined as essential mineral nutrients [Arnon and Stout, 1939]. Essential elements are divided into two distinct groups as macronutrients (N, P, K, S, Mg, Ca) and micronutrients (Fe, Mn, Zn, Cu, Ni, Mo, B) according to the required amounts of elements in plant metabolism [Taiz and Zeiger, 1991], [Epstein, 1999]. It is known that inadequate supply of nutrients not only affects plant growth but also diminishes yield which is described as the proportion of harvested plant parts to total biomass. The nutritional status of plants plays an important role in biomass production by affecting processes such as photosynthesis, leaf senescence and respiration [Marschner, 2011]. Therefore, a proper fertilization regime must be applied in agricultural systems to grow healthy plants and receive maximum yield.

1.3. Boron

Boron (B) belongs to third group of periodic table along with other metalloid elements such as silicon (Si) and germanium (Ge), displaying intermediate properties between the metals and non-metals [Princi et al., 2016]. Boron is distributed in soil solution unevenly as H_3BO_3 and $\text{B}(\text{HO})_4^-$ and predominantly taken up by plants in the form of H_3BO_3 [Princi et al., 2016], [Brdar-Jokanovic, 2020].

The primary natural sources of B are borosilicate mineral tourmaline, volatile volcano leaks and water sources such as groundwater, geothermal streams or seawater. Tourmaline is a stable crystal mineral occurring as combinations of different elements formed with boron silicate, and it accumulates in sedimentary rocks. The boron is not accessible for plants before it is degraded from boron silicate into bioavailable boric acid molecules. Apart from rock weathering, seawater evaporation contributes to the B concentration in saline soils [Brdar-Jokanović, 2020]. Main anthropogenic sources of boron release are agricultural practices; coal and oil use in electricity generation; mining, processing, and leaching of borates and sewage disposal [Landi et al., 2019].

Boric acid (H_3BO_3) is highly soluble therefore soil boron is prone to leaching, B deficiency is very common in humid areas which gets more frequent precipitation [Brdar-Jokanović, 2020], [Tanaka and Fujiwara, 2008]. On the other hand, B excess rather occurs in arid or semiarid regions and it may become toxic for plants since the range between deficiency and toxicity is extremely narrow for boron [Landi et al., 2019], [Paull et al., 1991]. Both deficiency and toxicity of B cause plant disorders that reduce crop quality and limit yield. Availability of the element for plants is regulated by their adsorption to soil particles, which is affected by pH, soil texture, salinity, Al and Fe oxide content, organic matter, and clay content [Princi et al., 2016], [Ozturk et al., 2010].

1.4. Boron in Plants

The knowledge on functions of B in plant metabolism is still lacking although the requirement for the element is the highest among micronutrients. The main reasons which slow down the improvements regarding to understand the role of B in plants are difficulties in determining low concentrations in cells or tissues, instability of B and its reversible binding ability to various molecules [Marschner, 2011].

The processes that boron contributes in plant metabolism include

- Cell wall synthesis and lignification
- Sugar transport
- Carbohydrate metabolism
- Preserving plasma membrane integrity
- Phenol metabolism
- The ascorbate/ glutathione cycle
- Indole acetic acid metabolism
- Pollen tube growth
- Respiration and photosynthesis
- Various enzymatic activities [Princi et al., 2016], [Marschner, 2011].

The main function of boron is thought to be the synthesis of cell wall and maintenance of its integrity and function. The amount of the element present in cell wall structure is changeable according to the species or organ but almost 90% of cellular boron are observed to be located in the cell wall [Loomis and Durst, 1992] [Goldbach et al., 2000], [Brdar-Jokanović, 2020]. Boron is taken up by plants predominantly in the form of uncharged boric acid molecules, although it is possible to be taken up as borate anions to a much lesser extent. These compounds have the capability to form complexes with diols and polyols, especially with cis-diols. Rhamnogalacturonan II is known to be the first boron-containing compound defined in plant kingdom, borate bridges two monomers by forming ester bonds between apiose residues of RG II [O'Neill et al., 1996], [Brdar-Jokanović, 2020]. When an

adequate amount of boron is supplied, dimerization of more than 90% of RGII takes place by borate ester bonds. Although the amount of RG-II in cell wall polysaccharides is relatively low, growing evidence suggests that RG-II and its borate crosslinking are important for plant growth and development by sustaining cell adhesion and mechanical strength [Funakawa et al., 2015]. Boron takes role in plant cell membranes by forming complexes with the main lipid raft components such as glycosyl inositol phosphoryl ceramides (GIPC) [Voxeur and Fry, 2014]. As a result, B helps to create GIPCs-B-RGII complexes, acting as a bridge between the cell plasma membrane and the cell wall [Wang et al., 2015], [Landi et al., 2019].

Inadequate B supply limits photosynthetic capacity and impairs the translocation of photosynthetic products [Wang et al., 2015], [Brdar-Jokanović, 2020].

Many physiological abnormalities have been reported as the results of B deficiency, and it has been predicted that B is involved in a wide range of metabolic processes. Boron and the ascorbate/glutathione cycle have been found to have a strong connection. Number of researches have highlighted that by restricting ascorbate peroxidase and glutathione reductase, B deficiency decreases ascorbate and glutathione levels considerably. Supplying plants with ascorbate partially compensated for the reduction in the root growth induced by B insufficiency [Lukaszewski and Blevins, 1996], [Cakmak and Römheld, 1997], [Brown, 2002].

1.5. Boron Toxicity

Boron toxicity is observed mostly in arid and semi-arid locations in plants growing on soils derived from marine parent material or irrigated with water that contains high levels of B [Nable et al., 1997], [Marschner, 2011].

High levels of B in soil causes various effects on very diverse processes in vascular plants; B toxicity impairs plant growth and development, alters plant metabolism, impairs root cell division, reduces leaf chlorophyll content and photosynthetic rate

and causes gradual necrosis in leaves, stems, and other abnormalities in fruits [Reid et al. 2004], [Landi et al., 2019], [Camacho-Cristóbal et al., 2008]. The macroscopic impairments of excess B occur mostly due to the three metabolic actions that B has:

- Disrupted cell division and development caused by binding to ribose (as free sugar or RNA component)
- Hindered primary metabolism caused by binding to ribose in ATP or NAD(P)H
- Altered protein synthesis due to reduced cytosolic pH [Reid et al. 2004], [Landi et al., 2019].

In many plants, boron accumulates from the leaf base to the tip, causing common toxicity symptoms such as marginal or tip chlorosis or/and necrosis on older leaves [Marschner, 2011], [Camacho-Cristóbal et al., 2008].

Root growth inhibition accompanied with reduced root dry weight are common consequences of increased accumulation of B in roots. Plants under B toxicity stress attempt to reduce B intake and limit toxicity by inducing modifications at the root level, such as alteration of root meristems, increased thickness of hypodermis, deposition of suberin in cortical cells, and root lignification [Choi et al., 2007], [Landi et al., 2019]. A study by Aquea et al. (2012) reported that B toxicity induced a water-stress response linked with root development inhibition, as evidenced by the repression of genes producing water transporters [Princi et al., 2016].

1.6. Boron Toxicity Tolerance

General knowledge indicates that B toxicity tolerance is defined by capabilities of plants such as reduction of B uptake and exudation of excessive B from roots, induced production of B-chelating compounds, stimulation of antioxidative response for alleviating oxidative stress caused by increased ROS accumulation, partition of B

into locations where it will be less damaging for cellular activities [Landi et al., 2019].

Genotypic differences cause a variation in B tolerance at both inter- and intra-specific levels [Ozturk et al., 2010]. Studies on wheat cultivars differing in B tolerance reported that root concentration of B was correlated to both shoot B concentration and shoot yield; indicating that main procedure of B tolerance is regulating the B concentrations in root tissues and limiting the accumulation of B in shoot, not tolerating high B levels in shoot tissues [Nable et al., 1997], [Marschner, 2011].

It is also possible that B tolerance is linked to the preferential distribution of accumulated B at the organ, tissue, or cellular level. Phloem mobility of B varies between species, B accumulation may occur in leaf margins (in phloem immobile plants) or fruits, cambial tissue and stems (in phloem mobile plants) [Brown and Hu, 1996], [Nable et al., 1997] Phloem immobility may be contributing the B tolerance by reducing B accumulation in critical metabolic sites [Nable et al., 1997].

Transpiration was observed to be affecting B accumulation; as water usage increases, so does boron transport and concentration in the leaf tips. A study on *Arabidopsis* showed that excessive external boron decreases water transport and transpiration, which might be a boron tolerance mechanism [Macho-Rivero et al., 2018], [Brdar-Jokanović, 2020].

The antioxidant apparatus, which includes both enzymatic and non-enzymatic antioxidants, is one of the most effective mechanisms against a variety of environmental factors that cause oxidative stress. Plants that have higher antioxidative activity, constitutive or induced, were observed to have higher B tolerance [Landi et al., 2019].

1.7. Beneficial Soil Fungi

Since the term symbiosis was first defined as "the coexistence of dissimilar organisms" a variety of symbiotic lifestyles have been established based on fitness

advantages or repercussions on macroscopic hosts and microscopic symbionts [De Bary, 1879], [Rodriguez et al., 2009]. Collective knowledge indicates that most, if not all, plants that grow in natural ecosystems form a symbiotic relationship with mycorrhizal fungi or other beneficial fungi [Rodriguez et al., 2009].

Arbuscular mycorrhizal fungi (AMF), several species grouped in phylum Glomeromycota, are microscopic fungi that colonize both the roots and the rhizosphere and extend out across several centimeters in fractal patterns through their filaments called hyphae [Bonfante and Genre, 2010]. AMF have great ecological importance since they are able to form symbiosis with wide range of terrestrial and a number of wetland plants [Xie et al., 2014]. Tree-like structures that are called arbuscules are formed within plant cells as a component of symbiotic interaction between AM and host plants; the primary site of nutrient exchange between the fungal and plant symbiotic partners is considered to be these structures. AMF also forms a connection between plants and hyphal network of fungi, which is designed for uptake of water and nutrient (primarily phosphate). In exchange for nutrients and water they supply to their host, AM fungi receive carbohydrates from plants [Finlay, 2008], [Parniske, 2008]. The possible benefits of AM are particularly noticeable when nutrient availability is restricted. Although the regulator mechanisms are still unclear, it is known that the colonization efficiency of AM decreases when nutrients are in abundance [Parniske, 2008].

Due to the vast surface area of their hyphae and their high-affinity uptake mechanisms, AM fungi are thought to improve the P nutrition of plants by scavenging the bioavailable P. There have been studies showing that AM may increase P availability by dissolving insoluble mineral phosphates through organic acid release. However, because AM are obligate symbionts, their potential as a phosphate biofertilizer is limited by their inability to be cultured *in vitro* [Gyaneshwar et al., 2002].

AM fungi were shown to improve plant growth and yield of plants under abiotic stress. It is estimated that AM may also benefit plants by taking roles as biofertilizers, biocontrol agents and bioprotectors [Jeffries and Barea, 2012]. AMF-promoted alleviation of oxidative stress, accelerated water and nutrient uptake, and

changes in gene transcription in signaling pathways or stress responses are all thought to be mechanisms enabling AM's protective effects in plants [Abdel Latef et al., 2016].

Piriformospora indica (*P. indica*), belonging to order Sebaciniales (Basidiomycota), is an axenically cultivable root endosymbiont that is recognized for its similar capabilities of AM fungi in improving growth and health of host plants [Varma et al., 1999], [Abdelaziz et al., 2017]. The fungus was discovered in rhizosphere samples of orchids in Thar desert [Verma et al., 1998]. *Piriformospora indica* is able to form symbiosis with a wide variety of monocotyledonous and dicotyledonous plants, including crop plants of great importance [Varma et al., 1999]. Fungal growth occurs in cortical tissues of root, often in the differentiation and root hair zone; pear-shaped chlamydospores are detected in colonized tissues. Colonization of older tissues is linked to the host cell death required for proliferation, but no detrimental effect on root growth or viability is observed [Deshmukh et al., 2006], [Achatz et al., 2010].

The most prominent impact of *P. indica* on plants is that it promotes vegetative growth, which has been demonstrated in a variety of plant families. The examined growth parameters revealed that *P. indica* contributes in the initial stages of plant development and also induces the transition to generative stages [Barazani et al., 2005], [Franken, 2012]. These effects are thought to be mostly dependent on enhanced root development [Franken, 2012]. Other than improved growth and biomass production, beneficial effects of *P. indica* such as increased secondary metabolite synthesis, improved nutritional status and stress tolerance was reported [Ansari et al., 2013]. One advantage of *P. indica* in comparison to AM fungi is that colonization occurs independently of nutrient availability [Varma et al., 1999]. Thus, it makes *P. indica* an alternative growth promoter that can be applied in soils which are rich in P or other nutrients [Achatz et al., 2010], [Franken, 2012].

