

T.R.
GEBZE TECHNICAL UNIVERSITY
GRADUATE SCHOOL

INVESTIGATION OF THE PHYSIOLOGICAL EFFECTS OF
***Piriformospora indica* ON BORON TOXICITY IN WHEAT**

ALİ KAVAL

A THESIS OF DOCTORATE
DEPARTMENT OF BIOTECHNOLOGY

ADVISOR: ASST. PROF. DR. ÜMİT BARIŞ KUTMAN

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LİSANSÜSTÜ EĞİTİM ENSTİTÜSÜ

BUĞDAYDA *Piriformospora indica*'NİN BORON
TOKSİSİTESİ ÜZERİNE FİZYOLOJİK ETKİLERİNİN
ARAŞTIRILMASI

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I wholeheartedly dedicate this dissertation to Prof. Dr. Ömer MUNZUROĞLU, who fundamentally and inwardly influenced numerous people in his too-short time here on Earth. He was the one, who had been a truly great inspiration and exemplar throughout my life period, after meeting him in 2008 and died suddenly in 2017. I am unequivocally convinced that the waves created by him in the universe are going to influence the generations despite his desperate absence with everlasting grief.

ABSTRACT

Boron (B) toxicity, which has been known as a prevalent global nutritional issue thus far, significantly impedes crop yield in the agricultural areas of many countries. Microbial symbionts such as arbuscular mycorrhizal (AM) fungi can boost plant growth through the uptake of plant nutrients, growth promotion, and resistance against abiotic and biotic stresses, thus they have been receiving ever-increasing attention. Besides that, the symbiotic fungus *Piriformospora indica* has been extensively researched as a fungal root endophyte with considerable potential concerning improving host plants' stress tolerance.

The first and second experiments were conducted under different B toxicity levels to unveil the ameliorative effects of *Piriformospora indica* on physiological and morphological aspects of two commercial wheat cultivars in sterile and native soil conditions and were terminated at vegetative and yield stages, respectively. In the first experiment, *P. indica* reduced MDA concentration by 18% in both tested cultivars, and REL by 94% and by 62% for bread and durum wheat, respectively. This experiment found that *P. indica*-inoculated shoot dry matter in bread and durum wheat with 21% and 14% increases, respectively. The shoot phosphorus (P) contents of bread and durum cultivars were improved by *P. indica* up to 14% and 5%, respectively. The results showed the positive effects of *P. indica* on the shoot antioxidative enzyme activities of bread wheat, as evidenced by the following increases: 9% (superoxide dismutase), 9% (peroxidase), and 12% (ascorbate peroxidase) when the plants were inoculated with *P. indica* as compared to the control plants.

The second experiment revealed *P. indica*-inoculated yield parameters in bread and durum wheat cultivars with 7% and 8% increases in grain numbers and 7% and 11% increases in straw dry matters, respectively in comparison to plants grown without *P. indica*.

The third experiment was conducted under different B toxicity and P supplementation levels to unveil comparatively the ameliorative effects of both *P. indica* and arbuscular mycorrhizal fungi on physiological and morphological aspects of the same cultivars in sterile soil and terminated at vegetative stage. The results found that the shoot P concentration was improved by *P. indica* up to 17% in durum. Compared with corresponding control groups, *P. indica*-inoculated bread and durum wheat cultivars displayed improved chlorophyll (11% and 9%) and carotenoid (15% and 10%) biosynthesis, respectively. This experiment also showed the ameliorative effects of *P. indica* as well as AM fungi on the cell membrane damage as evidenced by the following reductions in *P. indica*-inoculated bread and durum wheat with 20% and 17% respectively, and in AM-inoculated durum wheat with 36%.

The current dissertation revealed major evidence that both *P. indica* and arbuscular mycorrhiza can significantly confer benefits to either cultivar in different manners. This research found that *P. indica* can be a potentially useful biotechnological tool under B toxic conditions found in many arid and semiarid regions. Nevertheless, more studies are needed to assess to the potential of *P. indica* under field conditions.

Keywords: *Serendipita indica*, Abiotic Stress, Boron Toxicity, Wheat, Endophytic Fungi, Plant-Microbe Interactions

ÖZET

Şu ana kadar yaygın bir küresel beslenme sorunu olarak bilinen bor (B) toksisitesi, birçok ülkenin tarım alanlarındaki mahsul verimine ciddi ölçüde engel olmaktadır. Arbusküler mikorizal (AM) mantarlar gibi mikrobiyal simbiyontlar, bitki besin maddelerinin alımı, büyüme teşviki ve abiyotik ve biyotik streslere karşı direnç yoluyla bitki büyümesini artırabilir, dolayısıyla giderek artan ilgi görmektedir. Ayrıca, simbiyotik mantar *Piriformospora indica*, konukçu bitkilerin stres toleransını iyileştirme konusunda önemli potansiyele sahip bir kök mantar endofiti olarak kapsamlı olarak araştırılmaktadır.

Birinci ve ikinci deneyler, *Piriformospora indica*'nın steril ve doğal toprak koşullarında iki ticari buğday çeşidinin fizyolojik ve morfolojik yönleri üzerindeki iyileştirici etkilerini ortaya çıkarmak amacıyla farklı B toksisitesi seviyelerinde yürütüldü ve sırasıyla vejetatif ve verim aşamalarında sonlandırıldı. Birinci deneyde *P. indica*, MDA konsantrasyonunu her iki test edilen çeşitte de %18 oranında azaltırken, REL ekmek ve makarnalık buğday için sırasıyla %94 ve %62 oranında azaldı. Mevcut deney, *P. indica* ile inoküle edilmiş ekmek ve durum buğdayında sürgün kuru maddesinde sırasıyla %21 ve %14 artışların olduğunu buldu. *P. indica* ile ekmek ve makarnalık çeşitlerin sürgün fosfor (P) içeriklerini sırasıyla %14 ve %5'e kadar iyileştirildi. Dahası sonuçlar, *P. indica*'nın ekmeklik buğdayın yeşil aksam antioksidatif enzim aktiviteleri üzerindeki pozitif etkilerini ortaya koymuş, bu da inoküle edilmemiş kontrol bitkilerine kıyasla takip eden artışlarla kanıtlanmıştır: %9 (süperoksit dismutaz), %9 (peroksidaz) ve %12 (askorbat peroksidaz).

İkinci deneyde, *P. indica* ile inoküle edilmiş ekmek ve makarnalık buğday çeşitleri *P. indica* olmadan yetiştirilen bitkilerle kıyaslandığında sırasıyla dane sayısında %7 ve % 8, saman kuru maddesinde ise % 7 ve % 11 artış olduğu ortaya çıktı.

Üçüncü deney, hem *P. indica* hem de arbusküler mikorizanın, steril toprakta mevcut bitki çeşitlerinin fizyolojik ve morfolojik yönleri üzerindeki iyileştirici etkilerini karşılaştırmalı olarak ortaya çıkarmak için farklı B toksisitesi ve P takviyesi seviyeleri altında gerçekleştirilmiş olup vejetatif aşamada sonlandırıldı. Sonuçlar, durum buğdayında sürgün P konsantrasyonunun *P. indica* ile %17'ye kadar iyileştirildiğini gösterdi. Karşılık gelen kontrol gruplarıyla karşılaştırıldığında, *P. indica* ile inoküle edilmiş ekmek ve durum buğdayı çeşitleri sırasıyla klorofil (%11 ve %9) ve karotenoid (%15 ve %10) biyosentezinde iyileşme gösterdi. Ayrıca bu deney, *P. indica*'nın yanı sıra AM mantarlarının hücre zarı hasarı üzerindeki iyileştirici etkilerini de göstermiş bu takip eden azalmalar ile kanıtlanmıştır; *P. indica* ile inoküle edilmiş ekmek ve durum buğdayı çeşitlerinde sırasıyla %20 ve %17 oranında ve AM ile inoküle edilmiş durum buğdayında ise %36 oranında.

Mevcut tez, hem *P. indica*'nın hem de arbusküler mikorizanın her iki buğday çeşidine de farklı şekillerde ve önemli ölçüde fayda sağlayabileceğine dair önemli kanıtlar ortaya çıkardı. Bu araştırma, *P. indica*'nın birçok kurak ve yarı kurak bölgede bulunan B toksik koşullarında potansiyel olarak yararlı bir biyoteknolojik araç olabileceğini buldu. Ancak *P. indica*'nın saha koşullarındaki potansiyelini değerlendirmek için daha fazla araştırmaya ihtiyaç bulunmaktadır.

Anahtar Kelimeler: *Serendipita indica*, Abiyotik Stress, Bor Toksisitesi, Buğday, Endofitik Fungus, Bitki-Mikrop Etkileşimleri

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LIST OF ABBREVIATIONS AND ACRONYMS

$(\text{CO}_3)^{-2}$	: Carbonate
(HCO_3^-)	: Bicarbonate
$(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$	: Ammonium heptamolybdate tetrahydrate
$(\text{SO}_4^-)_2$	: Sulphate
$^1\text{O}_2$	: Singlet oxygen
Act	: Activity
AMF	: Arbuscular Mycorrhizal fungi
ANOVA	: Analysis of variance
APX	: Ascorbate peroxidase
ASC	: Ascorbate
B	: Boron
BHT	: Butylated hydroxytoluene
BOREN	: National Boron Institute
BS	: Boron supply
Ca	: Calcium
$\text{Ca}(\text{NO}_3)_2$	: Calcium nitrate
CAT	: Catalase
chl	: Chlorophyll
Cl^-	: Chloride
cm	: Centimeter
CO_2	: Carbon dioxide
Conc.	: Concentration
Cont.	: Content
Cu	: Copper
$\text{CuSO}_4 \cdot 5(\text{H}_2\text{O})$	: Copper sulphate pentahydrate
cv.	: Cultivar
Da	: Decare
DAS	: Days After Sowing
DF	: Degrees of freedom
dH_2O	: Distilled water
dS	: Deci Siemens
DW	: Dry weight
EC	: Electrical Conductivity
EC_e	: Saturated Paste Extract

ESP	: Exchangeable Sodium Percentage
FAO	: Food and Agriculture Organization
Fe	: Iron
FeEDTA	: Iron ethylenediaminetetraacetate
FW	: Fresh weight
g	: Gram
GB	: Glycine Betaine
GR	: Glutathione reductase
GSH	: Reduced glutathione
GSSG	: Oxidized Glutathione
h	: Hour
H ₂ O	: Water
H ₂ O ₂	: Hydrogen peroxide
H ₃ BO ₃	: Boric acid
HCl	: Hydrochloric acid
HI	: Harvest index
HNO ₃	: Nitric acid
HSD	: Tukey's honestly significant difference
ICP-OES	: Inductively coupled plasma optical emission spectrometry
K	: Potassium
K ₂ SO ₄	: Potassium sulphate
KCl	: Potassium chloride
kg	: Kilogram
KH ₂ PO ₄	: Potassium dihydrogen phosphate
KI-I ₂	: Potassium iodide - Iodine
K-P	: Potassium phosphate buffer
l	: Liter
LSD	: Fisher's least significant difference
m	: Meter
MDA	: Malondialdehyde
mg	: Miligram
Mg	: Magnesium
MgSO ₄	: Magnesium sulphate
min	: Minute
ml	: Milliliter
mM	: Millimolar
Mn	: Manganese
MnSO ₄ .H ₂ O	: Manganese sulphate monohydrate
MPa	: Megapascal

N	: Nitrogen
<i>n.s.</i>	: not significant
Na	: Sodium
NaCl	: Sodium chloride
NADPH	: Nicotinamide adenine dinucleotide phosphate
NADPH- Na_4	: Dihydronicotinamide adenine dinucleotide phosphate tetrasodium salt
NBT	: Nitro blue tetrazolium chloride
NH_4^+	: Ammonium
NO_3^-	: Nitrate
O_2^-	: Superoxide radical
OH^-	: Hydroxyl radical
P	: Phosphorus
pH	: Power of hydrogen
ppm	: parts per million
Pr	: Probabilities
REL	: Relative electrolyte leakage
RG-II	: Rhamnogalacturonan II
ROS	: Reactive Oxygen Species
S	: Salinity
SOD	: Superoxide dismutase
Sp	: Specific
TBA	: Thiobarbituric acid
TCA	: Trichloroacetic acid
TGW	: Thousand grain weight
Tox	: Toxicity
Zn	: Zinc
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	: Zinc sulphate heptahydrate

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1. INTRODUCTION

Abiotic stresses are already regarded as crucial factors (i.e., drought, salinity, freezing, heat, mineral deficiencies, B toxicity, and heavy metal toxicities) in limiting worldwide crop production [Ahuja et al., 2010; Calanca, 2017]. Climate change is probably going to exacerbate this situation further. In order to boost agricultural production and food security, finding useful strategies to improve crop tolerance against abiotic stress factors will be vital especially in the light of expected growth in world population and food demand [Calanca, 2017]. B toxicity has long been considered a problem across the world. Since the range between critical concentrations of B deficiency and B toxicity is quite narrow, the yield and quality of many crops are influenced by B deficiency or toxicity [Ben-Gal and Shani, 2002; Tlili et al., 2019]. B deficiency can be overcome by B-enriched fertilizer treatments; on the other hand, improper fertilization, and use of irrigation water with high B can result in B toxicity [Nable et al., 1997; Landi et al., 2012]. In this sense, beneficial soil microorganisms may substantially confer cost-effective, eco-friendly, and sustainable solutions.

Microbial symbionts, like arbuscular mycorrhizal fungi (AMF), are able to enhance the growth of plants via nutrient uptake, growth promotion, and protection against biotic and abiotic stresses [Bajaj et al., 2018]. Beneficial root associates belonging to the basidiomycete order Sebaciniales which is a ubiquitous and ecologically diverse group of fungi are less known. Across a broad spectrum of plant species, they universally form endophytic, and in some cases mycorrhizal-like associations [Weiss et al., 2011; Weiss et al., 2004; Selosse et al., 2002]. Hitherto, it has been demonstrated that they improve nutrient uptake [Barazani et al., 2005; Weiss et al., 2016] while boosting resistance to a range of biotic and abiotic stresses [Waller et al., 2008]. As a root endophytic Sebacinaceae, *Piriformospora indica* was first documented in the Thar Desert of India [Verma et al., 1998].