Piriformospora indica was shown to increase tolerance to abiotic stress and promote growth under stress conditions through mechanisms such as regulating defense system and metabolism to compensate for the limited photosynthesis and alleviate the oxidative damage induced by stress. Studies on salinity and drought

reported increased antioxidative activity, particularly for ascorbate-glutathione (ASH-GSH). The Induced activity of other several antioxidative enzymes like superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (AP), glutathione reductase (GR), peroxidase (POD), mono-dehydroascorbate reductase (MDHAR) and dehydroascorbate reductase (DHAR) were also observed [Varma et al., 2013].

1.8. Effects of Abiotic Stress and Endophytes on Root System Architecture (RSA)

Root system architecture (RSA) refers to the spatial arrangement of plant roots, which includes primary roots, branch roots, root hairs, root meristems, and root caps [Khan et al., 2016]. Significant changes in root morphology including branching, surface area, number of lateral roots and root hairs were reported under abiotic stress [Liu et al., 2019]. Symbiosis with beneficial endophytes may be a coping mechanism of plants for alleviating those stress-related alterations. Colonization of fungal endophytes enhances nutrient solubilization, uptake, and water absorption efficiency of plants by expanding root surface area, eventually improving plant fitness [Lamont 2003], [Yakti et al. 2018], [Verma et al., 2021]. Alteration of root morphology by AM and *P. indica* was reported by a few studies even though the results are changeable depending on the plant and stress factors [Abdelaziz et al., 2017], [Hosseini et al., 2017], [Price et al.; 1989], [Berta et al., 1993], [Schroeder and Janos, 2005]. AM is known to alter root morphology especially when P is limited [Berta et al., 1993].

With the development of novel root phenotyping tools, demonstrating changes in root architecture induced by stress or fungal colonization has become easier [Verma et al., 2021]. Imaging systems and specialized image analysis softwares such as WinRhizo are used for measuring parameters related to root morphological traits.

1.9. Wheat

Wheat is a major agricultural crop in many parts of the world, including Turkey. Turkey, which is part of the wheat center of origin, offers a favorable yet varied environment for wheat cultivation. As a result, Turkey has a potential in the creation of high-yielding, high-quality wheat varieties, which is a crucial crop for the country [Gummadov et al., 2015].

Durum wheat (*Triticum durum*, tetraploid) and bread wheat (*Triticum aestivum*, hexaploid) are closely related species with distinct grain and end-use characteristics (pasta and bakery products, respectively) [Mastrangelo and Cattivelli, 2021]. These two species display different characteristics in terms of yield and stress tolerance and durum wheat is assumed to be more stress tolerant [Marti and Slafer, 2014].

Several studies have demonstrated substantial differences in resistance of wheat cultivars to B toxicity. In wheat, B toxicity causes reduction in shoot growth and yield, malfunction in development and reproduction. Damage in roots and decrease in lateral root growth are also reported [Nable, 1988], [Brdar-Jokanović, 2020].

There have been numerous researches that investigate the mechanisms and the benefits of the symbiosis between AM colonization and wheat cultivars. Advantages in the uptake of nutrients such as P, N, Zn and Se in wheat plants colonized with AM were reported [Blackwell et al., 2015], [Ryan et al., 2008], [Luo et al., 2019], [Ganugi et al., 2019]. Studies on the effects of AM colonization on abiotic stress tolerance of wheat plants proved that AM stimulates growth and increases tolerance against salinity, drought and heavy metal stress [Ganugi et al., 2019]. Similar to these studies, the effects of *P. indica* inoculation on growth, yield and stress tolerance of wheat plants have been investigated. Increased Fe and P concentration, increased tolerance to salinity, drought and Cd toxicity are amongst the reported benefits of *P. indica* colonization [Baghaie and Aghilzafreei, 2020], [Taghinasab et al., 2018], [Zarea et al., 2012], [Hosseini et al., 2017], [Shahabivand et al., 2016].

2. MATERIAL AND METHODS

2.1. Materials and General Information

Documented experiments were conducted on bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak).

Both wheat cultivars were tested and proved suitable to be cultivated in pseudo-hydroponic conditions. Nusrat is a bread wheat cultivar which was verified in 2015, it is known for its great yield potential and expected to meet the need of qualified wheat bread for flour industry [Web 1]. Saricanak is a durum wheat cultivar that was verified in 1998, it has high yield capacity in areas with sufficient precipitation or well-irrigation; the cultivar is widely used in the southeastern Anatolia region in Turkey [Web 2].

High B level condition were created by adding excess boric acid (H_3BO_3) into nutrient solution that was used for irrigating subject plants.

Phosphorus was added as KH_2PO_4 form into standard full-strength nutrient solution; 0.2 mM for adequate level and 0.02 mM for deficiency conditions.

Shubhodoya Mycorrhizal Bio-Fertilizer, a commercial product, was used as inoculation agent for colonization with AM.

Piriformospora indica was axenically cultured in Kaefer medium prepared according to [Hill and Kafer, 2001]. The fungus was grown in solid medium for 19 days at 28 °C, then scratched off the surface of the solid medium and transferred into 200 mL liquid medium. *Piriformospora indica* was then grown in liquid medium for 15 days at 28 °C and 200 rpm in an incubator shaker.

2.2. Plant Growth Facilities and Growing Medium

All experiments were conducted in a greenhouse illuminated with natural daylight. The temperature inside the greenhouse was controlled by heating and evaporative cooling systems to be kept between 15-25°C.

The pseudo-hydroponic system was used for germinating and growing subject plants. Fine vermiculite (2-4 mm) was used as solid substrate. Standardized full strength nutrient solution which was used for daily irrigation contained 0.6 mM K₂SO₄, 4 mM Ca(NO₃)₂·4H₂O, 0.75 mM MgSO₄·7H₂O, 0.1 mM KCl, 75 µM Fe (in the form of FeEDTA), 2 µM H₃BO₃, 2 µM MnSO₄·H₂O, 3 µM ZnSO₄·7H₂O, 0.6 µM CuSO₄·5H₂O and 0.5 µM (NH₄)₆Mo₇O₂₄·4H₂O. P and excess B were added according to the experimental plan.

Plants were irrigated daily with full strength nutrient solution till exceeding water holding capacity and the overflowed solution was removed.

2.3. Experimental Designs and Applied Methods

2.3.1 First Experiment with Arbuscular Mycorrhiza Colonization

In this experiment, bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants were grown in pots filled with vermiculite, irrigated with nutrient solution, and kept in controlled environmental conditions.

The experiment was consisted of 64 pots in total (4 replicates x 2 cultivars x 2 inoculation status x 2 P levels x 2 B levels); there were 8 single plants in each pot.

Plants were supplied with two P levels: 0.02 mM and 0.2 mM in the form of KH_2PO_4 . In addition, two levels of B were applied into nutrient solution: 2 μM and 1000 μM in the form of H_3BO_3 .

Colonization with AM was initiated 7 days after sowing (DAS) and performed by adding approximately 3.58 gr Shubhodoya Mycorrhizal Bio-Fertilizer into nutrient solution for 7 days (25 gr in total). The same amount of AM product was autoclaved at 121 °C for 30 minutes and applied to pots of control (non-inoculated) treatment. B toxicity treatment (1000 mM B) was first applied 14 DAS.

The experiment was terminated 35 DAS. Fully expanded youngest and second youngest leaves were taken as fresh samples for Relative Electron Leakage (REL) analysis. Three plants from each pot were harvested and dried for dry weight measurement and element analysis. Fresh samples from desired leaves of four plants of each pot were cut and shocked by immersing in liquid nitrogen, the frozen samples were stored at -80°C later on. These samples were used for antioxidative activity assays.

2.3.2 Second Experiment with Arbuscular Mycorrhiza Colonization

In this experiment, bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants were grown in pots filled with vermiculite, irrigated with nutrient solution, and kept in controlled environmental conditions.

The experiment was consisted of 112 pots in total (7 replicates x 2 cultivars x 2 inoculation status x 2 P levels x 2 B levels); there was one single plant in each pot. Plants were supplied with two P levels: 0.02 mM (deficient) and 0.2 mM (adequate) in the form of KH_2PO_4 . In addition, two different levels of B were applied into nutrient solution: 2 μM (adequate) and 1000 μM (high) in the form of H_3BO_3 .

Colonization with AM was initiated 7 days after sowing (DAS) and performed by adding approximately 1.8 gr Shubhodoya Mycorrhizal Bio-Fertilizer into nutrient solution for 7 days (12,5 gr in total). The same amount of AM product was autoclaved at 121 °C for 30 minutes and applied to pots of control (non-inoculated) treatment. B toxicity treatment (1000 mM B) was first applied 14 DAS.

The experiment was terminated 21 DAS. Shoots of 3 replicates were harvested and dried for dry weight measurement. Fresh roots of 3 replicates were taken as whole, washed with distilled water, and used for scanning with WinRhizo morphological analysis system at the day of harvest. Scanned roots were dried later on for weighing root dry weight.

2.3.3 First Experiment with *P. indica* Colonization

In this experiment, bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants were germinated in plastic seedbeds, then grown in pots filled with vermiculite later on, irrigated with nutrient solution, and kept in controlled environmental conditions.

The experiment was designed as 4 treatments and 32 pots in total (4 replicates x 2 cultivars x 2 inoculation status x 2 B levels); 8 single plants were planted in each pot. Two levels of B were applied into nutrient solution: 2 µM (adequate) and 1000 µM (high) in the form of H₃BO₃.

Seeds were sown and germinated in plastic seedbeds. Germinated plants were transferred into pots 7 DAS. During the transplantation process, 100 ml of solution with *P. indica* were added to each pot of inoculation treatment group. The solution was prepared with the harvested and centrifuged *P. indica* pellets (318.2 g) and distilled water. B toxicity treatment (1000 mM B) was first applied 14 DAS, 7 days after transfer and inoculation.

The experiment was completed 35 DAS. Fully expanded second youngest leaves were taken as fresh samples for REL analysis. Three plants from each pot were harvested and dried. Fresh samples from desired leaves of four plants of each pot were cut and shocked by immersing in liquid nitrogen; the frozen samples were stored at -80°C later on. These samples were used for antioxidative activity assays.

Root tip cuttings (3 cm) from each pot were taken and used for staining and scanning of roots to investigate *P. indica* spores.

2.3.4 Second Experiment with *P. indica* Colonization

In this experiment, bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants were grown in pots filled with vermiculite, irrigated with nutrient solution, and kept in controlled environmental conditions.

The experiment was designed as 4 treatments and 32 pots in total (4 replicates x 2 cultivars x 2 inoculation status x 2 B levels); one single plant was planted in each pot. Two levels of B were applied into nutrient solution: 2 µM (adequate) and 1000 µM (high) in the form of H₃BO₃.

Inoculation was initiated 7 DAS as described at 2.3.3. Boron toxicity treatment was applied 14 DAS.

The experiment was terminated 21 DAS. Shoots of each plant were harvested, dried, and weighed. Fresh roots were taken as whole, washed with distilled water, and used for scanning in WinRhizo morphological analysis system at the day of harvest. Scanned roots were dried later on for weighing root dry weight.

2.4. Relative Electron Leakage (REL) Analysis

Selected leaf samples were cut and placed in tubes filled with 50 ml deionized water in a way that whole sample was immersed in water. The first measurement was done 6 hours after sampling by an EC meter for obtaining EC₁ value. Measured samples were then kept in a water bath that was set to 95 °C for 1 hour and the second measurement for obtaining EC₂ value was done after samples reached room temperature. REL was calculated as below:

$$REL = (EC_1/EC_2)*100\%. \quad (2.1)$$

2.5. Root Morphology Analysis

After harvest, root samples were washed with deionized water for the removal of the substrate. Epson Perfection V700 scanner (Seiko Epson Corporation, Nagano, Japan) was used for imaging of roots. Obtained images were analyzed with WinRHIZO (Regent Instruments Inc., Ville de Quebec, Canada) to determine root length, root surface area, average diameter, root volume and the number of tips.

2.6. Element Analysis

Dried shoot samples were processed with an electric coffee grinder and ground into powder. Around 0.2 g of ground samples were weighed and placed in microwave digestion tubes. 2 ml of 30% hydrogen peroxide (H₂O₂) and 5.0 ml of nitric acid (HNO₃) were added in digestion tubes and samples were digested in a

closed-vessel microwave system (MarsExpress; CEM Corp., Matthews, NC, USA). The sample solution was completed to 20 ml with double-deionized water when the samples drop to room temperature. Sample solutions were filtered through an analytical filter paper and placed in 25 ml tubes. Concentrations of macro (except for N) and micronutrients in samples were measured by an inductively coupled plasma optical emission spectrometry (ICP-OES) (Vista-Pro Axial, Varian, Australia).

2.7. Antioxidative Enzymes and Total Protein Analysis

2.7.1. Superoxide Dismutase (SOD) Analysis

The determination of superoxide dismutase activity was carried out according to the method by Giannopolitis and Ries, 1977; 3.2 ml of 50 mM K-P extraction buffer was mixed with 0.6 ml of 50 mM Na₂CO₃, 0.6 ml of 13 mM L-methionine, 0.6 ml of 63 µM NBT, 0.04 ml of crude sample extract (diluted 1:10) and 0.6 ml of 1.3 µM riboflavin in glass tubes. Since compounds were light-sensitive, they were kept in the dark during preparation. The glass tubes were kept under light for 8 minutes after riboflavin was added. Measurements were performed at a wavelength of 560 nm via a sipper.

2.7.2. Glutathione Reductase (GR) Analysis

Glutathione reductase activity was determined according to the method of Carlberg and Mannervik, 1985. 0.7 ml of 50 mM K-P buffer, 0.1 ml of 0.5 mM Oxidized Glutathione (GSSG), and 0.1 ml of crude sample extract (diluted 1:10) were mixed and as the final step, 0.1 ml of 0.45 mM NADPH-Na₄ was added to this

mixture. The mixture was transferred to the quartz cuvette. The activity was measured spectrophotometrically at 340 nm and the absorbance readings were monitored for 2 minutes to estimate the average rate of NADPH-Na⁴ degradation.

2.7.3. Ascorbate Peroxidase (AP) Analysis

Ascorbate peroxidase activity was determined according to the method by Nakano and Asada, 1981. 0.7 ml of 50 mM K-P buffer was mixed with 0.1 ml of 12 mM H₂O₂ and 0.1 ml of crude sample extract (diluted 1:40); finally 0.1 ml of mM ascorbic acid (C₆H₈O₆) were added with the mixture in a quartz cuvette. The activity was measured spectrophotometrically at 290 nm and the absorbance readings were monitored for 2 minutes to estimate the average rate of L- ascorbic acid degradation.

2.7.4. Catalase (CAT) Analysis

Catalase activity was determined according to the method by Chance and Maehly, 1955. 0.8 ml of 50 mM K-P buffer was mixed with 0.1 ml of 100 mM H₂O₂ and 0.1 ml of crude extract of sample extract (diluted 1:40). The activity was measured spectrophotometrically at 240 nm and the absorbance readings were monitored for 2 minutes to estimate the average rate of H₂O₂ degradation.

2.8. Root Staining

Root samples were stained according to a modified version of method by Kumar et al., 2009. 3 cm-long root tips were immersed in 10% KOH for 15 minutes and 1 M HCl for 10 minutes. Root samples were treated with 0.05% trypan blue solution for overnight. For de-staining, stained root samples were kept in 50% Lactic acid for 2 hours. Stained roots were observed with light microscopy and photographed with Swift Imaging Software (Swift Optical Instruments Inc., USA) or KameraM Micro Structure Imaging Software (Micro Sistem, Istanbul, Turkey).

2.9. Statistical Analysis

JMP software was used for statistical analysis. The significance of the effects of the treatments and their interactions on the reported traits was evaluated by analysis of variance (ANOVA) at 5% significance. Significant differences between means were then determined using Fisher's least significant difference test.

3. RESULTS

3.1. First Experiment with Arbuscular Mycorrhiza Colonization

This experiment was conducted to investigate the effects of the interaction of inoculation with AM fungi with P deficiency and B toxicity on growth, shoot mineral accumulation and stress responses in two wheat cultivars, bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak), grown in pseudo-hydroponic system and provided nutrients in a fully controlled manner.

Severe symptoms of P deficiency such as inhibited growth and changes in leaf color were observed in the first week of the experiment. (Figure 3.1.)



Figure 3.1.: Effects of P deficiency, B toxicity and inoculation with AM fungi on 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two levels of P (Low: 0.02 mM KH_2PO_4 ; Control: 0.2 mM KH_2PO_4) and high level of B (1000 μM H_3BO_3).

One week after first application of B toxicity, chlorosis and necrosis on leaf tips and margins were visible. It was also observed that inoculation with AM alleviated chlorosis on mature leaves under B toxicity. (Figure 3.2.)

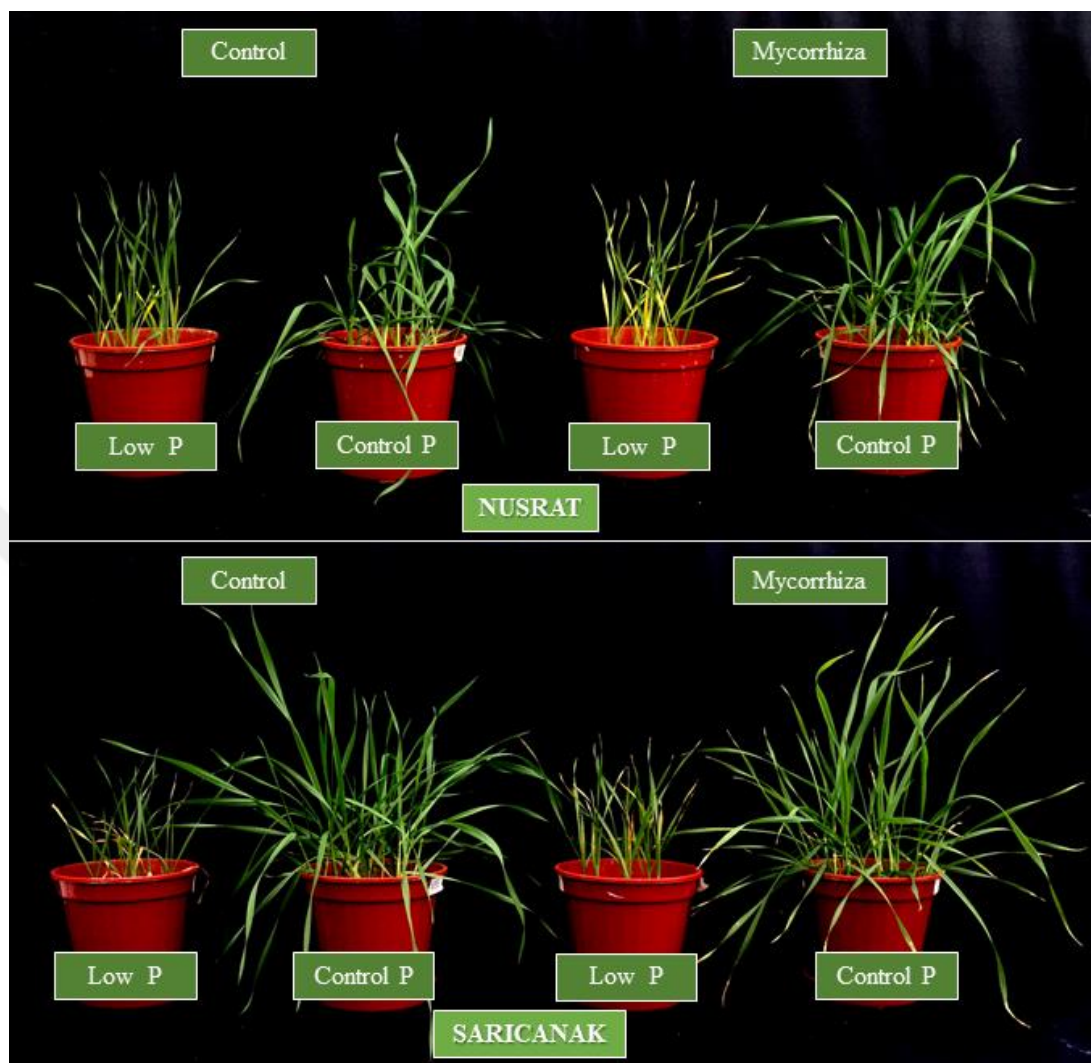


Figure 3.2.: Effects of inoculation with AM fungi on 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) supplied with an inadequate level of P (0.02 mM KH_2PO_4) and two levels of B (Control:2 μM H_3BO_3 ; High:1000 μM H_3BO_3).

Shoot DW was significantly affected by P deficiency in both cultivars but the decrease in DW of durum wheat (*Triticum durum* cv. Saricanak) was significantly higher than bread wheat (*Triticum aestivum* cv. Nusrat) even though durum wheat (*Triticum durum* cv. Saricanak) performed better when supplied with adequate P (Table 3.1). Mycorrhizal inoculation and B toxicity showed no significant effect on DW values of both cultivars.

Table 3.1: Shoot dry weight data of 5-week-old non-inoculated (Control) or inoculated (AM) bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Inoculation	Cultivar	Boron	Phosphorus	Shoot DW (mg.plant ⁻¹)		
Control	Nusrat	Control	Deficient	113	±	8
			Adequate	228	±	34
		High	Deficient	91	±	13
			Adequate	258	±	21
	Saricanak	Control	Deficient	71	±	10
			Adequate	354	±	48
AM	Nusrat	Control	Deficient	96	±	29
			Adequate	238	±	46
		High	Deficient	111	±	7
			Adequate	199	±	27
	Saricanak	Control	Deficient	74	±	8
			Adequate	304	±	22
		High	Deficient	73	±	13
			Adequate	360	±	23

Shoot DW LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; 17; n.s.; 17; n.s.; n.s.; n.s.; n.s.; 24; n.s.; n.s.; n.s.; n.s.; 49)

Relative electron leakage from youngest fully expanded leaves was significantly increased with B toxicity treatment in both Nusrat and Saricanak. (Table 3.2). ANOVA results indicated that higher levels of P and B supply significantly increased relative electron leakage. (Table 3.3). Although B toxicity had no significant effect, P deficiency was observed to be increasing REL from second youngest leaves. The highest REL values were observed in Saricanak supplied with insufficient level of P. Inoculation with AM had no significant effect on REL either from first or second youngest leaves.

Table 3.2: Relative electron leakage (%) from first (REL-1) and second (REL-2) youngest leaves of 5-week-old non-inoculated (Control) or inoculated (AM) bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak). Data points represent the means and standard deviations of four replicates.

Inoculation	Cultivar	Boron	Phosphorus	REL-1 (%)		REL-2 (%)	
Control	Nusrat	Control	Deficient	5.1	± 0.5	6.6	± 0.5
			Adequate	5.3	± 1.4	7.2	± 1.2
		High	Deficient	7.2	± 1.0	9.4	± 2.2
			Adequate	15.6	± 12.8	14.6	± 6.9
	Saricanak	Control	Deficient	5.7	± 2.4	27.4	± 13.1
			Adequate	4.8	± 0.3	4.4	± 0.7
AM	Nusrat	Control	Deficient	5.8	± 0.5	5.9	± 0.6
			Adequate	5.1	± 1.0	5.1	± 0.4
		High	Deficient	7.7	± 2.2	9.8	± 1.5
			Adequate	10.4	± 3.8	17.0	± 1.8
	Saricanak	Control	Deficient	5.1	± 0.6	40.9	± 12.4
			Adequate	4.5	± 0.6	4.0	± 0.3
		High	Deficient	4.6	± 0.6	6.7	± 0.5
			Adequate	10.0	± 1.9	13.7	± 3.5

REL-1 LSD_{0,05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; n.s.; 1,9; 1,9; n.s.; n.s.; n.s.; n.s.; 2,7; n.s.; n.s.; n.s.; n.s.; n.s.)
REL-2 LSD_{0,05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; 2,6; n.s.; 2,6; n.s.; 3,7; n.s.; 3,7; 3,7; 3,7; 5,3; n.s.; n.s.; 5,3; n.s.)

Table 3.3: Analysis of variance of the main effects of AM inoculation, P deficiency, B toxicity and their interaction on dry weight (DW), relative electron leakage from youngest leaves (REL-1) and second youngest leaves (REL-2) and mineral status of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

Source of Variation	Shoot DW	REL-1 (%)	REL-2 (%)	B Conc.	P Conc.
Inoculation (I)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Cultivar (CV)	***	<i>n.s.</i>	***	***	**
Boron Supply (B)	<i>n.s.</i>	***	<i>n.s.</i>	***	**
Phosphorus Supply (P)	***	*	*	***	***
I*CV	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	***	<i>n.s.</i>
I*B	<i>n.s.</i>	<i>n.s.</i>	*	<i>n.s.</i>	<i>n.s.</i>
I*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	**	*
CV*B	<i>n.s.</i>	<i>n.s.</i>	***	***	<i>n.s.</i>
CV*P	***	<i>n.s.</i>	***	<i>n.s.</i>	<i>n.s.</i>
B*P	<i>n.s.</i>	**	***	***	*
I*CV*B	<i>n.s.</i>	<i>n.s.</i>	**	***	<i>n.s.</i>
I*CV*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	**
I*B*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	**	<i>n.s.</i>
CV*B*P	<i>n.s.</i>	<i>n.s.</i>	***	*	<i>n.s.</i>
I*CV*B*P	**	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Source of Variation	Mg Conc.	S Conc.	K Conc.	Fe Conc.	Zn Conc.
Inoculation (I)	***	***	<i>n.s.</i>	***	**
Cultivar (CV)	**	***	<i>n.s.</i>	<i>n.s.</i>	**
Boron Supply (B)	**	***	<i>n.s.</i>	<i>n.s.</i>	*
Phosphorus Supply (P)	**	***	***	***	***
I*CV	<i>n.s.</i>	*	**	<i>n.s.</i>	**
I*B	<i>n.s.</i>	**	<i>n.s.</i>	**	<i>n.s.</i>
I*P	*	<i>n.s.</i>	<i>n.s.</i>	**	**
CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	**	<i>n.s.</i>
CV*P	***	***	**	*	**
B*P	**	<i>n.s.</i>	*	<i>n.s.</i>	<i>n.s.</i>
I*CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
I*CV*P	**	<i>n.s.</i>	***	**	***
I*B*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	*	<i>n.s.</i>
CV*B*P	*	*	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
I*CV*B*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

n.s. Not significant; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

A significant decrease in P concentration was observed in both cultivars under P deficiency. (Table 3.4). Colonization with AM had no significant effect on P concentration of Saricanak; but in Nusrat, inoculation caused a significant increase in P concentration of plants supplied with inadequate P.