Wheat, besides being one of the most broadly grown and consumed crops, is one of the most important staple foods across the world and is a substantial source of calories, minerals, and plant-originated protein in human food, therefore, its

contribution to both human and livestock diets is indisputable [Tadesse et al., 2019; Shewry and Hey, 2015; Cakmak, 2008; Curtis et al., 2002].

In this dissertation, taking into account all these facts tackled precede page, the following hypotheses were tested: (1) that the deleterious effects of B toxicity could be ameliorated by *P. indica* in terms of plant growth and yield losses, and whether B tolerance of wheat cultivars tested in this study could cause different physiological and enzymatic responses in the presence of *P. indica*; (2) that removing the soil microorganisms through autoclaving could enhance plant growth and yield and ease the effects of B toxicity; (3) that inoculation of wheat plants with arbuscular mycorrhizae and/or *P. indica* could enhance plant growth in a sterile soil by promoting nutrient uptake and other stress-alleviating mechanisms and high P could confer/promote beneficial effects (i.e., stress alleviation and plant growth promotion) of either fungus against B toxicity. In the final accounting, this dissertation is aimed to ascertain B tolerance mechanisms in AM and *P. indica*-inoculated plants and also to compare the interactive antioxidant effects of these fungi on plant growth.

1.1. Boron in Plant Biology

Plants need essential macro and micronutrients, for normal growth and development. When nutrient supply is inadequate, it leads to loss of yield and quality in agricultural plants [Nable et al., 1997; Marschner, 2011]. Boron (B), which is a member of group 13 on the periodic table (and a member of the metalloid subgroup III), exhibits intermediate properties between metals and non-metals and may produce both bases and acids [Marschner, 2011]. B has low abundance in nature, is dispersed widely in both the lithosphere and the hydrosphere, and its concentrations range from 5 to 10 mg kg⁻¹ in rocks [Shorrocks, 1997], from 3 to 30 µg kg⁻¹ in rivers [Power and Woods, 1997], and from 4 to 5 mg L⁻¹ in the ocean [Lemarchand et al., 2000]. Because of its chemical properties and functions in plant, B is a unique chemical that serves as a micronutrient and is therefore essential for the normal growth and development of plants [Gupta, 1979; Dunn et al., 2005]. Its essentiality was first noted in 1923 [Warrington, 1923] and revealed that the availability of this nutrient in the soil and irrigation water is crucial in agricultural production [Tanaka and Fujiwara, 2008].

Boron plays various functions in the physiological processes of plants [Marschner, 2011]. It has substantial functions that are mostly if not exclusively associated with its structural role in cell walls and the maintenance of plasma membrane functions [Brown et al., 2002; Wimmer et al., 2009; Hajiboland et al., 2015]. Compared to the vegetative stage, the reproductive stage of plants is far more sensitive to B deficiency [Ahmad et al., 2009]. Furthermore, B is also required for a plethora of processes, such as the growth of meristematic tissues, calcium utilization, cell division, water relations, disease resistance, membrane function, assimilate partitioning, nitrogen (N) metabolism, and the flowering/reproductive phase, e.g., pollen germination [Sprague, 1951; Goldbach and Wimmer, 2007; Ahmad et al., 2009].

The composition of cell wall components, including cellulose, hemicellulose, pectin, and lignin, is altered by B deficiency. Water-soluble pectin levels are reduced while cellulose, hemicellulose, and uronic acid concentrations are enhanced [Zehirov and Georgiev, 2003]. With the diols group in the *cis* position, boric acid and borate can interact, and borate esters readily form with monosaccharides which include those with the β -D-erythrofuranose structure. The rhamnogalacturonans I and II (RG-I and RG-II) and homogalacturonans, as well as pectin and polysaccharide complexes, are essential parts of the cell wall [Kobayashi et al., 1997; Reuhs et al., 2004]. Through B-diester linkages, boron and the RG-II complex serve as connection sites for pectic polysaccharides [Matoh, 1997], and cell wall stabilization is facilitated by borate-diester bonds. Borate creates a cross-link with the apiose residues in RG-II and contributes to the formation of a three-dimensional pectic network [O'Neill et al., 2004]. RG-II is regarded as the fundamental infrastructure for cell wall stabilization due to its extremely complicated structure and the appearance of its genes during the early phases of cell wall formation [Matsunaga et al., 2004]. The expansion and structure of primary and secondary cell walls depend heavily on the RG-II-B complex; the interaction of B with RG-II also takes place beyond cell wall cross-linking [Ryden et al., 2003; Kohorn et al., 2006]. Small structural changes can affect the stability of the RG-II complex; consequently, a higher B/RGII ratio is necessary for complex formation [Goldbach and Wimmer, 2007].

B is needed for the proper function of enzymes and other proteins located in the cell membrane; moreover, maintenance of membrane integrity, regulation of transport

through the membrane, and prevention of potential damage to the membrane structures from ROS [Cakmak and Römheld, 1997; Goldbach et al., 2001; Goldbach and Wimmer, 2007]. The deficiency of B causes changes in the membrane potential. This happens as follows: a proton gradient is created across the plasma membrane as a result of increased proton pumping ATPase activity, and the activity of plasma membrane-bound oxidoreductase is inhibited [Blaser-Grill et al., 1989; Obermeyer et al., 1996; Wimmer et al., 2009]. Because of interacting with glycolipids, B is required for the integrity and stability of membrane function [Brown et al., 2002]. Owing to the modulation of the auxin-mediated activity of cell membrane-bound H⁺-ATPases and the accumulation of phenolics, B deficiency swiftly changes ion fluxes [Cakmak and Römheld, 1997].

In soil solution, H₃BO₃ can be readily leached under conditions of heavy rainfall [Shorrocks 1997; Yan et al., 2006] causing deficiencies in the plants that grow there (e.g., many regions in Japan, China, USA, and Brazil). It is widely known that B deficiency, in vascular plants, leads to various effects on a wide range of processes. The formation of pollen tubes, sugar translocation, carbohydrate metabolism, indoleacetic acid (IAA) oxidase activity, and root elongation are all some examples of these processes [Blevins and Lukaszewski, 1998; Goldbach and Wimmer, 2007]. Over and above, plasmalemma-bound enzymes and ion fluxes across membranes, membrane potential [Blaser-Grill et al., 1989; Goldbach et al., 2001], cytoskeletal proteins [Yu et al., 2003], nitrogen metabolism [Camacho-Cristobal and Gonzalez-Fontes, 2007], and accumulation of phenolics and polyamines [Camacho-Cristobal et al., 2008], inter alia, are processes in which B may also play a role. Boron deficiency leads to membrane leakage because of a loss of membrane functional integrity [Cakmak and Römheld, 1997]. A sufficient supply of B may induce plasma membrane H⁺-pumping activity via enhancing the driving power for K⁺ influx [Cakmak and Römheld, 1997]; inhibition of plasma membrane-bound [oxidoreductase] activities suggest a direct interaction between membranes and B [Goldbach and Wimmer, 2007].

Boron toxicity or deficiency does not specifically affect photosynthesis. Nevertheless, B deficiency has an indirect impact on photosynthesis via reducing the photosynthetic area and changing leaf constituents [Dell and Huang, 1997; Wang et al., 2007]. Chlorophyll and soluble protein contents in the leaves are decreased by B

deficiency which leads to a loss in photosynthetic enzyme activity that prevents the Hill reaction and lowers net photosynthesis [Sharma and Ramchandra, 1990], [Cakmak and Römheld, 1997; Brown et al., 2002]. There is a finding that B deficiency disrupts the electron transport chain (ETC) leading to photo-inhibition, however, the exact mechanism is uncertain [Goldbach and Wimmer, 2007]. Boron deficiency can result in the downregulation of photosynthesis by impairing the plant phloem transport, lowering sink capacity, and increasing sugar accumulation in the leaves [Wimmer and Eichert, 2013]. B deficiency lowers photosynthetic activity as a result of reducing the ability of the plant to capture light [Cakmak and Römheld, 1997]. Under B-deficient circumstances, plants show poor leaf expansion which indirectly inhibits photosynthesis [Furlani et al., 2003]. In more detail, because low stomatal frequencies and smaller stomatal apertures occur in B-deficient leaves, which both decrease stomatal conductance to CO₂ [Sharma and Ramchandra, 1990].

Boron, which is an integral part of the sugar-borate complex, facilitates sugar transport [Westmark et al., 1996; Dordas and Brown, 2000]. Because sink capacity is reduced in B-deficient environments, assimilate translocation from source to sink diminishes, changing the distribution of assimilates in roots and shoots [Dugger, 1983; Marschner, 2011]. Sugars and starch accumulate in B-deficient source leaves as a result of the diminished assimilates translocation from the leaves to other organs [Kastori et al., 1995]. Reduced assimilate translocation to growing regions is responsible for the most prominent effects of B deficiency which are increased leaf fraction and reduced stem and fruit fractions [Dixit et al., 2002]. With sufficient B nutrition, the rate of assimilate redistribution to new growth regions, especially fruits, increased [Hellal et al., 2009]. As a result of B deficiency, however, sucrose transport from mature to younger leaves diminished [Marschner, 2011]. For optimal assimilate partitioning to developing grains, sufficient B nutrition is required at grain filling [Hussain et al., 2012]. Boron transporters have been found in pollen, flowers, and possibly the upper stem and contribute to B distribution and partitioning [Yoshinari and Takano, 2017]. A study revealed that because of B deficiency, plant tissues show elevated N concentration and fluid accumulation [Shelp, 1988]. Additionally, under B-deficient conditions, N partitioning as well as the relative composition of the amino acids [glutamine, alanine, and asparagine] is affected [Shelp, 1990].

Historically, it was found that plant vegetative growth is less sensitive to B deficiency than plant reproductive growth [Dell and Huang, 1997; Uraguchi and Fujiwara, 2011; Rerkasem and Jamjod, 2004]. As a result, B deficiency is regarded to decrease grain set more than vegetative growth in field crops. For some field crops, yet, growing in B-deficient calcareous soils, this was not the case e.g., wheat [Rashid et al., 2002a; Rashid et al., 2011], rapeseed [Rashid et al., 2002b], and rice [Rashid et al., 2002a; Rashid et al., 2005]. Boron deficiency in rice lowered the production of straw biomass more than paddy yield [Rashid et al., 2002a; Rashid et al., 2005].

As a result of B deficiency, plants form empty, shrivelled, and malformed pollen grains or normal pollen that lack starch reserves [Dell and Huang, 1997]. Moreover, as monitored in rice, pollen germination and pollen tube growth are disrupted under B deficiency conditions [Wang et al., 2003; Lordkaew et al., 2013]. In pollen cell walls, B is expected in the form of borate–RG-II complex which serves as a binding site for B in the plant cell wall [Matoh, 1997; Matoh and Kobayashi, 1998]. It was reported that under B deficiency, abnormal stomata and distorted guard cells are formed at the reproductive stage [Blevins and Lukaszewski, 1998; Huang et al., 2000]. As a result, B has important roles during anther development, pollen tube formation, seed set and seed germination [Wang et al., 2003].

Considering global crop production, B deficiency is known to be the most common microelement problem [Shorrocks et al., 1997]. B has a quite narrow range between deficiency and toxicity and the yield and quality of many crop species are gravely hampered by the narrow adequacy zone of B [Tlili et al., 2019; Ben-Gal and Shani, 2002]. Unlike B deficiency, which can be rectified relatively easily with proper soil and foliar fertilization applications, B toxicity is more challenging to manage.

1.2. Boron Concentrations and Requirements in Plant Parts

Depending on the species, different plants have various requirements and responses to applied B [Rashid et al., 2002c]. When compared to monocots, the internal B requirement of dicots is 4–7 times higher (5–10 mg kg⁻¹ and 20–70 mg kg⁻¹, respectively). It is explained by the differences in the extensibility and composition of the cell walls caused by the level of pectin. Therefore, monocots are less tolerant of excess B and have relatively lower B requirements [Brown and Hu, 1996;

Marschner, 2011; O'Neill et al., 2004; Miwa and Fujiwara, 2010; Chormova and Fry, 2016]. Namely, the primary cell wall of graminaceous plants contain small amounts of pectin (only 3–10 $\mu\text{g g}^{-1}$ dry weight) and therefore monocots have comparatively lower B requirements than dicots with more pectin and considerably higher tissue B requirement (20–30 $\mu\text{g g}^{-1}$) **[Darvill et al., 1980; Gupta, 1993].** For example, it was found that B deficiency symptoms are generally associated with leaf B concentrations of less than 1 mg kg^{-1} dry matter in monocots such as wheat compared to less than 10 mg kg^{-1} dry matter in dicots **[Bell, 1997].**

A study reported that there was a much greater B requirement for both reproductive and vegetative growth in rice and elaborated that B concentrations still increased both in anthers ($>20 \text{ mg kg}^{-1}$) and in flag leaves (3 mg kg^{-1}) at anthesis. Reproductive and vegetative growths are significantly decreased below this concentration **[Lordkaew et al., 2013].** According to experimental inputs of wheat for grain set and leaf growth, critical B deficiency concentrations were determined at 10 mg kg^{-1} (in the anther) and $< 1 \text{ mg kg}^{-1}$, respectively; nevertheless, the critical concentration was found at 7 mg kg^{-1} for normal pollen development and function in maize **[Rerkasem et al., 1997; Lordkaew et al., 2011].** Other studies also reported that 3 mg kg^{-1} dry matter of B concentration is adequate during the vegetative stage in wheat **[Reuter and Robinson, 1997; Marten and Westermann, 1991].** Moreover, among the reproductive parts of wheat, the male reproductive organs required more B for pollen germination than the female reproductive organs for gamete development **[Rerkasem et al., 1997].**

1.3. Boron Uptake

Several forms of Boron can be found in the soil solution, but the most abundant form is boric acid (H_3BO_3) at widespread soil pH values (5.5–7.5). It is widely suggested that B is predominantly taken up from the soil in the form of boric acid and it is the only plant nutritional element that is not absorbed from the soil as an **ion** **[Marschner, 2011; Brown et al., 2002; Tanaka and Fujiwara, 2008].** Depending on B availability, uptake of boric acid by roots can be performed by three different molecular mechanisms: passive diffusion through lipid bilayer; facilitated permeation via major intrinsic protein (MIP) channel; and lastly, an energy-dependent high-affinity transport system induced in answer to low B supply

mediating via BOR transporters **[Tanaka and Fujiwara, 2008]**. In conditions of sufficient B supply, the element uptake is carried out by roots through a passive procedure (without protein catalysis and energy consumption) that involves mostly the diffusion across the lipid bilayers, and B transport rate is determined by the concentration gradients, accordingly. This passive diffusion occurs partly thanks to the high permeability of boric acid through lipid bilayers because boric acid is a small, soluble, undissociated, and non-charged molecule **[Brown and Shelp, 1997; Brown et al., 2002; Tanaka and Fujiwara, 2008]**.