Shoot K concentration was significantly affected by P supply. Higher K concentration was measured in plants supplied with adequate P. Colonization with AM had no significant effect on K concentration but ANOVA results indicated a triple interaction between inoculation, cultivar and P level. Shoot K concentration was not significantly affected by B toxicity.

Inoculation, B toxicity and P deficiency significantly decreased shoot Mg concentrations (Table 3.4). Non-inoculated Nusrat supplied with sufficient P was observed to have the highest concentration of Mg. Along with their main effects, P deficiency and B toxicity were detected to have a significant additive effect on Mg concentration.

Shoot S concentration showed significant differences caused by inoculation, cultivar, B toxicity and P deficiency (Table 3.4). Inoculation with AM significantly increased S concentration while B toxicity and P deficiency caused significant reduction. Higher concentration of S was observed in Saricanak in both P deficiency and B toxicity conditions.

Table 3.4: Concentrations of selected macronutrients (K,P,Mg,S) of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants with two inoculation statuses (Control; Arbuscular Mycorrhiza); supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Control														
CV	Boron	Phosphorus	Shoot Concentrations (%)											
			K			P			Mg			S		
N	Control	Deficient	4.2	±	0.2	0.07	±	<0.01	0.12	±	0.01	0.17	±	0.02
		Adequate	5.3	±	0.5	0.29	±	0.06	0.14	±	0.01	0.32	±	0.03
	High	Deficient	4.3	±	0.2	0.07	±	<0.01	0.11	±	0.01	0.16	±	<0.01
		Adequate	5.8	±	0.1	0.34	±	0.02	0.14	±	0.00	0.32	±	0.02
S	Control	Deficient	5.3	±	0.5	0.08	±	0.01	0.14	±	0.01	0.24	±	0.01
		Adequate	5.0	±	0.1	0.28	±	0.01	0.13	±	0.01	0.47	±	0.05
	High	Deficient	5.1	±	0.2	0.07	±	<0.01	0.13	±	0.01	0.25	±	0.01
		Adequate	5.2	±	0.1	0.29	±	0.02	0.13	±	<0.01	0.42	±	0.04
Arbuscular Mycorrhiza														
CV	Boron	Phosphorus	Shoot Concentrations (%)											
			K			P			Mg			S		
N	Control	Deficient	5.0	±	0.3	0.10	±	0.01	0.12	±	<0.01	0.25	±	0.01
		Adequate	5.2	±	0.2	0.28	±	0.03	0.13	±	<0.01	0.37	±	0.03
	High	Deficient	5.1	±	0.6	0.12	±	0.02	0.11	±	0.01	0.20	±	0.03
		Adequate	5.3	±	0.7	0.31	±	0.04	0.12	±	0.01	0.32	±	0.05
S	Control	Deficient	4.6	±	0.2	0.07	±	0.01	0.14	±	0.01	0.27	±	0.01
		Adequate	4.7	±	0.2	0.27	±	0.03	0.12	±	<0.01	0.49	±	0.04
	High	Deficient	4.5	±	0.1	0.06	±	<0.01	0.12	±	0.01	0.25	±	0.01
		Adequate	5.2	±	0.1	0.30	±	0.01	0.12	±	0.01	0.42	±	0.02

Shoot K Conc. LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; n.s.; n.s.; 1,7; 0.24; 0.24; n.s.; n.s.; 0.24; n.s.; n.s.; 0,34; n.s.; n.s.; n.s.)
Shoot P Conc. LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; 0,01; 0,01; 0,01; n.s.; n.s.; 0,02; n.s.; n.s.; 0,02; n.s.; 0,02; n.s.; n.s.; n.s.)
Shoot Mg Conc. LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (0,003; 0,003; 0,003; 0,003; n.s.; n.s.; 0,004; n.s.; 0,004; 0,004; n.s.; 0,006; n.s.; 0,006; n.s.)
Shoot S Conc. LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (0,01.; 0,01; 0,01; 0,02; 0,02; n.s.; 0,02; n.s.; n.s.; n.s.; 0,03; n.s.)

High B supply significantly increased B concentration in both cultivars. (Table 3.5)

Shoot B concentration in Nusrat was significantly increased in AM-inoculated plants when P was deficient. It was also observed that, in both cultivars, shoot B concentration was decreased in P-deficient plants when B toxicity was applied. In Saricanak, shoot B concentration was increased with mycorrhizal colonization under B toxicity.

Shoot Fe concentration was significantly decreased under P deficiency. In Saricanak, shoot Fe concentration was decreased with high B supply; and, in both cultivars, Fe concentration was lower in AM-inoculated plants under B toxicity.

In Nusrat, shoot Zn concentration was markedly higher in AM-inoculated plants than in non-inoculated plants under P-deficient conditions; however, a similar Zn response was not observed in Saricanak. Boron toxicity had no effect on shoot Zn concentrations of Nusrat and Saricanak.

Table 3.5: Concentrations of selected micronutrients (B, Fe, Zn) of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants with two inoculation statuses (Control; Arbuscular Mycorrhiza); supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Control											
CV	Boron	Phosphorus	Shoot Concentrations (mg.kg ⁻¹)								
			B			Fe		Zn			
N	Control	Deficient	4	±	<1	48	±	4	12	±	1
		Adequate	6	±	1	93	±	7	23	±	2
	High	Deficient	306	±	25	50	±	6	11	±	<1
		Adequate	545	±	28	132	±	29	22	±	2
S	Control	Deficient	9	±	<1	79	±	12	18	±	1
		Adequate	6	±	2	97	±	5	23	±	3
	High	Deficient	469	±	34	65	±	4	17	±	<1
		Adequate	709	±	22	92	±	6	21	±	2
Arbuscular Mycorrhiza											
CV	Boron	Phosphorus	Shoot Concentrations (mg.kg ⁻¹)								
			B			Fe		Zn			
N	Control	Deficient	8	±	1	56	±	11	18	±	2
		Adequate	7	±	1	79	±	14	22	±	2
	High	Deficient	461	±	60	49	±	4	17	±	3
		Adequate	541	±	59	68	±	5	22	±	2
S	Control	Deficient	8	±	<1	61	±	21	17	±	2
		Adequate	7	±	1	97	±	22	23	±	1
	High	Deficient	403	±	46	50	±	6	16	±	1
		Adequate	606	±	33	73	±	7	22	±	1

Shoot B Conc. LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; 16; 16; 16; 22; n.s.; 22; 22; n.s.; 22; 32; 32.; n.s.; 32.; n.s.)
 Shoot Fe Conc. LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (6; n.s.; n.s.; 6; n.s.; 9; 9; 9; 9; n.s.; n.s.; 13; 13; n.s.; n.s.)
 Shoot Zn Conc. LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (1; 1; 1; 1; 1; n.s.; 1; n.s.; 1; n.s.; n.s.; 2; n.s.; n.s.; n.s.)

Total GR activity in Nusrat was significantly increased in AM-inoculated plants under P deficiency. (Table 3.7). Also, B toxicity caused a significant decrease in total GR activity. In Saricanak, total GR activity was significantly decreased with B toxicity in non-inoculated plants; however, same response was not observed in AM-inoculated plants.

Total activity of AP was significantly decreased with inoculation in both cultivars. In Nusrat, total AP activity was increased under B toxicity. P deficiency showed no significant effect in Nusrat; but in Saricanak, it was significantly increased when P was deficient.

In Nusrat, total CAT activity was significantly lower in AM-inoculated plants under both control and B toxicity conditions; however, in Saricanak, total CAT activity was increased with inoculation in control B group. Total activity of CAT was significantly decreased with B toxicity in Nusrat.

Total AP activity was significantly higher in Saricanak and the reverse was observed in total CAT activity. ANOVA results also indicated a significant effect of interaction between inoculation, cultivar, and B. (Table 3.6)

Table 3.6: Analysis of variance of the main effects of AM inoculation, P deficiency, B toxicity and their interaction on total and specific enzymatic activities of ascorbate peroxidase (AP), glutathione reductase (GR) and catalase (CAT) of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

Total Enzyme Activity	Source of Variation	AP	GR	CAT
	Inoculation (I)	***	<i>n.s.</i>	<i>n.s.</i>
	Cultivar (CV)	***	<i>n.s.</i>	**
	Boron Supply (B)	<i>n.s.</i>	*	**
	Phosphorus Supply (P)	**	<i>n.s.</i>	<i>n.s.</i>
	I*CV	<i>n.s.</i>	<i>n.s.</i>	***
	I*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	I*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	CV*P	**	<i>n.s.</i>	<i>n.s.</i>
	B*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	I*CV*B	<i>n.s.</i>	*	<i>n.s.</i>
	I*CV*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	I*B*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	CV*B*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	I*CV*B*P	*	<i>n.s.</i>	<i>n.s.</i>
Specific Enzyme Activity	Source of Variation	AP	GR	CAT
	Inoculation (I)	***	***	***
	Cultivar (CV)	***	***	*
	Boron Supply (B)	<i>n.s.</i>	**	**
	Phosphorus Supply (P)	**	***	***
	I*CV	***	***	<i>n.s.</i>
	I*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	I*P	*	**	<i>n.s.</i>
	CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	CV*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	B*P	<i>n.s.</i>	*	<i>n.s.</i>
	I*CV*B	<i>n.s.</i>	**	<i>n.s.</i>
	I*CV*P	<i>n.s.</i>	*	<i>n.s.</i>
	I*B*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	CV*B*P	<i>n.s.</i>	*	<i>n.s.</i>
	I*CV*B*P	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Table 3.7: Total antioxidative enzyme; ascorbate peroxidase (AP), glutathione reductase (GR) and catalase (CAT) activities of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants with two inoculation status (Control; Arbuscular Mycorrhiza); supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Total Activity					
Control					
Cultivar	Boron	Phosphorus	GR	AP	CAT
			(-μmol [NADPH] g ⁻¹ FW min ⁻¹)	(-μmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)	
Nusrat	Control	Deficient	1.5 ± 0.1	20 ± 2	2.8 ± 0.4
		Adequate	2.1 ± 0.6	23 ± 4	2.7 ± 0.2
	High	Deficient	2.0 ± 0.7	26 ± 6	1.9 ± 0.4
		Adequate	1.9 ± 0.3	25 ± 4	1.8 ± 0.4
Saricanak	Control	Deficient	2.6 ± 0.6	45 ± 7	1.1 ± 0.2
		Adequate	2.6 ± 0.9	29 ± 4	1.4 ± 0.4
	High	Deficient	1.7 ± 0.3	35 ± 6	1.3 ± 0.6
		Adequate	1.9 ± 0.1	34 ± 4	1.2 ± 0.5
Arbuscular Mycorrhiza					
Cultivar	Boron	Phosphorus	GR	AP	CAT
			(-μmol [NADPH] g ⁻¹ FW min ⁻¹)	(-μmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)	
Nusrat	Control	Deficient	1.9 ± 0.7	15 ± 7	1.6 ± 0.5
		Adequate	2.7 ± 0.5	14 ± 4	1.7 ± 0.6
	High	Deficient	1.4 ± 0.3	17 ± 6	1.3 ± 0.5
		Adequate	1.7 ± 0.3	18 ± 4	1.6 ± 0.5
Saricanak	Control	Deficient	2.2 ± 0.7	26 ± 6	1.8 ± 0.5
		Adequate	2.2 ± 0.2	21 ± 3	1.6 ± 0.2
	High	Deficient	2.2 ± 0.8	29 ± 4	1.0 ± 0.4
		Adequate	1.9 ± 0.3	19 ± 2	1.9 ± 0.6

GR Activity LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; n.s.; 0.3; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; 0.6; n.s.; n.s.; n.s.; n.s.)
AP Activity LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (3; 3; n.s.; 3; n.s.; n.s.; n.s.; n.s.; 4; n.s.; n.s.; n.s.; n.s.; n.s.; 7)
CAT Activity LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; 0.2; 0.2.; 0.3; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.)

Specific activity of GR was decreased under P deficiency conditions in both cultivars. (Table 3.8) In Nusrat, specific GR activity was significantly higher in inoculated plants in control conditions; however, it was decreased with inoculation under B toxicity. In Saricanak, inoculation with AM decreased specific GR activity in both control and B toxicity conditions.