Both theoretical **[Raven, 1980]** and experimental **[Dordas and Brown, 2000; Dordas et al., 2000; Stangoulis et al., 2001b]** calculations of the lipid permeability coefficient for boric acid support the notion that B can move across membranes passively to meet plant B requirements **[Brown et al., 2002]**. Moreover, physiological studies evidently reported that under low B supply, an active B absorption can be carried out by roots **[Dannel et al., 2000; Dannel et al., 2002; Stangoulis et al., 2001b]**. The fact that B uptake was prevented by both metabolic inhibitors and low and high-temperature treatments (2-42°C) in roots supports this active B uptake **[Brown et al., 1994; Pfeffer et al., 1999; Dannel et al., 2000]**.

In the presence of B deficiency, B demands cannot be met by passive diffusion, therefore; borate exporters and boric acid channels are required for both uptake and translocation to growing tissues **[Brdar-Jokanović, 2020]**. Borate transporters (BOR) working under B deficient conditions were firstly determined in *Arabidopsis* **[Tanaka and Fujiwara, 2008; Takano et al., 2002]** and its homologues have been reportedly proposed to be involved in the efficient B uptake into the root cells for wheat, rice, maize, and other plant species thus far **[Nakagawa et al., 2007; Tanaka and Fujiwara, 2008; Diehn et al., 2019]**. Moreover, seven BORs have been determined in *Arabidopsis* thus far. In detail, BOR1 is required for boron transport from the roots to the xylem, and then its translocation from the xylem to the leaves, working with NIP5;1, and NIP6;1, respectively. Through its involvement in the crosslinking of RGII molecules, BOR2 promotes root development **[Miwa et al., 2013; Chormova et al., 2014]**. However, BOR4 predominantly contributes to the tolerance via exclusion from tissues in presence of excessive boron and studies reportedly found that there are BOR4 homologues in barley, wheat, and other

agricultural plants [Miwa et al., 2006; Sutton et al., 2007; Miwa et al., 2007; Pallotta et al., 2014; Reid, 2014].

In excessive B conditions, exporters are also involved in the exclusion process. Nodulin 26-like intrinsic proteins (NIPs) are the subfamily of the major intrinsic protein (MIP) family transporting boric acid. NIP5;1 was the first such channel identified in *Arabidopsis* functioning in the root cells under B deficit conditions, and when the level of external B increases, its expression is down-regulated [Takano et al., 2006; Takano et al., 2010; Tanaka et al., 2011; Wang et al., 2017]. Subsequently, NIP6;1 and NIP7;1 were identified to be involved in boron transfer from the xylem to phloem and in microsporogenesis, respectively [Li et al., 2011]. Moreover, other studies reported that there are homologous channels of NIP in barley, rice, and maize [Schnurbusch et al., 2010; Durbak et al., 2014; Hanaoka et al., 2014].

1.4. Boron Allocation and Mobility in Plants

Boron is unique among nutrients due to the significant differences in boron mobility between plant species. B is considerably and swiftly phloem-mobile in plant species that polyols (such as sorbitol, mannitol, and dulcitol) are the main products of photosynthesis and the B concentration disperses uniformly throughout the leaves of phloem-mobile species. Significant phloem mobility shows itself as a higher concentration in early leaves and fruits [Hu et al., 1997; Stangoulis, et al., 2010].

Once Boron has been taken up by root cells, this micronutrient is loaded into the xylem for upward transport to shoots. This process is carried out by passive mechanisms in well B-supplied plants that include B diffusion through the lipid bilayers and facilitated transport of boric acid via the MIPs channel [Dannel et al., 2002]. Nevertheless, in response to a low B supply, a high-affinity transport system that is energy-dependent and mediated by BOR transporters is activated [Tanaka and Fujiwara, 2008]. BOR1 is involved in the process of xylem loading and accumulated in the plasma membrane of pericycle cells under low B supply. Moreover, plant roots and shoots have been detected to have the mRNA of the B-specific transporter, BOR1, which is actively associated with the regulation of B transport [Takano et al., 2006].

After being loaded into xylem, B is transported by this vascular system to shoot in a mechanism assisted by the transpiration stream, which is necessary for the long-distance translocation of solutes in the xylem [Raven, 1980; Shelp et al., 1995]. Because the accumulation of B occurs at the site of a high transpiration rate (i.e., margins of large mature leaves), this long-distance transport has additionally been regarded as passive. Along the transpiration stream, B is transported and accumulates at its end; as a result, the element is significantly more concentrated in leaf tips and margins compared to the rest of the leaf [Brown and Hu, 1996; Brown and Shelp, 1997]. B can also be transported through phloem to both vegetative and reproductive tissues [Shelp et al., 1995; Matoh and Ochiai, 2005]; however, this capacity differs between species [Brown and Shelp, 1997].

The species (i.e., wheat, barley, canola, and walnut) translocating sucrose in their phloem display low B mobility, therefore; older leaves of these species have relatively higher B concentrations. However, other species including those belonging to the *Prunus*, *Malus*, and *Pyrus* genera (i.e., peach, almond, pear, cherry, apricot, apple, plum, prune), as well as olive, coffee, asparagus, onion, cauliflower, carrot, pea, cabbage, celery, and bean translocate significant amounts of sugar alcohols in their phloem; therefore, have considerable phloem B mobility due to the formation of B-polyol complexes. As a matter of fact, B can easily bind to the cis-hydroxyl groups of the sugar alcohols, enabling B to transport via phloem [Shelp et al., 1995; Brown and Hu, 1996; Hu et al., 1997; Stangoulis et al., 2010].

In detail, B-polyol complexes from the phloem sap of *Apium graveolens* have been isolated and characterized [Hu et al., 1997]. Moreover, it was found that tobacco transgenic plants with increased sorbitol levels exhibited better phloem B transport capacity and B deficiency tolerance [Bellaloui et al., 1999; Brown et al., 1999]. Nevertheless, B transport through phloem, particularly to young tissues, also takes place in plants that are unable to produce these kinds of carbohydrates [Stangoulis et al., 2001a; Takano et al., 2001; Matoh and Ochiai, 2005]. A study has reported that B is transported via phloem in white lupin from mature leaves to actively growing reproductive organs [Huang et al., 2008]. Therefore, it is still unclear the molecular mechanism underlying this B-phloem transport.

B deficiency rarely occurs in phloem-mobile species, owing to their ability to re-translocate internal B. For that reason, in order to enhance B utilization efficiency,

the increased synthesis of polyols may be useful tactic. This can be carried out either by genetically selecting for genotypes with high polyol synthesis or by transformation (for example, rice or tobacco) [Bellaloui and Brown, 1998; Brown and Shelp, 1997; Bellaloui et al., 2003].

1.5. Boron Toxicity

Boron toxicity has long been identified as a common nutritional problem on a global scale which attracts increasing interest. It significantly reduces crop yield in agricultural areas of Australia, North Africa, and West Asia and is associated with alkaline and saline soils with very limited leaching and low rainfall. It is therefore more common in arid and semi-arid areas [Reid, 2007; Tanaka and Fujiwara, 2008; Brdar-Jokanović, 2020]. Additionally, B-rich soils can also develop as a result of excessive fertilization and/or irrigation with water including high levels of B [Singh et al., 1990; Nable et al., 1997; Havlin et al., 1999; Kaur et al., 2006]. Particularly, Türkiye, South Australia, Russia, Italy, Egypt, Iraq, Libya, Jordan, Syria, Morocco, India, Chili, and California regions/countries have B toxicity problems in their agricultural areas [Yau et al., 1995; Tanaka and Fujiwara, 2008; Brdar-Jokanović, 2020].

Türkiye Soil Map of Boron, which is shown in **Figure 1**, was firstly made by The Republic of Türkiye Ministry of Agriculture and Forestry Soil, Fertilizer and Water Resources Central Research Institute in cooperation with the National Boron Institute (BOREN), the Center for Soil Fertilizers and Water Resources Research Institute. The Boron Map, the Boron State Detection and Mapping Project of the Turkish soil was terminated in 2010 with the final report, and the Boron map of Türkiye's soils was made according to this report and updated in 2017, subsequently. For this map, sample points were analysed, and different colours were used to indicate differences in B amount of Türkiye soils. Moreover, in the Central Anatolian Plateau of Türkiye, B toxicity is regarded as one of the two most significant micronutrient problems along with zinc deficiency [Sillanpaa, 1982; Yau and Ryan, 2008].

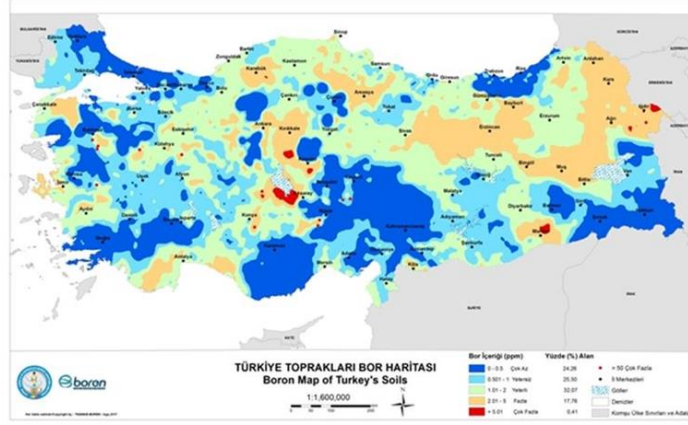


Figure 1: Boron Map of Türkiye's Soils

Studies have shown that B toxicity causes different effects on very diverse functions in vascular plants, i.e., altered cell wall component, loss of membrane functional integrity, changed metabolism, diminished root cell division, reduced lignin, and suberin levels, decreased leaf chlorophyll contents and photosynthetic rates [Nable et al., 1997; Ghanati et al., 2005; Reid, 2007; Hajiboland et al., 2015]. Therefore, plants exposed to high B levels typically exhibit diminished growth of shoots and roots [Nable et al., 1990b]. B accumulation, in many plants, leads to a pattern from leaf base to tip and this follows typical toxicity symptoms on older leaves which can be monitored as chlorosis (i.e., tip and/or marginal) and necrosis. In addition to chlorosis, necrosis, and reduced root and shoot elongation, the other most noticeable symptoms of B toxicity are dark brown elliptical spots, and brownish leaf tips [Marschner, 2011; Roessner et al., 2006; Rehman et al., 2018]. Excessive B restricts the production of starch or creates B-carbohydrate complexes resulting in delayed grain formation [Das, 2014] and because of the negative effects of excessive B on pollen development and pollen tube growth, seed and fruit set may be hampered [Fang et al., 2016].

In general, symptoms in barley appear earlier and more severely than those in wheat [Yau et al., 1997]. When B availability increases with soil depth, the symptoms appear later, and the yield is decreased less [Brennan and Adcock, 2004]. Leaf area, width, or lengths are not affected by extreme B [Yau and Saxena, 1997]. A study conducted hydroponically with wheat and barley reported that B causes general root weakness and reduces the growth of lateral roots [Nable, 1988]. Among genotypes, the severity of all B toxicity symptoms differentiates. For instance, depending on the

B treatment, plants have a lower, the same, or higher dry matter weight compared to the control group [Torun et al., 2006]. In wheat and barley, B toxicity reduced the yield and was monitored to cause significantly different effects among genotypes regarding both yield reduction and visual symptoms [Paull et al., 1988b; Cartwright et al., 1984; Yau and Saxena, 1997; Paull et al., 1990; Yau et al., 1997; Yau and Ryan, 2008]. Moreover, in spite of pronounced toxicity symptoms and elevated shoot B concentration, there are genotypes with constant yields in the presence of high soil B [Avci and Akar, 2005].

It was reported that excessive B concentration reduced emergence in tomato, maize, alfalfa, and carrot [Bañuelos et al., 1999]. For phloem-mobile *Prunus*, *Malus*, and *Pyrus* species, it was found that young shoot tip cessation, brown corky lesions along stems and petioles, leaf axil gumming, and bud abscission occurs. In the presence of toxic B concentrations, celery displays irregular stem shape and deformed young leaves as B toxicity symptoms [Brown and Shelp, 1997]. There are considerable differences with regard to plant B concentrations in the literature data that may be explained by plant species (B mobility), organ (for instance, in comparison to grains, wheat flag leaf has a B concentration that is almost ten times greater), stage of life (more B is found in older plants), B treatment, genotype and experimental methods, [Furlani et al., 2003; Nable et al., 1990b; Turan et al., 2018]. Typically, in wheat, the majority of plant B is found in the leaves (68%), followed by the roots (16%), the spikes (10%), and the stems (6%), respectively [Subedi et al. 1999].

Under B-toxic conditions, some studies evidently showed that B toxicity increased lipid peroxidation [Karabal et al., 2003] while others showed that antioxidant enzymes activities' trends are not consistent in different taxa under such conditions [Reid et al., 2004; Cervilla et al., 2007]. Even though the physiological basis for B toxicity is unclear, three major reasons have been suggested considering our knowledge of B chemistry: (i) alteration of cell wall composition; ii) metabolic disruption through attaching the ribose components of molecules like adenosine triphosphate (ATP), the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH), or, the reduced form of nicotinamide adenine dinucleotide (NADH); and finally (iii) prevention of cell division and growth through attaching to ribose, either to the free sugar or within RNA molecule [Reid et al., 2004].

1.6. Boron Toxicity Tolerance

In comparison with non-tolerant ones, B-tolerant varieties of some species exhibit a decreased B concentration in their leaf tissues [Nable et al., 1990a], which is likely a result of less B being absorbed into the roots and shoots. In this regard, the basis of B-tolerance has been described by its high ability to efflux B, and namely, an anion (borate) exchange or an anion channel are the two models that have been explained for this mechanism of active B efflux [Hayes and Reid, 2004]. Moreover, it was found that tobacco transgenic plants with increased sorbitol levels exhibited better phloem B transport capacity and B deficiency tolerance [Bellaloui et al., 1999; Brown et al., 1999].

It is widely suggested that lower B concentrations are found in all organs in tolerant barley and wheat genotypes, independent of the type of experiment or B concentrations in the soil or nutrient solution [Bellaloui and Brown, 1998; Paull et al., 1988a; Paull et al., 1988b; Rehman et al., 2006; Ghaffari et al., 2015]. Nevertheless, it was reportedly found that there is variability regarding shoot B concentration of barley, durum and bread wheat genotypes which are tolerant to excess B [Yau et al., 1997]. Thus, certain genotypes absorb or translocate less B, while others can tolerate higher tissue B concentrations.

For instance, after the investigation of 70 durum wheat genotypes, tolerant and sensitive genotypes with high and low shoot boron concentrations, respectively, were characterized [Torun et al., 2006]. A laboratory study was conducted with 40 bread wheat genotypes to investigate the B tolerance at a seedling stage; however, no correlation was determined between parameters of tolerance and plant B concentration. Contrarily, the tolerant genotypes exhibited a significantly higher B content, which was accounted for by the sensitive genotypes' lower absorption of all nutrients, including B [Brdar-Jokanović et al., 2013; Brdar-Jokanović, 2020].

1.7. Wheat as a Strategic Staple Crop

Wheat, a staple food throughout the world, is one of the most extensively grown and consumed crops [Shewry et al., 2012; Tadesse et al., 2019], and it is indisputable that wheat is economically significant and contributes to both human and livestock diets [Shewry and Hey, 2015]. The first cultivation of wheat happened ca. 10,000

years ago in the "Neolithic Revolution" that witnessed a passage from gathering and hunting for food to settled agriculture. Moreover, it is found that earliest cultivated forms of wheat originated from the south-eastern part of Türkiye [Heun et al., 1997; Nesbitt, 1998; Dubcovsky and Dvorak, 2007]. In developing countries, cereal crops are a critical source of protein and minerals. As an illustration, in many Central and West Asian nations wheat accounts for approximately 50% of the daily calorie intake on average and that is probably rising to more than 70% in the rural areas [Cakmak, 2008]. *Triticum aestivum*, a hexaploid species, is mainly referred to as "common" or "bread" wheat, the main species of wheat grown worldwide. On the other hand, *T. turgidum* var. durum, a tetraploid species, is used for making durum and is commonly called either "durum wheat" or "durum wheat". Moreover, the latter species is adapted to the hot, dry environments surrounding the Mediterranean Sea and similar climates in other areas and it makes up roughly 35–40 million tonnes of global wheat production. Only a small portion of other wheat species are cultivated, either for cultural reasons or to expand the market in terms of healthy foods [Shewry and Hey, 2015].

According to Food and Agriculture Organisation of the United Nations (FAO), 775.1 and 778.1 million tonnes of wheat were produced in 2021 and 2022, respectively. Moreover, as it can be noted from these statistics, 2022 wheat production is ~0.4% above 2021 production and more importantly an all-time high. Considering the latest assessment of wheat production, there is currently sufficient wheat supply to meet demand on a global scale [Web 1, 2023]. However, future wheat production must rise more than 50% over present levels by 2050 to meet demand because the world's population is anticipated to go beyond 9 billion by that time [Tadesse et al., 2019]. Additionally, to achieve the objectives of the "hidden hunger", changes to the grain quality requirements and finally growing the consumption of wheat products across many countries in Asia require elevated crop production [Shewry and Tatham, 2016].

The studies showed that 3 mg kg⁻¹ dry matter of B concentration is adequate during the vegetative stage in wheat [Reuter and Robinson, 1997; Marten and Westermann, 1991]. However, carpels and anthers have higher B requirements than leaves in wheat [Rerkasem and Jamjod, 2004]. In wheat, among the reproductive parts, the male reproductive organs required more B for pollen germination than the

female reproductive organs for gamete development. In detail, it was credibly reported that pollen needs 8–10 mg kg⁻¹ during reproductive growth to prevent grain yield losses due to sterility [Rerkasem et al., 1997]. Boron concentration of wheat grain can be increased by more than 20 times without causing an adverse impact on seed germination or seedling growth [Paull et al., 1992]. It was found that B deficiency symptoms are generally associated with leaf B concentrations of less than 1 mg kg⁻¹ dry matter in monocots such as wheat compared to less than 10 mg kg⁻¹ dry matter in dicots [Bell, 1997], and the critical toxicity concentrations in wheat range from 100 to 270 mg kg⁻¹ dry matter [Paull et al., 1992]. According to the literature, durum wheat species were reported to be more sensitive to B toxicity than most bread wheat cultivars although cultivars showed some variation [Kalayci et al., 1998; Karaman et al., 2012]. Moreover, it is worth recalling that phloem mobility of B is very low in wheat [Brown et al., 2002].

1.8. Arbuscular Mycorrhizae and *Piriformospora indica* as Beneficial Fungi

In the majority of terrestrial plants, associations of plant roots with beneficial fungi are quite prevalent. Mutualistic soil fungi assisted ancestors of today's vascular plants in colonizing the earth approximately 400 million years ago and the associations are based on the net benefit of both partners, [Selosse and Le Tacon, 1998; Reinhardt, 2007].

The term mycorrhiza, defining many and various root-fungus associations, and consists of a combination of the Greek words for “fungus” and “root” [Bonfante and Anca, 2009]. The “roots” of fungi are called hyphae, and mycorrhizal fungi thrive through the soil by using their hyphae. Hyphae are exceptionally filamentous, and they explore every interstice between and into soil particles to extract water, absorb mineral nutrients and hydrolyze organic matter. With respect to the nature of the physical contact between mycorrhizal fungi and their host plants, mycorrhizae are divided into two major types: ectomycorrhizae and endomycorrhizae. In ectomycorrhizae (ECMs), there are namely two main characteristics which are intercellular hyphal invasion of the root cortex and dense mycelial sheaths around the roots. Moreover, ECMs are limited to generally temperate forest trees and shrubs. In contrast, in endomycorrhizas, i.e., arbuscular (AMs), orchid, monotropoid, and

ericoid mycorrhizas, the fungi form external hyphal networks in the soil and penetrate the root cells intracellularly to establish symbiosis regardless of the plant host. Orchid and ericoid mycorrhizas are limited to the family Orchidaceae and, the order Ericales, respectively [Smith and Read, 2010]; however, AMs are common among various plant taxa [Bonfante and Genre, 2008].

AMs, belonging to the phylum Glomeromycota, are known to colonize the roots of more than 90% of vascular plants and over 80% of land plants. Therefore, AM symbiosis is considered to be a largely promiscuous association [Wang and Qui, 2006; Smith and Read, 2010]. Moreover, AM fungi are soil-inhabiting and nutrient-gathering obligate symbionts of most plant species. Hereby, they can only be cultured in the presence of their host. To establish a symbiotic interaction, AM fungi intracellularly form a unique structure called arbuscules located inside the root cortical cells of plants [Harrison, 2012; Gutjahr and Parniske, 2013]. In AM fungi, the diameter of the hyphae ranges from 2–20 μm , and they can be extended up to 25 cm from the root surface for increasing the nutrient and water uptake efficiency. AM fungi are highly variable in their characteristics but typically also quite adaptable to diverse soil and climatic conditions [Jansa et al., 2003; Munkvold et al., 2004].

The primary role of AMs is considered to contribute to plant nutrition, especially phosphorus (P) which is often the main controlling factor in the plant–fungal relationship and micronutrients, particularly in nutrient-depleted hostile environments. By increasing uptake of relatively immobile phosphate ions, AMs provide primary benefit to their host thanks to the ability of the fungal extraradical mycelium (i.e., extensive network within the soil) to enlarge beyond the phosphate depletion zone developing quickly around the roots [Koide and Li, 1991; Marschner, 2011]. For example, Das et al. have reportedly found that in rhizosphere, alkaline phosphatase and acid phosphatase enzyme levels were increased by *P. indica* which results in higher phosphate uptake by plants [Das et al., 2014]. Other than P, AMs increase the uptake of other macronutrients such as N, K, and Mg [Clark and Zeto, 2000; Hodge et al., 2001; Gosling et al., 2006]. In return, AM fungi receive carbon (C) provided by the host. Secondary roles of the fungus to the host have been characterized as follows: improved drought resistance [Augé et al., 1994], increased tolerance of salinity and heavy metals [Feng et al., 2002;

Mohammad et al., 2003], increased resistance to soil pathogens [**Lingua et al., 2002; Pozo et al., 2002**] and increased resistance to foliar-feeding insects [**Gange and West, 1994**]. Moreover, AMs have been indicated to have a major role in maintaining soil aggregate stability [**Tisdall, 1997; Degens et al., 1996**].

Piriformospora indica (synonym *Serendipita indica*) is analogous to AMs in many functional, morphological, and growth-promoting characteristics but it is not classified among AM species [**Rai and Varma, 2005; Varma et al., 2012**]. As a root-colonizing fungus, the filamentous fungi *P. indica* can be easily cultured axenically in contrast to AM fungi and grows on a wide range of media [**Varma et al., 1999**]. *P. indica* asexually forms pyriform spores called chlamydospores. Aptly named '*Piriformospora indica*' is based on its typical chlamydospores (pyriform means pear-shaped) [**Verma et al., 1998**]. *P. indica*, a multifunctional and versatile root endophytic fungus, belongs to the order Sebaciniales from the mushroom-forming Basidiomycetes (Agaricomycotina) and was originally found in the Indian Thar desert which is the western part of Rajasthan-a typical desert region of the Indian subcontinent- in the year 1997 [**Verma et al., 1998; Weiss et al., 2004; Hibbett et al., 2007**].

Plants colonized by *P. indica* show a wide range of beneficial effects including improvement of growth [**Lanza et al., 2019; Kundu and Vadassery, 2022**], enhancing yield [**Waller et al., 2005; Gosal et al., 2009**], plant tolerance to major biotic and abiotic stresses [**Waller et al., 2005; Oelmüller et al., 2009; Ghorbani et al., 2018**], water and nutrient uptake [**Goh et al., 2013**], and enhanced adventitious root formation in cuttings [**Zuccaro et al., 2011**].

1.9. Structure, Life Cycle, Host Range and Growth Conditions of *P. indica*

Even though *P. indica* displays physiological, morphological, functional, and serological similarities with AM fungal genera (i.e., *Glomus* and *Gigaspora*), it is not included among zygomycetous AM fungi. This is because the large subunit rRNA sequence is different and dolipore septa are present [**Varma et al., 2013**]. As a root-colonizing fungus, the filamentous fungus *P. indica* can be easily cultured axenically and grows on a wide range of synthetic simple and complex media in contrast to AM fungi. In other words, without the requirement of the living organs/tissues of the host

plant, mass cultivation of *P. indica* is facilitated via the media [Varma et al., 1999; Hill and Kaefer, 2001]. *P. indica* morphologically forms hyphae which possess thin walled, irregularly septate, multinucleate (i.e., multiple nuclei in each hyphal segment), white, and almost hyaline characteristics. Hyphae of *P. indica* frequently display anastomosis which is defined as fusion between branches of the different or same hyphae and typically have different diameters between 0.7 and 3.5 μm . At their tips and/or at the intercalary regions, the branching hyphae bear -either individually or in clusters- multinucleate (8–15 nuclei), large pear-shaped chlamydospores which are propagated in the root cortical cells and later occur in soil. The chlamydospores were measured 16–25 μm in length and 10–17 μm in width. Nevertheless, the clamp connections, hyphal knots, and sexual structures of this fungus have not to be documented so far [Varma et al., 2001; Varma, 2008].

The chlamydospores that germinate in the soil around the host roots lead to infection of the host roots. Inter and intracellular hyphae formations take place in the root cortex, and these hyphae formations frequently differentiate into dense hyphal coils and chlamydospores in higher plants. Similar to AM fungi, hyphae of *P. indica* grow and spread only within the host cortical tissues, and never crossing the endodermis. Moreover, the aerial parts of the plant -stem and leaves- are not invaded by the hyphae [Singh and Varma, 2000; Varma et al., 2001].

Being a versatile root endophyte, *P. indica* can infect a variety of host plants, including dicots and monocots such as herbaceous plants, shrubs, and tree species like poplar [Pham et al., 2004; Prasad et al., 2005]. *P. indica* infects plants which are economically important cash crops including wheat, maize, and sugarcane [Waller et al., 2005; Baltruschat et al., 2008] and medicinally important plants such as *Aloe vera*, *Artemisia*, *Bacopa*, *Adhatoda*, *Coleus*, *Abrus*, *Chlorophytum*, *Withania*, *Spilanthes*, and *Tridax* [Rai et al., 2001; Kumari et al., 2004; Oelmüller et al., 2009]. Moreover, legume crops (e.g., *Glycine max*, *Pisum sativum*, and *Nicotiana tobaccum*), Chenopodiaceae and Brassicaceae family members (e.g., spinach, mustard, and cabbage) and even the non-mycorrhizal model species *Arabidopsis thaliana* are infected and benefited by *P. indica* [Kumari et al., 2003; Peškan-Berghöfer et al., 2004].

Depending on the nutrient composition of the culture medium and the cultivation conditions (solid or liquid media), the mycelium morphology of the root endophyte

highly varies. Much of the mycelium of the endophyte grows under the surface of the agar media. When cultivation is carried out using solid media, *P. indica* forms only a few aerial hyphae. *P. indica* concentrically forms the mycelium that covers homogenously the agar media. Moreover, in Petri dishes, the growth of the mycelium sometimes occurs as rhythmic rings. The optimum temperature and pH of fungal growth are 28-30°C and 5.8, respectively and in a broad sense, the temperature range is between 20 and 35°C. Within cultures, the mycelium is not known to form morphologically fruit bodies or basidia [Varma et al., 2013].