Specific AP activity was significantly decreased under P deficiency in Nusrat. Inoculation with AM significantly decreased specific AP activity when P was adequate; but, the same trend was not observed under P deficiency. Specific activity of AP was significantly decreased with P deficiency and AM-inoculation in Saricanak.

In Nusrat, both P deficiency and B toxicity significantly decreased specific CAT activity. Inoculated plants had lower CAT activity except for high B group supplied with adequate P. In Saricacak, specific activity of CAT was significantly decreased under P deficiency; also, specific CAT activity was significantly lower in AM-inoculated plants.

A significant difference in specific activities of antioxidative enzymes was observed between cultivars; AP and GR showed higher level of activity in Saricanak while the activity of CAT was higher in Nusrat.

Table 3.8: Specific antioxidative enzyme; ascorbate peroxidase (AP), glutathione reductase (GR) and catalase (CAT) activities of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants with two inoculation status (Control; Arbuscular Mycorrhiza); supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Specific Activity						
Control						
Cultivar	Boron	Phosphorus	GR	AP	CAT	
			(-nmol [NADPH] mg ⁻¹ Prt.min ⁻¹)	(-μmol H2O2 mg ⁻¹ Prt.min ⁻¹)		
Nusrat	Control	Deficient	60 ± 6	0.8 ± 0.1	108 ± 19	
		Adequate	117 ± 27	1.3 ± 0.2	150 ± 4	
	High	Deficient	64 ± 18	0.8 ± 0.2	63 ± 12	
		Adequate	116 ± 9	1.3 ± 0.3	107 ± 35	
Saricanak	Control	Deficient	145 ± 38	2.5 ± 0.4	63 ± 15	
		Adequate	289 ± 37	2.9 ± 0.5	138 ± 30	
	High	Deficient	124 ± 36	2.7 ± 0.7	92 ± 36	
		Adequate	175 ± 9	3.1 ± 0.4	105 ± 44	
Arbuscular Mycorrhiza						
Cultivar	Boron	Phosphorus	GR	AP	CAT	
			(-nmol [NADPH] mg ⁻¹ Prt.min ⁻¹)	(-μmol H2O2 mg ⁻¹ Prt.min ⁻¹)		
Nusrat	Control	Deficient	100 ± 51	0.8 ± 0.3	83 ± 35	
		Adequate	140 ± 33	0.8 ± 0.2	97 ± 27	
	High	Deficient	61 ± 18	0.7 ± 0.2	54 ± 23	
		Adequate	94 ± 19	1.0 ± 0.1	90 ± 29	
Saricanak	Control	Deficient	62 ± 15	0.7 ± 0.1	49 ± 21	
		Adequate	107 ± 11	1.0 ± 0.3	79 ± 16	
	High	Deficient	72 ± 29	0.9 ± 0.3	34 ± 15	
		Adequate	71 ± 9	0.7 ± 0.3	71 ± 22	

GR Activity LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (14; 14; 14; 14; 20; n.s.; 20; n.s.; n.s.; 20; 28; 28; 28; n.s.; n.s.)
AP Activity LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (0.2; 0.2; n.s.; 0.2; 0.2; n.s.; 0.2; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.)
CAT Activity LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (14; 14; 14; 14; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.)

Mycorrhizal components were found in stained root samples of AM-inoculated plants; contamination in control group was not observed. (Figure 3.3)

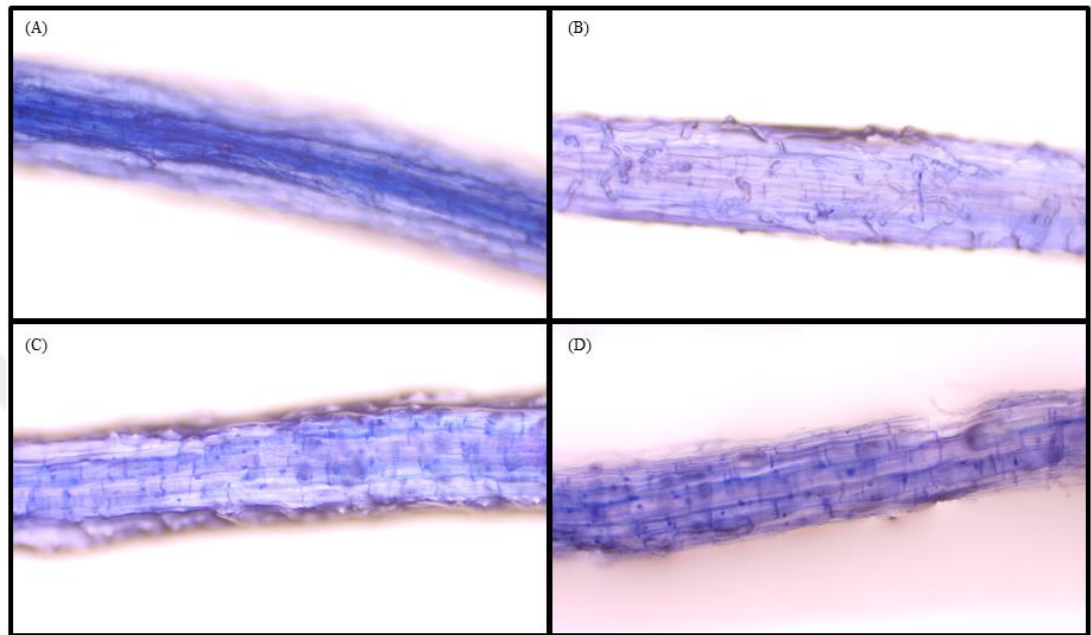


Figure 3.3: Mycorrhizal colonization in root samples of wheat plants, stained with trypan blue and observed under light microscope: A, B, non-inoculated (10x resolution); C, D, inoculated with AM (10x resolution).

3.2. Second Experiment with Arbuscular Mycorrhiza Colonization

The second experiment with AM colonization was conducted to study the effects of the interaction of mycorrhizal inoculation with P deficiency and B toxicity on root and shoot growth and morphological traits of roots such as root length, root surface area, root volume, the average diameter of roots and number of root tips in two wheat cultivars, bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak), grown in pseudo-hydroponic system and provided nutrients in a fully controlled manner. Significant effects of inoculation and P deficiency was detected according to two-way ANOVA. (Table 3.9)

Table 3.9: Analysis of variance of the main effects of AM inoculation, P deficiency, B toxicity and their interaction on shoot dry weight (DW) and root/shoot ratio of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

Source of Variation	Shoot DW	Root DW	Root/Shoot Ratio
Inoculation (I)	**	**	n.s.
Cultivar (CV)	**	***	***
Boron Supply (B)	n.s.	n.s.	n.s.
Phosphorus Supply (P)	***	n.s.	***
I*CV	n.s.	n.s.	n.s.
I*B	n.s.	n.s.	n.s.
I*P	n.s.	n.s.	n.s.
CV*B	n.s.	*	n.s.
CV*P	**	*	***
B*P	*	n.s.	n.s.
I*CV*B	n.s.	n.s.	n.s.
I*CV*P	n.s.	n.s.	n.s.
I*B*P	n.s.	n.s.	n.s.
CV*B*P	n.s.	n.s.	n.s.
I*CV*B*P	n.s.	n.s.	n.s.

Inoculation with AM fungi significantly decreased both shoot and root DW (Table 3.10). Phosphorus deficiency significantly reduced DW without causing any significant change in root DW; therefore root/shoot ratio was significantly higher in P deficient conditions. Saricanak had higher shoot DW when supplied with sufficient P, but the reduction in DW was significantly greater than Nusrat when P was deficient. Boron toxicity showed no significant effect on either shoot DW, root DW or root/shoot ratio. (Table 3.9)

Table 3.10: Effect of colonization with AM on shoot dry weight, root dry weight and root/shoot ratio of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants. Data points represent the means and standard deviations of three replicates.

Inoculation	Cultivar	Boron	Phosphorus	Shoot DW	Root DW	Root/Shoot ratio
Non-inoculated	Nusrat	Control	Deficient	73 ± 7	54 ± 3	0.74 ± 0.04
			Adequate	115 ± 15	75 ± 7	0.65 ± 0.04
		Stress	Deficient	66 ± 13	46 ± 10	0.71 ± 0.13
			Adequate	96 ± 24	56 ± 15	0.58 ± 0.01
	Saricanak	Control	Deficient	66 ± 10	47 ± 7	0.71 ± 0.02
			Adequate	145 ± 5	47 ± 2	0.32 ± 0.01
		Stress	Deficient	75 ± 1	54 ± 3	0.73 ± 0.05
			Adequate	120 ± 19	44 ± 3	0.37 ± 0.03
Arbuscular Mycorrhiza	Nusrat	Control	Deficient	57 ± 7	47 ± 5	0.82 ± 0.02
			Adequate	95 ± 6	59 ± 6	0.62 ± 0.08
		Stress	Deficient	67 ± 7	49 ± 6	0.73 ± 0.05
			Adequate	79 ± 19	48 ± 17	0.60 ± 0.09
	Saricanak	Control	Deficient	57 ± 9	44 ± 6	0.77 ± 0.05
			Adequate	125 ± 27	38 ± 4	0.31 ± 0.03
		Stress	Deficient	56 ± 12	39 ± 9	0.69 ± 0.06
			Adequate	120 ± 6	41 ± 5	0.35 ± 0.05

Shoot DW LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (8; 8; n.s.; 8; n.s.; n.s.; n.s.; n.s.; 11; 11; n.s.; n.s.; n.s.; n.s.; n.s.)

Root DW LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (4; 4; n.s.; n.s.; n.s.; n.s.; n.s.; 7; 7; n.s.; n.s.; n.s.; n.s.; n.s.)

Root/Shoot Ratio LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; 0,03; n.s.; 0,03; n.s.; n.s.; n.s.; n.s.; 0,05; n.s.; n.s.; n.s.; n.s.; n.s.)

Table 3.11: Analysis of variance of the main effects of AM inoculation, P deficiency, B toxicity and their interaction on root length and root surface area, root volume, average diameter of roots and the number of root tips of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

Source of Variation	Length	Surface Area	Average Diameter	Number of Tips
Inoculation (I)	**	*	n.s.	**
Cultivar (CV)	***	*	***	***
Boron Supply (B)	**	**	n.s.	n.s.
Phosphorus Supply (P)	***	***	**	***
I*CV	n.s.	n.s.	n.s.	*
I*B	n.s.	n.s.	n.s.	n.s.
I*P	*	*	n.s.	n.s.
CV*B	n.s.	n.s.	n.s.	n.s.
CV*P	n.s.	n.s.	n.s.	***
B*P	n.s.	*	*	n.s.
I*CV*B	n.s.	n.s.	n.s.	n.s.
I*CV*P	n.s.	n.s.	n.s.	n.s.
I*B*P	n.s.	n.s.	n.s.	n.s.
CV*B*P	n.s.	n.s.	n.s.	n.s.
I*CV*B*P	n.s.	n.s.	n.s.	n.s.

Inoculation with AM fungi decreased root length (Figure 3.4) and root surface area (Table 3.12). Phosphorus deficiency and B toxicity were also shown to cause a significant reduction in root length and surface area in both Nusrat and Saricanak. ANOVA results indicated a significant interaction between inoculation and P level on both traits. A significant increase in average root diameter was observed when P was deficient; although B toxicity had no significant effect on its own, it caused a significant increase when combined with P deficiency. The number of root tips was also significantly decreased under the effect of inoculation, B toxicity or P deficiency. Nusrat had significantly higher values of root length, root surface area, root volume and average root diameter while Saricanak had a significantly higher number of root tips.

Table 3.12: Root morphological traits of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) plants with two inoculation statuses (Control; Arbuscular Mycorrhiza) supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H₃BO₃; High 1000 μ M H₃BO₃) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of three replicates.

Control											
Cultivar	Boron	Phosphorus	Surface Area (cm2)			Average Diameter (mm)			Number of Tips		
N	Control	Deficient	54	±	3	0.42	±	0.03	396	±	39
		Adequate	110	±	10	0.42	±	0.01	607	±	96
	High	Deficient	41	±	10	0.50	±	0.05	317	±	102
		Adequate	83	±	8	0.39	±	0.08	559	±	98
S	Control	Deficient	55	±	12	0.32	±	0.01	563	±	126
		Adequate	109	±	8	0.31	±	0.01	1517	±	112
	High	Deficient	62	±	3	0.34	±	0.01	594	±	71
		Adequate	90	±	7	0.31	±	0.01	1253	±	316
Arbuscular Mycorrhiza											
Cultivar	Boron	Phosphorus	Surface Area (cm2)			Average Diameter (mm)			Number of Tips		
N	Control	Deficient	50	±	3	0.44	±	0.05	333	±	56
		Adequate	88	±	10	0.43	±	0.03	542	±	169
	High	Deficient	51	±	10	0.46	±	0.04	281	±	66
		Adequate	71	±	23	0.41	±	0.05	542	±	265
S	Control	Deficient	57	±	8	0.34	±	0.02	469	±	111
		Adequate	90	±	11	0.33	±	0.01	1051	±	74
	High	Deficient	52	±	14	0.34	±	0.01	489	±	134
		Adequate	88	±	9	0.33	±	0.00	979	±	208

Surface Area LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (6; 6; n.s.; 6; n.s.; n.s.; 9; n.s.; n.s.; 9; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.)