1.10. The Effects of Arbuscular Mycorrhizae and *Piriformospora indica* on ROS Generation and Scavenging under Stress Conditions

Under biotic and abiotic stress conditions, AMF, and *P. indica* have the capability to enhance plant vigour by altering mainly the chemical flexibility via utilizing reactive oxygen species (ROS) generation-scavenging system [Beneventi et al., 2013; Goh et al., 2013; Hashem et al., 2016; Mo et al., 2016]. Reactive oxygen species can be generated in normal metabolic processes, for example, as a result of electron transport chains in chloroplast and mitochondria. Both radical and non-radical forms of ROS are present. Nevertheless, under unfavourable conditions encompassing abiotic and biotic stresses, ROS generation can considerably be enhanced at the cellular level [Apel and Hirt, 2004; Gill and Tuteja, 2010; Rasool et al., 2013]. Superoxide radical ($\cdot\text{O}_2^-$), per-hydroxyl radical ($\cdot\text{HO}_2$), and alkoxy radicals (RO) can be included as major radical forms of ROS whereas hydrogen peroxide (H_2O_2) and singlet oxygen ($^1\text{O}_2$) are present in non-radical molecular form. Because of their highly reactive nature, radical forms of ROS are relatively more aggressive than non-radical ones [Gill and Tuteja, 2010; Sewelam et al., 2016].

Reactive oxygen species scavenging system possesses two main components in plants which are enzymatic and non-enzymatic systems. The main enzymatic components can be listed as catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), ascorbate peroxidase (APX), peroxidase (POX), guaiacol peroxidase (GPX), and finally monodehydroascorbate reductase (MDHAR). Furthermore, the main non-enzymatic components encompass glutathione (GSH) and ascorbic acid (AsA) which are defined as antioxidant metabolites [Apel and Hirt,

2004; Gill and Tuteja, 2010; Rasool et al., 2013]. In plants, the principal components of ROS generation system particularly are NADPH oxidases and respiratory burst oxidase homologs [Suzuki et al., 2013; Kadota et al., 2015]. The studies reported that the initial generation of ROS occurs to perform as long-distance signals in response to stress to induce the plants for stress adaptation [Mittler et al., 2011; Sewelam et al., 2016]. Additionally, during the initial stages of symbiotic interactions between roots and mycorrhizal fungi, ROS are credibly reported to be generated [Fester and Hause, 2005; Tanaka et al., 2006; Puppo et al., 2013; Espinosa et al., 2014; Kiirika et al., 2014]. The accurate balance between ROS production and ROS-scavenging pathways must be maintained in plants in order to efficiently benefit from ROS as a signalling molecule, and finally alleviate the potentially toxic and destructive effects of ROS [Mittler, 2002; Baxter et al., 2014]. Redox homeostasis, antioxidant signalling, and constant production/scavenging of ROS are cellularly among the stress signals of plants [Bose et al., 2014; Jajic et al., 2015]. Nevertheless, plants' neutralization capacity of excess ROS can be hampered by the intensity or prolonged duration of biotic and abiotic stresses, thereby leading to oxidative stress and ultimately impair crucial metabolic processes and viability at the cellular level [Gill and Tuteja, 2010; Barna et al., 2012; Nath et al., 2016].

Reports of *Piriformospora indica*-mediated stress tolerance have been presented for numerous crops, encompassing wheat, maize, barley, tomato, and lentil [Waller et al., 2005; Deshmukh and Kogel, 2007; Serfling et al., 2007; Kumar et al., 2009; Sarma et al., 2011; Dolatabadi et al., 2012]. Moreover, it was suggested that crucial mechanisms behind *P. indica*-mediated enhanced stress tolerance in wheat, barley, and maize were the control of ROS production and the modulation of critical antioxidant defense system components [Waller et al., 2005; Serfling et al., 2007; Kumar et al., 2009]. Baltruschat et al. focused particularly on antioxidants as they investigated the biochemical pathways underlying *P. indica*-mediated salt tolerance in barley [Baltruschat et al., 2008]. According to the results of this study, under salinity stress conditions, the plants inoculated with *P. indica* showed relatively higher plant growth, increased ascorbic acid, and elevated antioxidant activities compared to the control group. Moreover, the findings from this study have suggested that plant tolerance (both inherited and endophyte-mediated) to salinity might be because of antioxidants as reported. Another study conducted to reveal the

mutualistic symbiosis of *P. indica* with barley showed that the endophytic fungus causes host cell death for proliferation in differentiated barley roots [Deshmukh et al., 2006]. Moreover, during initial microbial invasion of host plants, one of the characteristics of the early host-defence mechanism is ROS generation. The hypersensitive reaction and cell death can additionally occur at the interaction region [Puppo et al., 2013].

An antioxidant is a substance that considerably prevents or delays oxidative damage to the oxidizable substrates from oxidizing agents (i.e., ROS) [Halliwell and Gutteridge, 2015; Halliwell, 1990]. Almost every component of living tissues, particularly proteins, lipids, carbohydrates, and DNA, is considered as an oxidizable substrate [Halliwell et al., 1995]. To evaluate a compound as an antioxidant, some criteria must be met in terms of chemical and biochemical aspects. Plants have evolved different phytochemicals and enzymes aiming to be equipped with effective antioxidant defence systems in order to scavenge the ROS and protect plants from harmful reactions, thereby sustaining growth and metabolism even under stressful conditions where ROS production is increased [Oven et al., 2002; Blokhina et al., 2003; Bhattacharjee, 2005; Smirnoff, 2005; Inze and Van Montagu, 2001; Arora et al., 2002; Mittler, 2002]. The above-mentioned systems encompass hydrophilic (ascorbate, glutathione, phenolic compounds, flavonoids, *inter alia*) and lipophilic (α -tocopherol, carotenoids, lycopene) metabolites with antioxidative properties, protective enzymes (POX, SOD, and CAT) that deal with toxic oxidants directly, and finally enzymes (MDHAR, DHAR, and GR) that assist to sustain the pool of antioxidants in their reduced state [Pandhair and Sekhon, 2006; Oven et al., 2002]. For instance, the Halliwell-Asada cycle, also known as the ascorbate–glutathione pathway, is catalysed by four enzymes and has been linked to a critical role in the plant's defence pathways [Foyer and Halliwell, 1976; Noctor and Foyer, 1998; Asada, 2006]. In the first step, the hydrogen peroxide, produced under oxidative stress conditions, is scavenged through the oxidation of ascorbate by the action of APX. This enzyme carries out the oxidation of ascorbate to monodehydroascorbate, which can be turned into ascorbate by the action of MDHAR, subsequently. In order to prevent monodehydroascorbate from escaping this cycle, it is swiftly turned into dehydroascorbate which is then converted back to ascorbate via the agency of DHAR. Finally, GSH is employed by DHAR to be converted to glutathione disulfide

(GSSG) which is ultimately used by GR to regenerate GSH [Foyer and Halliwell, 1976; Murshed et al., 2008].

Total antioxidative capacity can be directly assayed via procedures based on the capacity to inhibit the oxidation of a substance. The Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) equivalent antioxidant capacity (TEAC) is considered one of the most common and direct procedures used for this purpose [Miller et al., 1993; Erel, 2004; Re et al., 1999; Rubio et al., 2016].

1.11. The Effects of Arbuscular Mycorrhizae and *Piriformospora indica* About Plant Growth-Promoting Activity and Nutrient Uptake under Stress Conditions

According to experimental input by Kumar et al., the plants inoculated with *P. indica* showed enhanced resistance against abiotic stress and under stress conditions, the plant growth promotion which is one of the typical effects of *P. indica* could also be monitored [Kumar et al., 2012]. Even prior to detectable root colonization, root growth promotion effect of *P. indica* was showed which may be due to the increased nutrient -notably P- availability and high amount of nutrient transfer to the aerial parts of the inoculated plant or seedlings [Shahollari et al., 2005]. Nevertheless, according to another study conducted by Barazani et al., the reason for visible improved vegetative or reproductive performance is not always because of the enhancement of nutritional status in *P. indica* inoculated plants (P and N status) [Barazani et al., 2005].

1.12. Nutrient Uptake

Thus far, under AM colonization, the uptake of several micro- and macro-nutrients has been monitored and reported to be increased, thereby improving the biomass production of the plants [Chen et al., 2017]. Association with AM does not only enhance the availability of macronutrients such as N, P, Potassium (K) and Calcium (Ca), but also that of some micronutrients such as Zinc (Zn) and Copper (Cu). As a result of that, the leaf surface area and photosynthetic activity may be increased [Jajoo and Mathur, 2021]. Even under adverse conditions, additional nutrients and water are provided by AM to root cells [Smith and Read, 2010]. The hyphal surface

area (ca. 100 folds) and length are considerably higher compared to the those of the plant's root system [Rabie, 2005].

Like AM fungi, the ability of *P. indica* to extract, mobilize, and transport two main macronutrients (i.e., P and N) as well as a number of micronutrients from the soil has been well documented [Yadav et al., 2010; Hibbett et al., 2007; Qiang et al., 2012; Weiss et al., 2004; Gosal et al., 2013]. *P. indica* actively carries out the transfer of these nutrients through plant-fungal interfaces to the infected-host plant [Gosal et al., 2013].

1.12.1. Macronutrient Uptake

Various studies have showed that AM can considerably increase the uptake of different nutrients especially P via hyphal structures and some other mechanisms encompassing the generation of enzymes like phosphatases [Miransari, 2011], so that availability of phosphorus is increased by AM to the host plants under P deficient conditions. For example, thanks to AM inoculation, maize plants displayed higher inorganic P absorption compared to corresponding control plants [Garces-Ruiz et al., 2017]. A research paper has reported that AM led to enhanced N, P, and Fe concentrations in *Pelargonium graveolens* L. under drought stress [Amiri et al., 2017]. These results are consistent with another research's results conducted on euonymus (*Euonymus japonica* Thunb.) plants under salinity stress. AM-colonized plant showed increased P, Ca, and K concentrations under such conditions [Gomez-Bellot et al., 2015]. Besides that, AM enhanced the seedling weight through enhancing water, intercellular CO₂, P, and N contents in *Leymus chinensis* so that it caused increase in photosynthesis, subsequently [Lin et al., 2017].

Arbuscular mycorrhizae play a fundamental role in the N cycle [Hodge and Fitter, 2010]. Inorganic N can be absorbed and assimilated by the extra-radical hyphae. A significant percentage of the total uptake of N (ca. 20–75%) can be transferred to their hosts via AM [Govindarajulu et al., 2005]. The plants inoculated with AM are rich in N content that ultimately culminate in enhanced chlorophyll content in their hosts [De Andrade et al., 2015]. Moreover, a study showed that a plant ammonium transporter, which is activated in the availability of AM fungi, has been identified.

This result indicated that the way of N transfer to the plant may be analogous to P transfer [Guether et al., 2009].

Because high amounts of phosphatase enzymes are produced by *P. indica*, this root endophyte has been accepted as a potential phosphate mobilizer and the mechanism of this enzyme is to hydrolyze insoluble polyphosphates and organic phosphates by cleaving phosphate ester bonds in short [Yadav et al., 2004]. Moreover, via interacting/communicating with a variety of rhizobacteria which are able to dissolve inorganic phosphate because of producing a range of organic acids, mobilization of P-reserves can be indirectly carried out by *P. indica* [Singh et al., 2009]. In contrast to AM, because it is known that nitrogen is preferentially absorbed as ammonium rather than nitrate from the soil to the host plants by AM, *P. indica* also mediates nitrate uptake from the soil [Sherameti et al., 2005].

1.12.2. Micronutrient Uptake

To sustain plant growth and reproduction, plants essentially require some elements in very small quantities. Those elements are called micronutrients and include boron, iron (Fe), Zn, manganese (Mn), Cu, molybdenum (Mo), and nickel (Ni) [Marschner, 2011]. Most micronutrients function as cofactors for various enzymes and enzyme complexes and play a greater role as crucial components of the proteins working the electron transport system [Varma et al., 2013]. Arbuscular mycorrhizae increase the availability of micronutrients (i.e., Zn and Cu) and regulates their translocation to avoid toxicity (i.e., Cadmium (Cd), Mn, and Zn) within the host plants [Latef et al., 2016; Li and Christie, 2001; Kamal et al., 2010], thereby contributing to metal homeostasis and overall plant performance [Liao et al., 2003]. On the other hand, in some cases, on heavy metal-contaminated soils, AM do not only enhance the transport of nutrients into the host but can also preserve their host plants from toxicity by reducing the uptake of heavy metals [Li and Christie, 2001; Kamal et al., 2010]. Extraction, mobilization, and translocation of the micronutrients from the soil such as iron, zinc, manganese, and copper, is carried out by *P. indica* similar to mycorrhiza. As a result, the root endophyte makes these micronutrients available to the plant [Gosal et al., 2010; Achatz et al., 2010; Gosal et al., 2008; Gosal et al., 2007].

1.13. The Effects of Arbuscular Mycorrhizae and *Piriformospora indica* on Osmolyte Accumulation

Osmolytes are composed of three major groups encompassing sugars and sugar alcohols (e.g., inositol and trehalose), ammonium compounds (e.g., glycine betaine and polyamines), and amino acids (e.g., proline and ectoine) [Omari Alzahrani, 2021]. Studies on the physiological, biochemical, and molecular levels conducted *in vivo* and *in vitro* demonstrate that osmolytes are crucial components of the plant's stress response system [Singh et al., 2015]. Under osmotic stress conditions, osmolytes accumulate in plant cells and enable continued water uptake and improved water balance through osmotic adjustment. As a result, the accumulation of osmolytes sustains cellular turgor [Hossain et al., 2019]. However, osmolytes do not only serve osmotic adjustment but may provide additional benefits for plants under stress, including but not limited to ROS scavenging, stabilization of membrane structures and protection of the hydration shells of proteins and thus protein structures [Dar et al., 2016; Adejumo et al., 2021; Hossain et al., 2019].