Average Diameter LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (n.s.; 0.02; n.s.; 0.02; n.s.; n.s.; n.s.; n.s.; n.s.; 0.03; n.s.; n.s.; n.s.; n.s.; n.s.)

Number of Tips LSD_{0.05} (I; CV; B; P; IxCV; IxB; IxP; CVxB; CVxP; BxP; IxCVxB; IxCVxP; IxBxP; CVxBxP; IxCVxBxP) = (86; 86; n.s.; 86; 123; n.s.; n.s.; n.s.; 123; n.s.; n.s.; n.s.; n.s.; n.s.; n.s.)

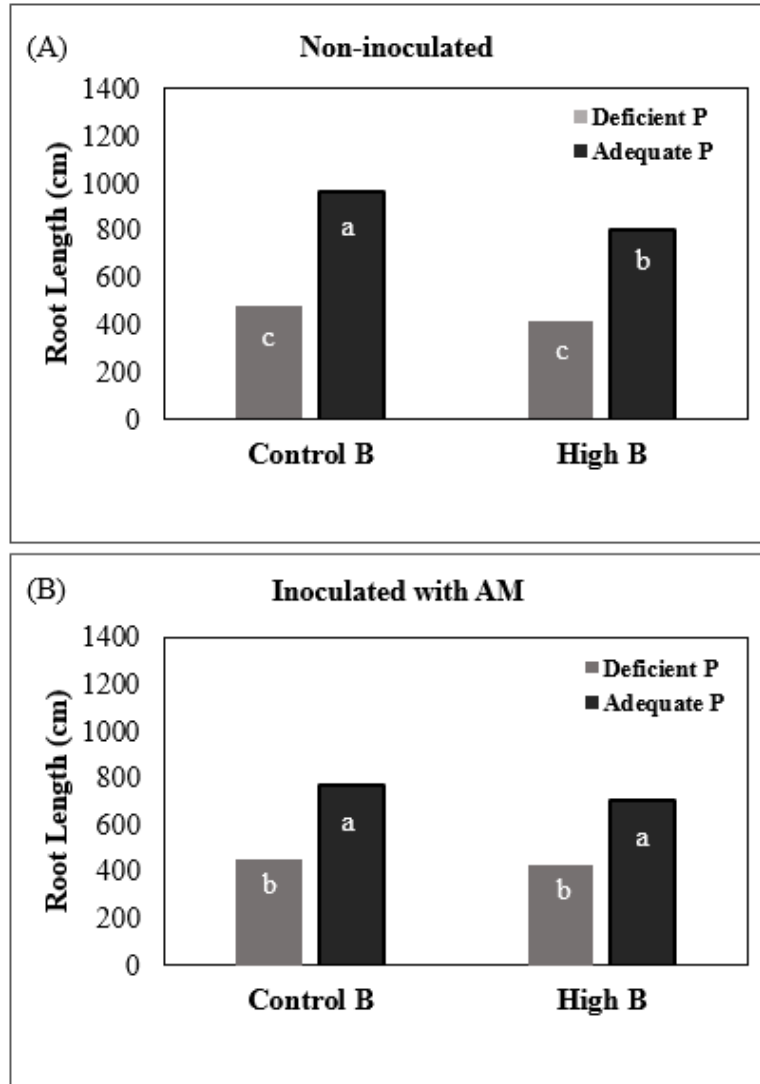


Figure 3.4: Effect of colonization with AM fungi (A: non-inoculated; B: inoculated) on total root length of 3-week-old wheat plants supplied with two different levels of P (Deficient: 0.02 mM; Adequate: 0.2 mM) and B (Control: 2 μ M H_3BO_3 ; High 1000 μ M H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions. Different letters indicate significant differences between means according to two-way ANOVA and Fisher's protected LSD test ($p \leq 0.05$).

3.3. First Experiment with *P. indica* Colonization

This experiment was conducted to investigate the effects of the interaction of inoculation with *P. indica* and B toxicity on growth, shoot mineral accumulation and stress responses in two wheat cultivars, bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak), grown in pseudo-hydroponic system and provided nutrients in a fully controlled manner.

One week after first application of B toxicity, necrosis on leaf tips and margins were visible; even though *P. indica* colonization did not show any difference in respect of necrosis on leaf tips, a great difference in growth was observed (Figure 3.5)



Figure 3.5: Effects of inoculation with *P. indica* on 5-week-old wheat plants supplied with two levels of B (control: 2 μM H_3BO_3 ; high: 1000 μM H_3BO_3).

Boron toxicity significantly decreased DW (Table 3.13) in both cultivars but *P. indica* colonization was observed to increase DW in both control and B toxicity conditions. (Figure 3.6). Dry weight values of Saricanak was significantly higher compared to Nusrat regardless of B level or inoculation status.

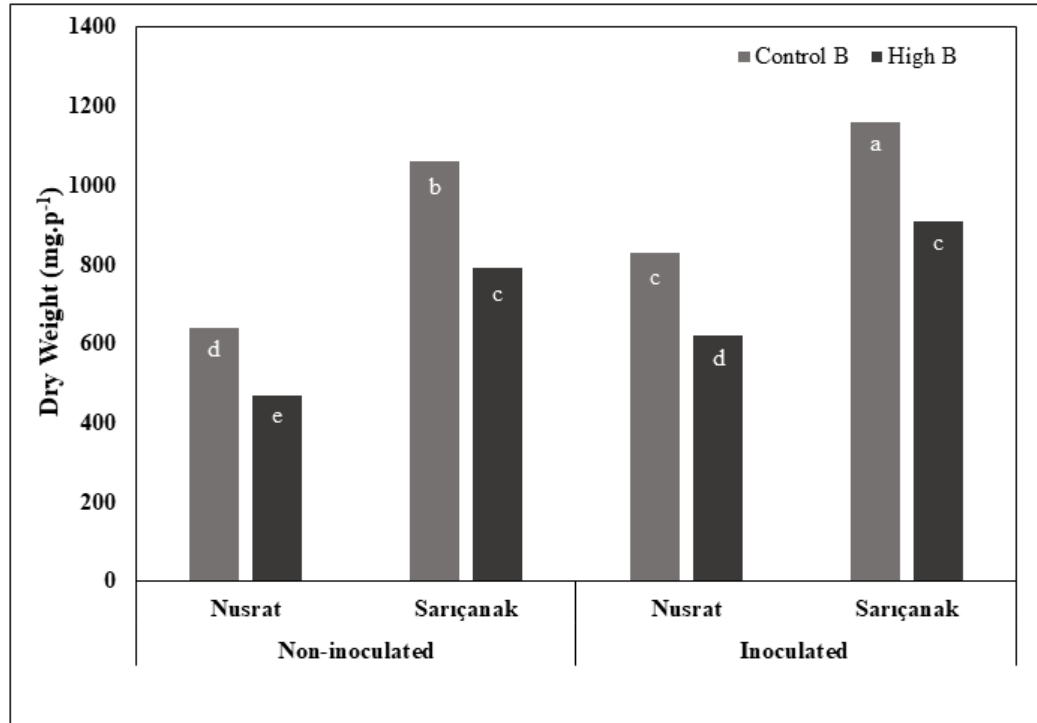


Figure 3.6: Effect of colonization with *P. indica* (A: non-inoculated; B: inoculated) on 5-week-old wheat plants supplied with two different levels of Boron (Control: 2 μM H₃BO₃; High 1000 μM H₃BO₃) grown in pseudo-hydroponic system under greenhouse conditions. Different letters indicate significant differences between means according to two-way ANOVA and Fisher's protected LSD test ($p \leq 0.05$).

According to ANOVA, inoculation or cultivar difference did not cause a significant change in REL (Table 3.14). In both cultivars, REL was significantly increased under B toxicity (Table 3.13)

Table 3.13: Relative electron leakage (%) from second youngest leaves of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two different levels of Boron (Adequate: 2 μ M H_3BO_3 ; High 1000 μ M H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Inoculation	Cultivar	Boron	Relative Electron Leakage (%)		
Non-inoculated	Nusrat	Adequate	5.56	±	1.39
		High	43.33	±	14.67
	Saricanak	Adequate	4.42	±	0.39
		High	23.84	±	3.07
Inoculated with <i>P. indica</i>	Nusrat	Adequate	4.44	±	0.68
		High	31.56	±	18.58
	Saricanak	Adequate	5.32	±	0.57
		High	30.45	±	12.41

REL LSD_{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; n.s.; 6.95; n.s.; n.s.; n.s.; n.s.)

Table 3.14: Analysis of variance of the main effects of *P. indica* inoculation and B toxicity and their interaction on dry weight (DW), relative electron leakage (REL) and mineral status of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

Source of Variation	Shoot DW	REL (%)	Shoot B Conc.	Shoot B Content
Inoculation (I)	***	<i>n.s.</i>	*	<i>n.s.</i>
Cultivar (CV)	***	<i>n.s.</i>	<i>n.s.</i>	***
Boron Supply (B)	***	***	***	***
I*CV	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
I*B	<i>n.s.</i>	<i>n.s.</i>	*	<i>n.s.</i>
CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	***
I*CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Source of Variation	Shoot K Conc.	Shoot P Conc.	Shoot Ca Conc.	Shoot Mg Conc.
Inoculation (I)	<i>n.s.</i>	**	<i>n.s.</i>	***
Cultivar (CV)	***	<i>n.s.</i>	<i>n.s.</i>	**
Boron Supply (B)	***	***	***	***
I*CV	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	***
I*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	**
CV*B	***	**	<i>n.s.</i>	*
I*CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	***

Source of Variation	Shoot S Conc.	Shoot Fe Conc.	Shoot Zn Conc.
Inoculation (I)	***	***	<i>n.s.</i>
Cultivar (CV)	<i>n.s.</i>	**	**
Boron Supply (B)	**	***	***
I*CV	**	<i>n.s.</i>	**
I*B	<i>n.s.</i>	***	<i>n.s.</i>
CV*B		*	**
I*CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

n.s. Not significant; * $0.01 \leq F \text{ Pr.} < 0.05$; ** $0.001 \leq F \text{ Pr.} < 0.01$; *** $F \text{ Pr.} < 0.001$

Shoot K, P, Mg and S concentrations were significantly increased under B toxicity stress in both cultivars. (Table 3.15). In Nusrat, shoot S concentration was increased under B toxicity; but, the same response was not observed in Saricanak. Shoot P and S concentrations were increased with *P. indica* inoculation in both cultivars. In Nusrat, shoot Mg concentration was lower in inoculated plants under both control and B toxicity conditions; however, in Saricanak Mg concentration was increased with inoculation under B toxicity.

Table 3.15: Concentrations of selected macronutrients (K,P,Mg,S) of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two different levels of Boron (Adequate: 2 μ M H₃BO₃; High: 1000 μ M H₃BO₃) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Control					
Cultivar	Boron	Shoot Concentrations (%)			
		K	P	Mg	S
Nusrat	Adequate	3.73 $\pm$ 0.14	0.19 $\pm$ 0.02	0.27 $\pm$ 0.01	0.29 $\pm$ 0.02
	High	5.22 $\pm$ 0.10	0.26 $\pm$ 0.01	1.21 $\pm$ 0.03	0.31 $\pm$ 0.03
Saricanak	Adequate	3.46 $\pm$ 0.16	0.20 $\pm$ 0.01	0.27 $\pm$ 0.01	0.28 $\pm$ 0.02
	High	4.16 $\pm$ 0.22	0.24 $\pm$ 0.02	1.10 $\pm$ 0.03	0.28 $\pm$ 0.02
<i>P. indica</i>					
Cultivar	Boron	Shoot Concentrations (%)			
		K	P	Mg	S
Nusrat	Adequate	3.67 $\pm$ 0.21	0.19 $\pm$ 0.01	0.19 $\pm$ 0.02	0.31 $\pm$ 0.02
	High	5.05 $\pm$ 0.10	0.28 $\pm$ 0.01	0.87 $\pm$ 0.03	0.38 $\pm$ 0.02
Saricanak	Adequate	3.46 $\pm$ 0.12	0.21 $\pm$ 0.01	0.24 $\pm$ 0.02	0.39 $\pm$ 0.03
	High	4.00 $\pm$ 0.04	0.27 $\pm$ 0.01	1.15 $\pm$ 0.08	0.38 $\pm$ 0.02

Shoot K Conc. LSD_{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; 0.11; 0.11; n.s.; n.s.; 0.15; n.s.)
Shoot P Conc. LSD_{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (0.01; n.s.; 0.01; n.s.; n.s.; 0.01; n.s.)
Shoot Mg Conc. LSD_{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (0.03; 0.03; 0.03; 0.04; 0.04; 0.04; 0.05)
Shoot S Conc. LSD_{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (0.02; n.s.; 0.02; 0.02; n.s.; 0.02; n.s.)