1.13.1. Biosynthesis and Accumulation of Proline in Plants

Proline, a proteinogenic amino acid with exceptional conformational rigidity, is regarded as one of the most effective osmoprotectants (i.e., compatible solutes) and signaling molecules and it is crucial for primary metabolism both in the form of free amino acids and as a component of proteins [Singh et al., 2015; Burritt, 2012; Hosseinifard et al., 2022]. Under various stress conditions, free proline accumulation is also advantageous to plants and in addition to being a great osmolyte and a signaling molecule, serves two crucial functions during such conditions i.e., as an antioxidative defense molecule, and a metal chelator [Dar et al., 2016; Adejumo et al., 2021]. According to numerous studies, proline accumulation and increased plant stress tolerance appear to be positively correlated [Sadeghipour, 2020; Elewa et al., 2017; Qirat et al., 2018]. Proline accumulation occurs in the cytosol without damaging cellular structures and this is an essential component of the physiological response in many plant species, under stress conditions [Hayat et al., 2012; Dar et al., 2016; Adejumo et al., 2021; Hosseinifard et al., 2022].

Proline can improve protein stability and maintains membranes integrity via binding to hydrogen bonds [Hossain et al., 2019]. Protection of cells can be carried out by

proline through improving water uptake potential and promoting activation of enzymes [Burritt, 2012]. In addition to aforementioned roles, the other roles of which are being a ROS scavenger and being an inhibitor of programmed cell death [Dar et al., 2016; Adejumo et al., 2021]. Many studies have shown that the stress tolerance of plants can be improved by exogenous proline through triggering stress prevention mechanisms [Butt et al., 2016; Orsini et al., 2018; Priya et al., 2019].

Under B toxicity, an increase in proline level and elevated H₂O₂ accumulation was considered a typical response of plants [Karabal et al., 2003]. Proline has also an activator role in the Krebs cycle reactions; therefore, it leads to an increase in the photosynthesis reactions and energy flow of plants, subsequently [Gunes et al., 2006]. For example, increased soil B levels were followed by a significant increase in total proline and total protein contents in grape (*Vitis vinifera* L.) plants [Sarabandi et al., 2019]. Besides that, high levels of B lead to proline accumulation in wheat [Gunes et al., 2007; Metwally et al., 2012]. *P. indica*-colonized wheat plants were reported to exhibit higher proline content under drought stress condition compared to the uncolonized plants [Zarea et al., 2012]. Similarly, under salinity stress, *P. indica*-colonized rice plants (*Oryza sativa* L.) were found to show elevated antioxidant activity and proline content [Bagheri et al., 2013].

1.13.2. Biosynthesis and Accumulation of Glycine Betaine (GB) in Plants

Glycine betaine (N,N,N-trimethylglycine), one of the many quaternary ammonium compounds found in many plants, is produced in the greatest amounts against abiotic stress, particularly in response to dehydration stress; therefore, this compatible solute has been defined as an effective osmolyte against such harsh environmental conditions [Venkatesan and Chellappan, 1998; Mansour, 2000], [Mohanty et al., 2002; Yang et al., 2003; Fariduddin et al., 2013]. Glycine betaine is a compound possessing water soluble and non-toxic characteristics [Yildirim et al., 2015]. GB is prevalent primarily in chloroplasts, where it is essential for thylakoid membrane adjustment and protection, thereby sustaining photosynthetic efficiency [Robinson and Jones, 1986; Genard et al., 1991]. Production of GB occurs in chloroplast in higher plants from serine via other compounds i.e., ethanolamine, choline, and betaine aldehyde [Hanson and Scott, 1980; Rhodes and Hanson, 1993]. The

production of GB is carried out by two enzymes i.e., choline monooxygenase (CMO), and betaine aldehyde dehydrogenase (BADH), respectively. The former is used to alter choline into betaine aldehyde which is subsequently altered to GB by the latter [Ashraf and Foolad, 2007]. Even though other pathways (e.g., direct N-methylation of glycine) have also been identified thus far, the above-mentioned pathway (i.e., choline to glycine betaine) has been reported in all GB-accumulating plant species [Weretilnyk et al., 1989].

Plant species have been found to exhibit diversity in their capacity of glycine betaine production. For example, as compared to the plants like rice (*Oryza sativa* L.), mustard (*Brassica* spp.), Arabidopsis (*Arabidopsis thaliana* (L.) Heynh.) and tobacco (*Nicotiana tabacum* L.) which naturally do not produce GB under stress or non-stress conditions [Rhodes and Hanson, 1993], numerous crop plants including sugar beet (*Beta vulgaris* L.), spinach (*Spinacia oleracea* L.), barley (*Hordeum vulgare* L.), wheat (*Triticum aestivum* L.), maize (*Zea mays* L.), cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme*), tomato (*Solanum lycopersicum* L.), and sorghum (*Sorghum bicolor* (L.) Moench) have been found to accumulate relatively higher level of GB in their chloroplast in response to stress [Weimberg et al., 1984; Fallon and Phillips, 1989; McCue and Hanson, 1990; Rhodes and Hanson, 1993; Yang et al., 2003; Ali et al., 2015; Al Hassan et al., 2015; Sozharajan and Natarajan, 2016; De la Torre-González et al., 2018]. By promoting the activities of various antioxidant enzymes i.e., POX, CAT and SOD, GB activates the plant's antioxidant system, which ultimately preserves the plant from oxidative damage [Yadu et al., 2017]. Thus far, it has been thoroughly researched and documented in numerous studies that GB can stimulate the antioxidant machinery in various plant species under numerous stressful conditions [Hisyam et al., 2017; Yadu et al., 2017]. Moreover, GB has been widely utilized in fortifying plants under a variety of stressful conditions i.e., drought, salinity, and heavy metals (HMs) [Khan et al., 2018; Liu et al., 2017; Ali et al., 2015; Farooq et al., 2016; Jabeen et al., 2016; Xalxo et al., 2017]. For instance, according to Nusrat et al., via stabilizing the proteins, scavenging ROS, and protecting the photosynthetic process, application of GB alleviated the HMs on pea plants (*Pisum sativum* L.) [Nusrat et al., 2014]. There are some studies conducted to investigate the effect of *P. indica* in terms of GB. For example, according to Ghorbani et al., *P. indica* caused to increase in proline and GB

accumulation in the roots thereby alleviating the toxic effects of salinity in tomato plants (*Solanum lycopersicum* L.) [Ghorbani et al., 2018]. Different research papers have also reported that *P. indica*-infected plants have relatively high levels of glycine-betaine, proline, and some plant hormones (i.e., salicylic acid, gibberellic acid) which enhances the plants' resistance to the negative effects of environmental stresses, particularly salinity [Ghorbani et al., 2018; Khalid et al., 2019]. There are studies showing that the colonization with *P. indica* inhibits osmotic stress-related damage via accumulation of glycine-betaine and proline thereby promoting plant growth and survival under stress conditions [Waller et al., 2005; Gill et al., 2016].

According to a study conducted on maize (*Zea mays* L.) plants, marked increases in leaf glycine-betaine and proline contents were induced by B stress [Nawaz et al., 2020]. However, another study conducted on two different wheat (*Triticum aestivum* L.) cultivars under B toxic conditions reported that proline and glycine betaine contents were not considerably altered in leaf and root tissues of both cultivars by B toxicity [Kayihan et al., 2017].

1.14. The Effects of Arbuscular Mycorrhizae and *P. indica* on Membrane Integrity

Malondialdehyde (MDA) content, one of the most frequently used oxidative damage biomarker, is formed from polyunsaturated fatty acids located in plant cellular membranes after degradation by ROS induced by stress; therefore, it causes downstream reduction in the content of unsaturated fatty acid. In other words, the degree of membrane damage and lipid peroxidation level are revealed by the MDA contents, and elevated levels of free radicals lead to overproduction of MDA in plant cells [Sun et al., 2010; Del Rio et al., 2005; Meloni et al., 2003; Verma and Mishra, 2005; Ma et al., 2015; Tyagi et al., 2022; Hu et al., 2012; Li et al., 2013; Yonny et al., 2017]. Moreover, because of its reaction with lipids, nucleic acids, glucose and proteins, MDA leads to decline in the membrane resistance and fluidity along with the content of unsaturated fatty acid. On the other hand, the electrolyte leakage level is increased, and electrolyte leakage is another indicator for membrane integrity loss [Beltrano and Ronco, 2008; Ma et al., 2015; Tyagi et al., 2022]. Consequently, in plant cells, the structure and function of cell membrane are harmed, and a plethora of physiological metabolisms are changed [Scandalios, 1993].

The link between *P. indica* or AMF and malondialdehyde content under stress conditions has been the subject of various studies thus far. For instance, a study conducted by Beltrano and Ronco showed that as compared to non-mycorrhizal plants, mycorrhizal wheat plants (*Triticum aestivum* L.) had significantly lower electrolyte leakage and thereby improving membrane integrity under water stress [Beltrano and Ronco, 2008]. According to the experimental input by Liu et al., the host plants inoculated with AMF (*Funneliformis mosseae*) exhibited significantly reduced level of MDA, H₂O₂, and O₂⁻ compared to corresponding control plants under chilling stress (8°C/4°C) [Liu et al., 2016]. To give another example, the latest studies also showed that *P. indica* inoculation of plants causes an increase in proline content and a reduction in MDA accumulation in corn (*Zea mays* L.) and rice (*Oryza sativa* L.) plants, respectively [Tsai et al., 2020; Xu et al., 2017]. Furthermore, studies reported that *P. indica* causes reductions in the level of electrolyte leakage under drought stress, thereby re-confirming the importance of this fungus in preventing oxidative damage induced by ROS [Tyagi et al., 2017; Ghabooli et al., 2020].

There are also studies conducted to investigate the effect of B toxicity in terms of lipid peroxidation and electrolyte leakage. For example, according to Kaya et al., B toxicity caused an increase in H₂O₂ levels, MDA content, and electrolyte leakage in leaves of maize (*Zea mays* L.) plants [Kaya et al., 2018]. In wheat, a significant increase in H₂O₂ and MDA contents was reported under B toxicity conditions [Eser and Aydemir, 2016]. Moreover, another study conducted by Karabal et al. to investigate the effect of B toxicity on the antioxidant response of sensitive and tolerant barley cultivars (*Hordeum vulgare* L.), showed that compared with controls, MDA contents and electrolyte leakage increased in a dose-dependent manner in leaves [Karabal et al., 2003]. Previously, lipid peroxidation and membrane damage were also reported in onion, tomato, and cucumber, under excessive soil B conditions [Inal and Tarakcioglu, 2001; Alpaslan and Gunes, 2001]. It has been demonstrated that elevated H₂O₂ and lipid peroxidation (MDA) in the leaves under toxic B levels cause oxidative stress [Gunes et al., 2006; Molassiotis et al., 2006; Wang et al., 2011].

1.15. The Relationship Between Fungal Symbionts (*P. indica* and AMF) and Phenylalanine Ammonia-lyase (PAL) Activity

Secondary metabolites (SMs), one of the alternative defence strategies developed by plants, are substances synthesized to cope with stressful conditions and maintain growth and metabolism properly [Bravo, 1998; Baslam et al., 2013]. They possess a significant role in terms of assisting plants to protect themselves against bacteria, viruses, other competing plants, and herbivores including insects, mollusks, and vertebrates [Edreva et al., 2008; Baslam et al., 2013]. Moreover, the synthesis of innumerable SMs, i.e., many phenolic compounds, can be induced and boosted under abiotic and biotic stress conditions including high or low temperatures, high light intensity, UV radiation, drought, mineral deficiencies, salinity, or pathogen infection [Bennett and Wallsgrove, 1994; Baslam et al., 2013].

Phenolic compounds, as one of the most common and widespread groups of substances in higher plants, are quite reactive and associated with a number of processes encompassing protection of cells from adverse conditions such as pathogens, wounds, heavy metals *inter alia* [Harborne, 1980; Dixon and Paiva, 1995; Bravo, 1998; Hamid et al., 2023]. In plants, phenolic compounds are required to fulfil various processes including pigmentation, UV sunscreens, resistance to pathogens, growth, and reproduction. Moreover, protection against oxidative damage is provided by those substances [Lattanzio et al., 2006; Cai et al., 2004; Fu et al., 2011; Tomás-Barberán et al., 2001].

As a result of the phenylpropanoid and flavonoid pathways, phenolic and polyphenol compounds are produced, and those two terms are usually used to refer to all secondary natural metabolites. Phenylalanine ammonia-lyase (PAL), which can be activated by various stresses, is the crucial enzyme in those biosynthetic pathways [McCallum and Walker, 1990; Bravo, 1998; Lattanzio et al., 2006]. In detail, the first committed step, deamination of L-phenylalanine (L-Phe) to generate trans-cinnamic acid, is catalysed by PAL in the biosynthesis of plant phenylpropanoid compounds including lignin, flavonoids and hydroxycinnamic acids [McCallum and Walker, 1990]. Therefore, accumulation of these secondary metabolites correlated with PAL activity in many cases [Zagoskina, 1990; Mizukami, 1993; Olenichenko and Zagoskina, 2005], and the experimental inputs from plethora of plant tissues

have demonstrated that there is a close relationship between the physiological or developmental status of a plant and PAL activity [Jones, 1984]. Antioxidant activity and the presence of phenols in common vegetables and fruits have been demonstrated to be clearly correlated [Cai et al., 2004; Fu et al., 2011; Tomás-Barberán et al., 2001]. It has been demonstrated that phenolics synthesis can be changed or improved by AMF colonization within various host plants such as strawberry (*Fragaria ananassa* Duch.), lettuce (*Lactuca sativa* L.), and olive tree (*Olea europaea* L.) [Ganz et al., 2002; Toussaint et al., 2007; Lee and Scagel, 2009; Castellanos-Morales et al., 2010; Baslam et al., 2013]. Similarly, a study showed that *P. indica* can enhance lignan accumulation with simultaneous improvement in PAL activity in *Linum album* [Kumar et al., 2012]. Moreover, infection of *Aloe vera* plantlets with *P. indica* enhances phenolic compounds (flavonol, flavonoid, and phenol content) of the plants, thereby decreasing the oxidative stress under salinity [Sharma et al., 2017].

The regulation of enzymes involved in phenol metabolism (such as PAL and peroxidase) is one of the essential roles of B in addition to its binding with RGII in the cell wall [Camacho-Cristóbal et al., 2002; Lewis, 1980; Wakabayashi et al., 1997]. A study on flax plants (*Linum usitatissimum* L.) showed that both deficient and toxic B conditions led to high PAL activity which was followed by elevated activity of the peroxidase enzyme [Heidarabadi et al., 2011]. Moreover, peroxidase-mediated cross-linking of several compounds, including lignin and phenolic monomers, which have been linked to a decrease in extensibility, results in cell wall rigidification, thereby causing deceleration of plant growth [Passardi et al., 2004]. According to Sarabandi et al., elevated soil B levels enhanced the content of phenolics and flavonoid in grape (*Vitis vinifera* L.) plants [Sarabandi et al., 2019].