Table 3.16: Concentrations of selected micronutrients (B, Fe, Zn) of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two different levels of Boron (Adequate: 2 μ M H_3BO_3 ; High: 1000 μ M H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Control				
Cultivar	Boron	Shoot Concentrations (mg.kg ⁻¹)		
		B	Fe	Zn
Nusrat	Adequate	5 ± 2	131 ± 10	18 ± 3
	High	1122 ± 76	658 ± 41	22 ± 1
Saricanak	Adequate	2 ± 1	113 ± 4	14 ± 3
	High	977 ± 56	538 ± 93	16 ± 1
<i>P. indica</i>				
Cultivar	Boron	Shoot Concentrations (mg.kg ⁻¹)		
		B	Fe	Zn
Nusrat	Adequate	6 ± 3	83 ± 6	15 ± 1
	High	876 ± 30	401 ± 43	22 ± 2
Saricanak	Adequate	7 ± 1	55 ± 8	17 ± 1
	High	913 ± 11	334 ± 22	19 ± 1

Shoot B Conc. LSD_{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (47; n.s.; 47; n.s.; 69; n.s.; n.s.)

Shoot Fe Conc. LSD_{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (24; 24.; 24; n.s.; 41; 41; n.s.)

Shoot Zn Conc. LSD_{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; 1; 1; 2; n.s.; 2; n.s.)

Table 3.17: Analysis of variance of the main effects of *P. indica* inoculation, B toxicity and their interaction on total and specific enzymatic activities of superoxide dismutase (SOD), ascorbate peroxidase (AP), glutathione reductase (GR) and catalase (CAT) of 5-week-old wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

Total Enzyme Activity	Source of Variation	SOD	GR	AP	CAT
	Inoculation (I)	<i>n.s.</i>	***	<i>n.s.</i>	*
	Cultivar (CV)	***	***	<i>n.s.</i>	**
	Boron Supply (B)	**	*	<i>n.s.</i>	<i>n.s.</i>
	I*CV	***	**	<i>n.s.</i>	<i>n.s.</i>
	I*B	<i>n.s.</i>	<i>n.s.</i>	*	<i>n.s.</i>
	CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	I*CV*B	<i>n.s.</i>	**	<i>n.s.</i>	<i>n.s.</i>
	Source of Variation	SOD	GR	AP	CAT
Specific Enzyme Activity	Inoculation (I)	<i>n.s.</i>	<i>n.s.</i>	*	<i>n.s.</i>
	Cultivar (CV)	<i>n.s.</i>	<i>n.s.</i>	***	<i>n.s.</i>
	Boron Supply (B)	<i>n.s.</i>	*	<i>n.s.</i>	*
	I*CV	*	<i>n.s.</i>	<i>n.s.</i>	**
	I*B	<i>n.s.</i>	*	<i>n.s.</i>	<i>n.s.</i>
	CV*B	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
	I*CV*B	<i>n.s.</i>	**	<i>n.s.</i>	<i>n.s.</i>

Table 3.18: Total antioxidative enzyme; superoxide dismutase (SOD), ascorbate peroxidase (AP), glutathione reductase (GR) and catalase (CAT) activities of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants with two inoculation status (Control; *P. indica*); supplied with two different levels of Boron (Control: 2 μM H_3BO_3 ; High: 1000 μM H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

			Total Activity				
Inoculation	CV	Boron	SOD	GR		AP	CAT
			(U g ⁻¹ FW)	(-μmol [NADPH] g ⁻¹ FW min ⁻¹)		(-μmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)	(-mmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)
Non-inoculated	N	Control	124 ± 36	0.98 ± 0.15		22 ± 10	1.0 ± 0.3
		High	82 ± 11	0.71 ± 0.11		18 ± 1	1.3 ± 0.9
	S	Control	381 ± 46	1.44 ± 0.51		22 ± 8	1.1 ± 0.4
		High	269 ± 49	2.07 ± 0.43		18 ± 6	1.7 ± 0.5
<i>P. indica</i>	N	Control	228 ± 24	1.34 ± 0.14		26 ± 7	1.0 ± 0.2
		High	207 ± 17	2.25 ± 0.22		28 ± 4	1.6 ± 0.1
	S	Control	237 ± 69	2.03 ± 0.19		9 ± 6	2.2 ± 0.9
		High	211 ± 29	1.94 ± 0.23		30 ± 18	2.5 ± 0.6

SOD Activity $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; 33; 33; 47.; n.s.; n.s.; n.s.)

GR Activity $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (0.22; 0.22; 0.22; 0.30; n.s.; n.s.; 0.45)

AP Activity $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; n.s.; n.s.; 10; n.s.; n.s.)

CAT Activity $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (0.4; 0.4; n.s.; n.s.; n.s.; n.s.; n.s.)

Total activities of SOD, GR and CAT were significantly increased in colonized plants but inoculation did not cause any significant change in total AP activity (Table 3.19). However, ANOVA results indicated an interaction between inoculation and B toxicity, total AP activity was significantly higher in colonized plants exposed to high levels of B. Boron toxicity significantly increased total GR activity; in contrast, SOD activity was significantly decreased.

Table 3.19: Specific antioxidative enzyme; superoxide dismutase (SOD), ascorbate peroxidase (AP), glutathione reductase (GR) and catalase (CAT) activities of 5-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants with two inoculation status (Control; *P. indica*); supplied with two different levels of Boron (Control: 2 μM H_3BO_3 ; High: 1000 μM H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Specific Activity						
Inoculation	CV	Boron	SOD	GRX	AP	CAT
			(U mg^{-1} prt. min^{-1})	(nmol [NADPH] mg^{-1} prt. min^{-1})	($\mu\text{mol H}_2\text{O}_2$ mg^{-1} prt. min^{-1})	($\text{mmol H}_2\text{O}_2$ mg^{-1} prt. min^{-1})
Non-inoculated	N	Control	12 $\pm$ 3	101 $\pm$ 14	1.1 $\pm$ 1.0	70 $\pm$ 13
		High	8 $\pm$ 2	71 $\pm$ 4	1.4 $\pm$ 1.0	102 $\pm$ 5
	S	Control	13 $\pm$ <1	70 $\pm$ 11	1.1 $\pm$ 0.3	63 $\pm$ 15
		High	13 $\pm$ 4	97 $\pm$ 12	0.9 $\pm$ 0.4	75 $\pm$ 10
<i>P. indica</i>	N	Control	13 $\pm$ 2	69 $\pm$ 13	1.5 $\pm$ 0.4	39 $\pm$ 28
		High	11 $\pm$ 2	120 $\pm$ 21	1.5 $\pm$ 0.4	83 $\pm$ 22
	S	Control	9 $\pm$ 3	59 $\pm$ 40	0.4 $\pm$ 0.2	89 $\pm$ 40
		High	9 $\pm$ 2	78 $\pm$ 17	1.2 $\pm$ 0.8	98 $\pm$ 17

SOD Activity $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; n.s.; n.s.; 3; n.s.; n.s.; n.s.)

GR Activity $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; n.s.; 16; n.s.; 22; n.s.; 33)

AP Activity $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (0.4; 0.4; n.s.; n.s.; n.s.; n.s.; n.s.)

CAT Activity $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; n.s.; 16; 22; n.s.; n.s.; n.s.)

The specific activity of AP was significantly decreased with *P. indica* inoculation while other enzymes were not affected by colonization (Table 3.20); specific activity of SOD were significantly higher in inoculated Saricanak plants compared to control group but the same trend was not observed in Nusrat. Specific activities of GR and CAT were significantly increased under B toxicity stress.

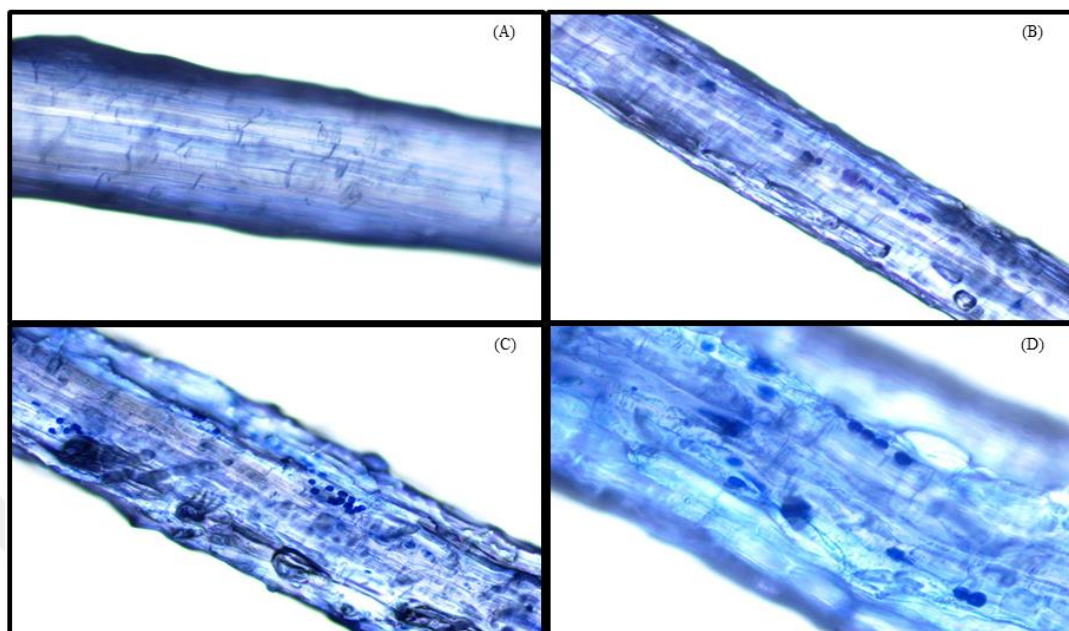


Figure 3.7: *P. indica* colonization in root samples of wheat plants, stained with trypan blue and observed under light microscope: A, non-inoculated (10x resolution); B, C, D, inoculated with *P. indica* (4x, 10x, 20x resolution, respectively).

3.4. Second Experiment with *P. indica* Colonization

The second experiment with *P. indica* colonization was conducted to study the effects of the interaction of inoculation and B toxicity on root and shoot growth and morphological traits of roots such as root length, root surface area, root volume, average diameter of roots and the number of root tips in two wheat cultivars, bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak), grown in pseudo-hydroponic system and provided nutrients in fully controlled manner.

Inoculation with *P. indica* or B toxicity had no significant effect on DW. (Table 3.20). Dry weights of Saricanak were significantly higher than Nusrat in both control and B toxicity conditions. (Table 3.21). Root DW was not significantly affected by inoculation but it was significantly reduced under B toxicity. Bread wheat (Nusrat) had significantly higher root/shoot ratio.

Table 3.20: Analysis of variance of the main effects of *P. indica* inoculation and B toxicity and their interaction on shoot dry weight (DW), root dry weight and root/shoot ratio of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

Source of Variation	Shoot DW	Root DW	Root/Shoot Ratio
Inoculation (I)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
Cultivar (CV)	***	<i>n.s.</i>	***
Boron Supply (BS)	<i>n.s.</i>	**	<i>n.s.</i>
I*CV	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
I*BS	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
CV*BS	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
I*CV*BS	<i>n.s.</i>	*	<i>n.s.</i>

Table 3.21: Effect of *P. indica* colonization on shoot dry weight, root dry weight and root/shoot ratio of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two different levels of Boron (Adequate: 2 μM H_3BO_3 ; High 1000 μM H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Inoculation	CV	Boron	Shoot DW (mg.plant ⁻¹)			Root DW (mg.plant ⁻¹)			Root/Shoot Ratio		
Non-inoculated	N	Adequate	217	±	40	136	±	22	0.63	±	0.03
		High	209	±	18	126	±	16	0.60	±	0.03
	S	Adequate	309	±	52	142	±	30	0.46	±	0.04
		High	274	±	57	95	±	29	0.34	±	0.06
Inoculated with <i>P. indica</i>	N	Adequate	248	±	50	146	±	27	0.62	±	0.21
		High	211	±	30	102	±	25	0.50	±	0.15
	S	Adequate	354	±	66	132	±	12	0.38	±	0.09
		High	315	±	27	119	±	17	0.38	±	0.03

Shoot Dry Weight $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; 33; n.s.; n.s.; n.s.; n.s.; n.s.)

Root Dry Weight $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; n.s.; 17; n.s.; n.s.; n.s.; 34)

Root/Shoot Ratio $\text{LSD}_{0.05}$ (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; 0,08; n.s.; n.s.; n.s.; n.s.; n.s.)

According to ANOVA, no significant effect of inoculation or B toxicity was observed on the total root length of both cultivars. (Table 3.22).

Table 3.22: Analysis of variance of the main effects of *P. indica* inoculation and B toxicity and their interaction on root length, surface area, root volume, average diameter and number of root tips of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and (*Triticum durum* cv. Saricanak) grown in pseudo-hydroponic system under greenhouse conditions.