1.16. The Effect of *P. indica* and AMF on the Photosynthetic Pigment Content under Stress

Pigments (such as chlorophylls and carotenoids), serving physiological functions, are run-of-the-mill constituents of plant cells and tissues. Chlorophylls (Chl) are photoreceptors found in chloroplasts that enable plants to absorb energy from light, thereby essential for photosynthesis. Chlorophyll *a* possesses an important role as the primary electron donor in the reaction centre of photosystems and is known as the

unique chlorophyll in higher plants. Moreover, Chl a also aids in transferring energy in the antenna complex together with chlorophyll *b* and carotenoids [Mulabagal et al., 2010; Baslam et al., 2013].

Carotenoids are known as pigmented substances that can be produced by higher plants, algae, fungi, and bacteria. In the course of photosynthesis process, the light is harvested and transferred to the reaction center of photosystems as absorbed energy by carotenoids which are located in the thylakoid membrane of chloroplasts. Furthermore, through dissipating the excess of energy and quenching reactive oxygen species generated under excess light, these organic pigments preserve chlorophyll from photooxidative damage [Demming-Adams and Adams, 1996; Baslam et al., 2013].

Plenty of studies have reported enhanced concentrations of total Chl in leaves of various crops such as onion (*Allium cepa* L.), strawberry (*Fragaria ananassa* cv. Senga Sengana), pepper (*Capsicum annuum* L. cv. Piquillo), and chickpea (*Cicer arietinum* L.) inoculated with diverse AM species from the genus *Glomus* (*G. mosseae*, *G. versiforme*, *G. intraradices*, *G. etonicatum* or *G. deserticola*) [Garmendia et al., 2004; Afek et al., 1990; Borkowska, 2002; Bolandnazar et al., 2007; Sohrabia et al., 2012]. Furthermore, thus far, experimental inputs have clearly demonstrated that metabolic pathways belonging to carotenoids can be stimulated by AMF, thereby enhanced carotenoid content [Walter et al., 2010; Strack and Fester, 2006; Walter et al., 2007; Mena-Violante et al., 2006]. A study conducted with rice plants to investigate the effect of *P. indica* infection under salt stress conditions (*Oryza sativa* L.) showed that *P. indica* colonization significantly enhanced Chl a, Chl b, and carotenoid content as compared to corresponding control plants [Jogawat et al., 2013]. Various papers have also demonstrated that photosynthetic improvements, including but not limited to improvement in chlorophyll levels and the photochemical efficiency of photosystem II, can be responsible for the observed positive effects of *P. indica* under salinity stress [Ghorbani et al., 2018; Zarea et al., 2012; Sharma et al., 2017; Abdelaziz et al., 2019].

2. MATERIALS AND METHODS

2.1. Materials and General Information

2.1.1. Wheat Cultivars Used in the Experiments

A summer bread wheat (*Triticum aestivum* L., cv. Nusrat) and a summer durum wheat (*Triticum durum* Desf., cv. Saricanak-98) were used in this study. Nusrat is a cultivar having white and semi-hard grains, it was bred by Sakarya Maize Research Institute and registered in 2015. Nusrat, with an average yield of 400–950 kg da⁻¹, has moderate rust and powdery mildew resistance. It is a mid-early cultivar which grows to 100–105 cm [Web 2, 2023].

Saricanak-98 was bred by GAP International Agricultural Research and Training Centre and registered in 1998. The cultivar has middle-height, waxy and firm stalk. The leaves have inclined leaf orientation and wide leaf structure. The cultivar has dirty white and yellow spikes and brown awns. Moreover, spikes of the cultivar are perpendicular, and sharp toward the end. Grains are amber (yellow), hard-textured, plump, and glassy. 1000–kernel weight (TKW) of the cultivar varies from 35 g to 45 g. Saricanak-98, with a good tillering habit, is a mid-early cultivar like Nusrat. Moreover, with an average yield of 500–800 kg da⁻¹ at optimum conditions, the cultivar is moderately resistant to yellow rust [Web 3, 2023].

2.1.2. Chemical and Physical Properties of Experimental Soil

The experimental soil that was obtained from location of Tepeören, Tuzla, Istanbul, had a clay-loam texture, low CaCO₃ content (0.3%), low organic matter content (0.9%) and slightly alkaline soil reaction (7.67) with a pH electrode in soil-dH₂O suspension. Saturation paste soil (prior to fertilizer addition) electrical conductivity (EC) was assessed to be 0.02% with an EC electrode in soil-dH₂O suspension. Nutritional status of the soil was as follows: very low N (0.04%), low P (P₂O₅, 0.55%), moderate K (K₂O, 2.53%), high Ca (0.43%), low Mg (0.014%), high Fe (8.55 ppm), sufficient Zn (1.26 ppm), sufficient Cu (6.83 ppm), and sufficient Mn (21.1 ppm).

2.1.3. Glasshouse Conditions

Soil experiments were conducted in the Research and Development Greenhouse at the Institute of Biotechnology of Gebze Technical University (Kocaeli, Türkiye) to study the effects of *P. indica* on the growth, grain yield and mineral balance of wheat grown under B toxicity stress. The greenhouse used in this study is a Venlo-type glasshouse located in Gebze at the following geographic coordinates: 40° 48' 40" N, 29° 21' 29" E. It has automatic climate control (evaporative cooling and heating) and complementary lighting. The daytime temperature was retained at 24±3°C and the nighttime temperature at 20±3°C in the glasshouse throughout the experiment.

2.1.4. Growth Chamber Conditions

Rooting steps of both wheat cultivars for all soil experiments were carried out in a growth chamber with specific climatic conditions (light/dark periods: 16/8 h; temperature (light/dark): 25°C/20°C; relative humidity (light/dark): 60%/70%; photosynthetic flux density: 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$) before being transferred to soil.

2.2. Experimental Designs and *P. indica* Treatments

2.2.1. Preparation of *P. indica* Inoculum for Soil Experiments

2.2.1.1. *P. indica* Growth Condition and Fungus Harvest

P. indica chlamydospores (DSM11827) were provided by Prof. Dr. R. Oelmüller, Friedrich Schiller University Jena (Thuringia, Germany). The chlamydospores were preserved in glycerine at -80°C. For the experiments, the fungus was maintained in axenic cultures and freshly re-isolated from barley and *Arabidopsis thaliana* roots, as described below. *P. indica* was cultured in a modified Kaefer agar medium (KM) according to Hill and Kaefer, incubated at 28°C in the dark for 1 month, and stored at +4°C [Hill and Kaefer, 2001]. In order to propagate the fungus in a liquid medium, 4 fungal plugs (1 cm diameter) were removed from the edge of active colonies and inoculated in Erlenmeyer flasks, including 200 ml of modified KM, composed of: peptone 2 g L⁻¹, casein hydrolysate 1 g L⁻¹, yeast extract 1 g L⁻¹, D-glucose 20 g L⁻¹, 50 ml L⁻¹ of macronutrients solution (NaNO₃ 12 g L⁻¹, KCl 10.4 g L⁻¹, MgSO₄·7H₂O 10.4 g L⁻¹, and KH₂PO₄ 30.4 g L⁻¹), 10 ml L⁻¹ of micronutrients solution [H₃BO₃ 1.1

g L⁻¹, MnSO₄.H₂O 0.37 g L⁻¹, ZnSO₄.7H₂O 2.2 g L⁻¹, CuSO₄.5H₂O 0.16 g L⁻¹, (NH₄)₆Mo₇O₂₄.4H₂O 0.11 g L⁻¹, CoCl₂.6H₂O 0.17 g L⁻¹], 1 ml L⁻¹ of Fe-EDTA solution (17.2 g L⁻¹), agar 10 g L⁻¹. Medium pH was adjusted to 6.5 with KOH (1 N) prior to sterilization (at 121°C for 20 min). Finally, 1 ml L⁻¹ of vitamin solution containing thiamine 100 g L⁻¹, glycine 0.4 g L⁻¹, nicotinic acid 0.1 g L⁻¹, and pyridoxine 0.1 g L⁻¹ were added. The fungus was incubated at 28 °C and 180 rpm for 14-21 days, in an orbital shaking incubator (ZWYR-D2401, LABWIT Scientific, Australia).

For fungus harvest, liquid cultures were centrifuged at 7500 rpm, 20°C for 10 min, and the supernatant was discharged to completely remove the medium. The pellet was resuspended in 3.2 L dH₂O, and the suspension stirred continuously, homogenous suspension aliquots were added by drenching to corresponding pots. Fresh cultures were routinely grown on liquid KM for all soil experiments [Hill and Kaefer, 2001; Das et al., 2012; Bertolazi et al., 2019].

2.2.1.2. Quantification of Spore Density Using Hemocytometer

Pear-shaped chlamydospores of *P. indica* were attached to the mycelium. The spores were dislodged by adding 200 µl of ‘Tween 80’ (2%) solution to 10 ml of the liquid culture, and vigorously vortexed for 2 min. Afterwards, the mixture was sonicated at 30% amplitude for 20 sec on and 30 sec off cycle on dry ice for total 5-8 min. After detachment of the spores, 2 ml of sonicated culture was transferred to Eppendorf (main stock) and gently vortexed. Prior to quantification of spore using a hemacytometer counting chamber (Neubauer improved, Isolab, Eschau, Germany), successive dilutions of the main stock were carried out to monitor and count the spores [Kumar et al., 2011; Singhal et al., 2017].

2.2.2. Maintaining “Root Colonization Efficiency” of *P. indica*

2.2.2.1. Periodic Transference and Re-isolation of *P. indica*

Periodic transference or sub-cultivation is a very straightforward and low-cost method which allows subcultures to live for a longer time (i.e., reaching a survival of 2–30 years depending on the strain). Even if some strains can adapt well to the next

transfer, the disadvantages of this technic are genetic, morphological, and physiological changes and risk of contamination in each sub-cultivation that are quite prevalent [Castellani, 1939]. Moreover, after repeated subculturing on synthetic medium, the root colonization efficiency, also known as the endophytic capacity, of *P. indica* is lost. To sustain its endophytic capacity, roots of a suitable host plants must be infected with the fungus (in soil or *in vitro*) regularly and, then re-isolated from the colonized roots [Johnson et al., 2011].

In this thesis, *P. indica* was re-isolated from *A. thaliana* plant roots grown *in vitro* and re-isolation procedure was described in the following parts.

2.2.2.2. Growth Conditions of *P. indica*

P. indica was cultured on KM. The medium was poured into sterile Petri dishes (30 ml per plate) and allowed to solidify. Fungal plugs were placed in the center of fresh KM plates. KM plates were incubated at 26-28°C in the darkness for 4 weeks in a temperature-controlled incubator. The fungus was sub-cultured on fresh KM plates every 4 weeks.

2.2.2.3. Seed Surface Sterilization and Growth Conditions of *Arabidopsis thaliana*

Seed surface sterilization was carried out according to Taghinasab et al. with following modifications [Taghinasab et al., 2018]: Seeds of *A. thaliana* were transferred to sterile 2 ml Eppendorf tubes and sterilized with 70% (v/v) ethanol for 1 min and followed by NaCl-Tween 20 solution in a mechanical shaker at 300 rpm for 15 min. In advance, the latter solution was prepared by adding 5 µl tween 20 solution to 20% (v/v) NaOCl (pure) and vortexed vigorously. The seeds were rinsed with sterile distilled water 4 times for 5 min and vortexed softly each time. Sterile seeds were transferred to 1.5 ml Eppendorf tubes prior to addition of sterilized 0.1% agar. A large number of seeds were not used in order not to have problems at sowing stage. To gain suspended and separated seeds in the agarose, the tubes were vortexed diligently and incubated at 4°C for 96 h for cold treatment to ensure uniform germination. The agarose was taken with sterile 0.5 ml Pasteur pipette, and the sterile seeds were slowly placed to seed germination medium one seed per point. The seed germination medium was half strength (2.2 mg/L MS) MS medium, and pH were

adjusted to 5.8 with NaOH prior to the addition of 0.8% agar (Murashige and Skoog 1962) and the medium was autoclaved at 121°C for 20 min. 8-10 sterile seeds were placed in a 500 cm³ transparent Petri dish containing 75 cm³ of MS medium (ca. 30 ml) and the plates were sealed with Parafilm strips. Finally, the cultures were incubated for 25 to 27 days under controlled climatic conditions [light/dark periods: 16/8h; temperature (light/dark): 25°C/20°C; relative humidity (light/ dark): 60%/70 %; photosynthetic flux density: 400 $\mu\text{mol m}^{-2} \text{s}^{-1}$]. Petri dishes were aerated in a laminar flow hood at 2 days intervals to provide oxygen for *Arabidopsis* seedlings.

2.2.2.4. Co-cultivation of *A. thaliana* and *P. indica*

Four 29-31day old (4°C for 96 h followed by 10-14 days in light) seedlings were transferred on the top of MS plates. The seedlings were kept 2 cm apart. 5 mm diameter fungal plugs or discs were made from 4-week-old fungal plates with a sterile cork borer or Pasteur pipette. 5 mm diameter of MS medium discs was cut with a sterile lancet prior the transfer of the seedlings. Afterward, the fungal plugs with the medium were placed to the 5 mm diameter gap about 1 to 1.5 cm away from the roots. Two or four seedlings were used per Petri dish in this study and fungal plugs were kept between the roots of the plants. The Petri dishes were sealed with Parafilm strips. The plates were incubated for 24 to 26 days under controlled climatic conditions as described above. Petri dishes were aerated at 2 days intervals to provide oxygen for both *Arabidopsis* seedlings and *P. indica* [Johnson et al., 2011].