Source of Variation	Length	Surface Area	Volume	Average Diameter	Number of Tips
Inoculation (I)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	**	*
Cultivar (CV)	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	*	***
Boron Supply (BS)	<i>n.s.</i>	**	**	**	<i>n.s.</i>
I*CV	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
I*BS	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>
CV*BS	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	**	<i>n.s.</i>
I*CV*BS	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>	<i>n.s.</i>

Boron toxicity significantly decreased root volume, surface area and average root diameter. (Table 3.23). A tendency to increase in root volume and surface area was observed with colonization, although the changes were insignificant. The average root diameter and the number of tips were significantly higher in colonized plants of both cultivars.

Table 3.23: Root surface area, average diameter, root volume and number of root tips of 3-week-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak) plants supplied with two different levels of Boron (Adequate: 2 μM H_3BO_3 ; High 1000 μM H_3BO_3) grown in pseudo-hydroponic system under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Control						
Cultivar	Boron	SurfArea (cm ²)	AvgDiam (mm)	Root Volume (cm ³)	Number of Tips	
Nusrat	Adequate	161 $\pm$ 38	0.402 $\pm$ 0.03	1.6 $\pm$ 0.5	985 $\pm$ 157	
	High	154 $\pm$ 16	0.407 $\pm$ 0.01	1.6 $\pm$ 0.1	766 $\pm$ 117	
Saricanak	Adequate	177 $\pm$ 32	0.415 $\pm$ 0.01	1.8 $\pm$ 0.3	1409 $\pm$ 59	
	High	141 $\pm$ 25	0.365 $\pm$ 0.02	1.3 $\pm$ 0.3	1359 $\pm$ 297	
<i>P. indica</i>						
Cultivar	Boron	SurfArea (cm ²)	AvgDiam (mm)	Root Volume (cm ³)	Number of Tips	
Nusrat	Adequate	193 $\pm$ 26	0.436 $\pm$ 0.02	2.1 $\pm$ 0.4	1096 $\pm$ 206	
	High	142 $\pm$ 28	0.415 $\pm$ 0.01	1.5 $\pm$ 0.3	1066 $\pm$ 239	
Saricanak	Adequate	177 $\pm$ 32	0.432 $\pm$ 0.01	1.9 $\pm$ 0.4	1730 $\pm$ 437	
	High	149 $\pm$ 22	0.387 $\pm$ 0.01	1.4 $\pm$ 0.2	1505 $\pm$ 99	
Root Surface Area LSD _{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; n.s.; 19; n.s.; n.s.; n.s.; n.s.)						
Root Average Diameter LSD _{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (0.02; 0.02; 0.02; n.s.; n.s.; 0.02; n.s.)						
Root Volume LSD _{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (n.s.; n.s.; 0.2; n.s.; n.s.; n.s.; n.s.)						
Number of Root Tips LSD _{0.05} (I; CV; B; IxCV; IxB; CVxB; IxCVxB) = (169; 169; n.s.; n.s.; n.s.; n.s.; n.s.)						

4. DISCUSSION

Phosphorus deficiency significantly inhibited shoot growth of wheat plants (Table 3.1) as reported in the literature [Rodríguez, 1998]. Symbiosis with AM fungi is expected to benefit plant, promote growth and alleviate abiotic stress [Latef et al., 2016]; however differences in responsiveness to AM between plant species are reported [Zhu et al. 2001]. A study by Hetrick et al. (1992) reported that modern bread wheat cultivars show less responsiveness to mycorrhizal colonization in respect of shoot growth [Hetrick et al., 1992], [Zhu et al.; 2001]. In our study, a significant effect of AM colonization on shoot growth was not observed but AM alleviated the visual symptoms of B toxicity such as chlorosis and necrosis.

Boron toxicity caused chlorosis and necrosis on leaf tips and margins (Figure 3.1). Boron is translocated through the xylem and accumulated at the end of the transpirational system in phloem-immobile species. This causes foliar symptoms like chlorosis or necrosis to be observed in leaf tips. The symptoms occur on the oldest leaves first, then spreads through the younger parts [Brdar-Jokanović, 2020].

In the experiments that were conducted to investigate the effects of *P. indica*, B toxicity caused necrosis in leaf tips and margins and decreased shoot growth in both cultivars. Inoculation with *P. indica* promoted growth in both control and stress conditions. *P. indica* is reported to enhance growth and yield in a wide range of plant species. Increased tolerance to various stress factors such as salinity, drought, heavy metal toxicity was also reported [Gill et al., 2016]. According to the literature, *P. indica* alleviates abiotic stress by modulating the defense system and metabolism to compensate for the restricted photosynthesis and reduce oxidative stress [Varma et al., 2013].

Boron is thought to play a major role in maintaining the structure and integrity of cell walls and membranes with its ability to form complexes with cis-diols. Effect of B on cell wall or membrane integrity and ion leakage was also highlighted in the

literature [Cakmak et al., 1997]. Significantly higher amount of electron leakage caused by B toxicity (Table 3.2) can be explained by the impaired integrity of cell walls along with the accumulation in the leaf tissues. P deficiency may also have contributed to increased electron leakage by second youngest leaves by limiting leaf area and indirectly increasing B concentration in older leaves. High B levels were reported to reduce P concentration in wheat leaves [Singh et al., 1990] and another study highlighted that improved P status may help to alleviate the detrimental effects of B toxicity [Kaya et al., 2009].

Similar to the results of experiments with AM, a significant increase in relative electron leakage from the leaves was observed under B toxicity and *P. indica* showed no significant effect on alleviating this stress symptom. The second youngest leaves were used for REL analysis and necrotic parts were also included. As mentioned before (Figure 3.3), *P. indica* promoted growth even under stress but there was no difference between control and colonized groups regarding to necrosis caused by B toxicity. We can conclude that cell wall integrity was already almost destroyed in necrotic parts, and *P. indica* played no part in preventing the leakage of accumulated ions.

Even though the literature is rich in studies that reports the positive effects of AM fungi such as improved growth, nutrient uptake and yield; there are very few studies that investigate the effects of AM on root growth or the changes in root morphology [Dal Cortivo et al., 2018]. Data on alterations of AM on root morphology are not consistent. There are studies that reported decreased root length, increased average diameter and increased branching [Price et al.; 1989], [Berta et al., 1993]; but the reduction in branching was also observed [Price et al. 1989]. A study by [Schroeder and Janos, 2005] reported increased specific root length, reduced surface area and average diameter.

Results reported in this study indicated a significant decrease in root DW, root length, surface area, and the number of root tips. Both P deficiency and B toxicity were observed to decrease root length, surface area and root volume. These may be

the symptoms of damages caused by combined abiotic stresses, or a tolerance mechanism for avoiding further toxicity.

Reports on the effects of *P. indica* colonization on root morphology are very limited. A study by [Hosseini et al., 2017] investigated the effects of *P. indica* on physiological characteristics and root morphology of wheat plants that were exposed to drought and mechanical stress, their results demonstrated that colonization alleviated the detrimental effects of combined stresses on roots and increased both root length and volume. In our case, any significant difference caused by B toxicity or colonization on root length was not detected. Root volume was decreased with the B toxicity and an increase in root volume values of colonized plants was observed even though it was not significant, similar to findings of [Hosseini et al., 2017]. Similar results were observed in root surface area, a tendency to increase in surface area was observed in plants inoculated with *P. indica*. Furthermore, a significant increase in average root diameter and number of tips were also observed. Similar findings, promotion of root growth and increase in average diameter, with *P. indica* colonization in roots of rapeseed (*Brassica napus*) was reported by [Wu et al., 2018]. These alterations may contribute to the increased shoot growth by increase in nutrient uptake or production of phytohormones.

AM is expected to improve shoot P status under deficiency [Baslam et al., 2013], [Xie et al., 2014]. A significant increase in P concentration was observed in Nusrat under P deficiency, as indicated in the literature. The same response was not observed in Saricanak. Increased S concentration by mycorrhizal colonization was reported on onion and maize [Guo et al., 2017], [Banerjee et al., 2013]. It is suggested that AM is able to obtain S from organic sources in soil [Allen and Hill, 2009]. It is also known that S is very mobile and prone to leaching [Eriksen and Askegaard, 2000]; an alternative mechanism by AM for increasing S concentration may be the prevention of S loss due to leaching by increased surface area for nutrient uptake through the spread of hyphae in growth media.

Besides the observed symptoms on aerial parts of plants, damage on root caused by B toxicity were reported by several studies [Choi et al., 2007], [Aqua et al.,

2012], [Esim et al., 2013], [Landi et al., 2019]. Morpho-anatomical alterations such as thickening of hypodermis, suberin deposition and increased lignification are considered mechanisms for the prevention of further B uptake [Choi et al., 2007], [Ghanati et al., 2002], [Landi et al., 2019]. Such alterations and increased tissue damage in roots may have affected uptake of other nutrients like Mg, Fe and Zn and cause significant decreases in shoot concentrations of those nutrients.

Inoculation with *P. indica* had no effect on shoot K concentrations, although it did increase P and S concentrations considerably (Table 3.16). Mg concentration, on the other hand, was significantly reduced with inoculation. Under B toxicity stress, shoot K, P, Mg, and S concentrations were higher in both cultivars. The concentrations of B and Fe were considerably lower in the colonized groups, but the concentration of Zn was unaffected. (Table 3.17). When plants were exposed to high B levels, there was a substantial rise in B, Fe, and Zn concentrations. Unlike the experiments with AM colonization, a significant increase in shoot growth was observed with *P. indica* inoculation. Since the nutrients for uptake were limited in media, a decrease in shoot nutrient concentrations is reasonable when shoot growth is greater. Again, increased concentrations of B, Fe and Zn can be explained by the reduced shoot growth under B toxicity. Lower concentrations of mentioned nutrients in colonized plants proves that colonization with *P. indica* compensated for the shoot growth loss caused by high B level.

Plants with higher antioxidative enzyme activity are considered more tolerant to B toxicity. However, reported responses of antioxidative enzymes to B toxicity are not consistent. Some studies have reported increased activity of AP [Landi et al., 2013], [Eraslan et al., 2007], CAT [Garcia et al., 2001], SOD [Garcia et al., 2001], [Landi et al., 2013] under B stress in different plant species. In contrast to that, in a study by [Eraslan et al., 2007] decreased activity of AP was observed. Similar to these, in some cases GR activity was increased [Wang et al., 2011], [Cervilla et al., 2007] and in others it was reduced [Ardic et al., 2009].

Increased antioxidative activity is reported by several studies for both AM [Wu et al., 2010], [Singh et al., 2011] and *P. indica* [Xu et al., 2017], [Verma et al., 2021] .

Suggested explanation of the mechanism is that AM colonization enhances antioxidative response by increasing SOD activity, catalyzes the reduction of $\bullet\text{O}_2^-$ (ROS) to H_2O_2 and limiting H_2O_2 accumulation through increased CAT, POX, and AP activities. However, there are also studies reporting no observed correlation between SOD, CAT activities and $\bullet\text{O}_2^-$ or H_2O_2 concentrations [Kapoor and Singh, 2017].

Inoculation with AM, P deficiency, and B toxicity led to a significant reduction in AP, GR, and CAT specific activities (Table 3.8). There was a significant difference in specific activities of antioxidative enzymes between cultivars; AP and GR activity were higher in Saricanak, whereas CAT activity was higher in Nusrat.

It is suggested that induction of antioxidative enzyme activity may be an indirect consequence of improved growth and P and N uptake [Ruiz-Lozano et al., 2012]. An increase in activity aided by increased uptake of Zn and Cu, which act as a cofactor for SOD, was also observed [Alguacil et al., 2003]. Impaired uptake of nutrients or increased accumulation of ROS due to B toxicity may have hindered the antioxidative regulation by AM.

Specific activity of AP was significantly reduced with *P. indica* inoculation while other enzymes showed no significant response (Table 3.20); specific activity of SOD was significantly higher in inoculated Saricanak plants compared to control group. Specific activities of GR and CAT were significantly increased under B toxicity stress.

Similar to our findings, induced antioxidative activity with *P. indica* colonization is reported on different plant species (maize, rice, barley) under various stress conditions (drought, salinity) [Baltruschat et al., 2008], [Bagheri et al. 2013], [Xu et al. 2017]. In maize, it was observed that *P. indica* colonization upregulated SOD and CAT activity and promoted shoot and root growth [Xu et al., 2017], [Verma et al., 2021].

5. CONCLUSION

Crop productivity is being hindered worldwide by the effects of diverse abiotic stress factors such as drought, salinity, ion toxicity, high temperatures, and pollution. Finding alternative solutions in order to enhance production is necessary for providing sufficient food for the increasing population. The use of plant-associated beneficial microorganisms are a promising way of promoting growth and increasing productivity. Therefore, understanding the mechanisms of plant-microbe interactions and integrating that knowledge with agricultural production is critical.

Results indicate that arbuscular mycorrhiza and *Piriformospora indica* have different effects on growth, nutritional status and stress tolerance. Alterations in root morphology were also varied depending on microorganisms and growth conditions. Further studies are needed for understanding the mechanisms behind the differences in responses and determine the effects of AM and *P. indica* on other stress tolerance parameters.

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