2.2.2.5. Root Surface Sterilization of *Arabidopsis* Seedlings and Re-isolation of *P. indica*

Roots of *Arabidopsis* seedlings were cut 3 cm long and washed with sterile distilled water to remove attached MS medium. The roots pieces were placed to 2 ml eppendorf tubes prior to addition of 70% (v/v) ethyl alcohol and kept 2 min. Afterwards, the root pieces were submerged in 20% (v/v) NaOCl for 20 min, and thoroughly rinsed with sterile distilled water 3 times for 5 min. Root pieces were placed on KM at 27±2°C for 14 days. Mycelial structures of re-isolated fungus were compared morphologically with original culture of *P. indica* and were found to be similar.

2.2.3. Preparation of Plant Material

Bread and durum wheat seeds were germinated (rooting) in perlite moisturized with 4 mM N in the form of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ solution for 5 days under controlled growth chamber conditions.

2.2.4. First Soil Experiment

In this experiment, the aim was to investigate the role of *P. indica* on B toxicity in 2 different wheat cultivars during vegetative growth in both sterile and non-sterile soils under greenhouse conditions. First of all, the experimental soil was thoroughly sieved to obtain finer soil from coarser particles (ca. 160 kg). One-half of the experimental soil and perlite were autoclaved at 121°C for 45 min and at 121°C for 20 min respectively to eliminate the native microorganisms and shortly after air-dried. Prior to the autoclave, the soil and the perlite were moisturized with dH_2O to increase the efficiency of the autoclave operation. Pots were filled with 2.1 kg of either non-autoclaved or autoclaved soil. Before the seedlings were sown, the following mineral nutrients were added to each pot as concentrated solutions and uniformly incorporated into the soil by mixing thoroughly (per kg air-dry soil): 250 mg N in the form of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 100 mg P in the form of KH_2PO_4 ; 10 mg Cl in the form of KCl; 1 mg (control), 10 mg (low), 20 mg (medium) and 30 mg (high) B in the form of H_3BO_3 . To loosen the soil, 150 g of either non-autoclaved and autoclaved perlite (at 121°C for 20 min) were added to corresponding pots and then mixed thoroughly. Transfer of 5-day-old seedlings to the pots and *P. indica* inoculation were carried out as follows: 250 ml of the medium from the top were dislodged and the surface was drenched with 100 ml of inoculum. The seedlings were diligently placed on the surface (10 seedlings /pot), 250 ml-medium was filled back to cover all the roots properly, and the top was drenched again with the same volume of inoculum. Fungus harvest and inoculum preparation for the soil applications were tackled in “*P. indica* Growth Condition and Fungus Harvest”. The soil experiment comprised a fully factorial design with 4 pot replicates per treatment (2 *P. indica* inoculation status, 2 soil sterilization status, 2 wheat cultivars, 4 B toxicity levels, 128 pots in total), and the pots were completely randomized in the greenhouse. Plants were checked daily and irrigated with fully dH_2O water every day or on alternate days throughout the experiment depending on the seasonal conditions

and the biomass of the plants. Pots were manually weeded when needed. The seedlings were thinned to 8 per pot after 14 days. 41 days after sowing (DAS), the experiment was terminated by harvesting the shoots and roots separately.

For elemental analysis, glycine-betaine (GB) determination, and dry matter weight, 4 plants from each pot (out of 8) were harvested by cutting them off at 1-2 cm above the soil level avoiding damage to the root systems. Harvested shoots were dried for 2 days at 70°C, ground, weighed, and analysed for mineral nutrient concentrations and GB determination as described below. Prior to harvest for those analyses, tip segments of the second youngest leaves from each pot were cut to execute relative electrolyte leakage (REL) measurement. The remaining four plants from each pot were used for the measurement of protein concentration and antioxidative enzyme activities; phenylalanine ammonia-lyase (PAL) activity; total chlorophyll and carotenoid amount; trolox equivalent antioxidative capacity (TEAC) activity; free proline content; thiobarbituric acid-malondialdehyde (TBA-MDA) activity as described below. Root samples from corresponding pots were used for both PCR analysis with specific primers and root staining in order to demonstrate *P. indica* colonization in the roots as described following parts.

2.2.5. Second Soil Experiment

This experiment was conducted to evaluate the potential of *P. indica* on grain yield and mineral balance of wheat cultivars under B toxic conditions. There were 3 B levels (1, 10 and 20 mg/kg), the pots were supplied with additional 150 mg N in the form of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$ 44 DAS. The remaining works were carried out in this experiment as described above for the first experiment. The seedlings were thinned to 5/pot 14 DAS. The experiment had a fully factorial design with 4 pot replicates per treatment, (2 inoculation status, 2 wheat cultivars, 3 B toxicity levels, 96 pots in total), and the pots were completely randomized in the greenhouse. The experiment was terminated when the grains reached complete maturity and the plants fully senesced (at least 80%, 111 DAS). The spikes, the shoot (straw) and the roots were harvested separately, and the spikes and the straw samples were dried at 60°C for 48 hours, to evaluate spike and straw dry matter production. Grains were separated from husk by using a thresher to determinate yield per plant, TKW and harvest index (HI), and then the husk samples were combined with the corresponding straw samples.

The grains and straw samples were acid-digested, and the concentrations of nutrients including B, P, K, Mg, S, Ca, Fe, Zn, Mn, and Cu were determined by ICP-OES, as described below. Finally, the roots were stained and monitored according to the procedure as described below.

2.2.6. Third Soil Experiment

In this experiment, the aim was to investigate the interactive effects of *P. indica* and arbuscular mycorrhizae (*Glomus* spp.) on B toxicity tolerance of wheat at vegetative stage in sterile soil. The same wheat cultivars as stated for first and second experiments were grown under greenhouse conditions. The experimental soil was sieved (ca. 320 kg) and the soil and perlite (ca. 20 kg) were autoclaved, and pots were filled with 2.1 kg of sterile soil. Before sowing the seedlings, the following mineral nutrients were added to each pot as concentrated solutions and uniformly incorporated into the soil by mixing thoroughly (per kg air-dry soil): 250 mg N in the form of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$; 20 mg (control) and 100 mg (high) P in the form of KH_2PO_4 ; 1 mg (control) and 20 mg (high) B in the form of H_3BO_3 ; 10 mg Cl in the form of KCl. After the addition of mineral nutrients, 32 gr of mycorrhizal inoculant (Shubhodaya, Cosme Biotech, India) were suspended in 3.2 L dH_2O . 50 ml of the suspension were applied to corresponding pots and mixed thoroughly (i.e., 1 kg soil was received 1 g of mycorrhizal inoculant). The pots were completed with sterile perlite (ca. 150 g), both mediums were thoroughly mixed, and the 5-day-old seedlings were transferred to the pots as described in the first soil experiment. *P. indica* soil application, plant growth and irrigation regime were carried out exactly the same as described for the first soil experiment. The third soil experiment consisted of a full factorial design with 4 pot replicates per treatment. (2 *P. indica* inoculation status, 2 mycorrhizal inoculation status, 2 wheat cultivars, 4 B toxicity levels, 128 pots in total), and the pots were completely randomized in the greenhouse. The seedlings were thinned to 8/pot right after 14 days. The pots were weeded manually when needed. 49 DAS, the experiment was terminated by harvesting the shoots and roots separately. After harvest, shoot and root samples were exposed to the same analyses carried out in the scope of the first soil experiment except for PCR analysis.

2.3. Applied Methods and Analyses

2.3.1. Relative Electrolyte Leakage (REL)

REL of the second youngest leaves from each pot was measured to find out the membrane stability in leaves according to a modified form of published procedures [Tyagi et al., 2017; Lutts et al., 1996]. The leaves were cut and washed under running dH₂O prior to the following step. In closed vials, the leaf segments with the same length were completely submerged in 50 ml of dH₂O for 6 h at 24-25°C; and electrical conductivity (EC₁) was measured by using an EC meter (Mettler Toledo, Switzerland). After disrupting the cell membrane integrity of the samples by boiling at 95°C for 1 h, the electrical conductivity (EC₂) was again measured. REL was calculated as follows: $REL (\%) = (EC_1/EC_2) \times 100$.

2.3.2. Dry Weight of Plants (Dry Matter Weight)

Plants were harvested by cutting them from 1-2 cm above soil level avoiding damage to the root systems. 4 out of 8 seedlings were harvested, dried at 70°C for 48 h, and preserved at 24-25°C until a constant weight was obtained (1-2 h) then weighed analytically.

2.3.3. Protein and Antioxidative Enzyme Assays

Approximately 0.5 g of pulverized (in liquid N₂) fresh leaf samples from the first fully expanded leaves were homogenized with 5 ml of a chilled 50 mM potassium phosphate (K-P) buffer using a Retsch MM 400 shaker-type ball mill at 30 Hertz for 30 sec. The K-P buffer was prepared by mixing 50 mM KH₂PO₄ and 50 mM K₂HPO₄ (pH:7.6), prior to adding 0.1 mM Na-EDTA (Titriplex-III) to this mixture for the extraction step. The homogenates were transferred to centrifuge tubes and centrifuged at 4°C in for 15 min and at 8,000×g. The supernatants were used for protein and enzyme analysis. Protein concentrations in the crude extracts were analyzed spectrophotometrically according to Bradford with some modifications as described below.

2.3.4. Protein Content Quantification by Bradford Method

One of the crude extract aliquots was used that was prepared for the purpose of measuring enzyme activities to carry out total protein content quantification. Total protein concentration was determined photochemically according to the method described by Bradford, modified and linearized by Zor and Selinger. 0.1g of Coomassie® Brilliant Blue G-250 was dissolved in 50 ml of 95% ethanol. 100 ml of 85% (v/v) ortho-phosphoric acid was added to this solution and the resulting solution was then reached to 0.6 L with distilled water. Prior to adding of 100 ml glycerine, the reagent was filtered through a filter paper (Whatman No. 1) and then made up to 1 L with distilled water. Bradford reagent was stored in an amber bottle at +4°C for 24 hours before use. A standard curve was prepared with successive dilutions (0, 100, 200, 400, 800 mg/ml) of bovine serum albumin (BSA), Fraction V in K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA. A sample blank was prepared with 1 ml of K-P buffer. 5 ml of Bradford reagent (1:1 v/v dilution by K-P buffer) was added to 100 µl of enzyme extract or standards and the reaction mixture was vortexed gingerly to avoid foaming. The enzyme extract and standards were measured 10 min right after the vortex. 1 ml of the reaction mixture was read at 590, 595, and 450 nm using a spectrophotometer versus a blank [Bradford, 1976; Zor and Selinger, 1996].

2.3.5. Superoxide Dismutase (SOD) Activity Assay

The SOD activity was assessed photochemically according to the method described by Giannopolitis and Ries. The original method was executed with slight modifications using the assay system consisting of methionine, riboflavin, and NBT (*p*-nitro blue tetrazolium chloride). The method is based upon the inhibition of the photochemical reduction of NBT by SOD. The reaction mixture was composed of 500 µl of 50 mM Na₂CO₃, 500 µl of 12 mM L-methionine, 500 µl of 75 µM NBT, 500 µl of 2 µM riboflavin and the appropriate volume of extract (50 to 100 µl). The total volume was finalized to 5 ml with K-P (pH 7.6) containing 0.1 mM Na-EDTA. After adding the riboflavin to the mixture, the glass vials were shaken diligently and placed under the lights to start the reaction. The vials containing the samples were illuminated for 8 min. A complete reaction mixture without enzyme, which formed maximum colour, served as control and separate controls were used in each set. In

order to stop the reaction, the light was switched off and the tubes placed into dark. A non-irradiated complete reaction mixture was used as a blank. The absorbance was recorded at 560 nm, and one unit of SOD activity was defined as the amount of enzyme which causes inhibition of NBT reduction by 50% in comparison with tubes lacking enzyme. The method is based the reduction of NBT by superoxide (O_2^-) radicals under illumination [Giannopolitis and Ries, 1977; Mengutay et al., 2013].

2.3.6. Glutathione Reductase (GR) Activity Assay

The GR activity was assayed by the decrease (in 30 sec intervals for 2 min) in absorbance at 340 nm according to Foyer and Halliwell with a few modifications. GR activity was determined by recording the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH). The reaction mixture contained 100 μ l of 0.5 mM oxidized glutathione (GSSG), 100 μ l of 0.12 mM NADPH, 50-100 μ l of the enzyme extract and 700-750 μ l of 50 mM K-P buffer (pH 7.6) with 0.1 mM Na-EDTA in a total volume of 1 ml. Corrections were made for the non-enzymatic oxidation of NADPH in the absence of GSSG by observing the decrease of absorbance at 340 [Foyer and Halliwell, 1976; Mengutay et al., 2013].

2.3.7. Peroxidase (POX) Activity Assay

According to the protocol by Hammerschmidt et al., the POX activity was determined. The reaction mixture consisting of 100 μ l of 10 mM H_2O_2 , 100 μ l of 0.25% (v/v) guaiacol, 50 μ l of the crude enzyme solution (1:4 diluted with K-P buffer) and 50 mM K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA was finalized to 1 ml total volume. For each sample, a sample blank was prepared by adding 100 μ l of H_2O_2 , 100 μ l guaiacol and the appropriate amount of K-P buffer. The POX activity was spectrophotometrically measured in 30 sec intervals for 2 min based on the changes in the absorbance at 470 nm and reported as units/mg protein/min [Hammerschmidt et al., 1982].

2.3.8. Ascorbate Peroxidase (APX) Activity Assay

The APX activity was assayed according to Nakano and Asada, based on the decrease in absorbance at 290 nm as ascorbic acid was oxidized. The reaction

mixture contained 650–750 μl of 50 mM K-P buffer, (pH 7.6, prior to addition of 0.1 mM Na-EDTA), 100 μl of 2.5 mM ascorbic acid, 100 μl of 12 mM H_2O_2 and 50-150 μl of the enzyme extract in a total volume of 1 ml. After addition of the enzyme or H_2O_2 , the reaction was started, and the absorbance fall was recorded in 30 sec intervals for 2 min right after addition. Corrections were made for the low, non-enzymatic oxidation of ascorbic acid by H_2O_2 [Nakano and Asada, 1981].

2.3.9. Catalase (CAT) Activity Assay

The CAT activity was determined according to the procedure from Feierabend et al. by monitoring the rate of decomposition of H_2O_2 at 240 nm. The reaction mixture consisting of 100 μl of 100 mM H_2O_2 , 50 μl of the crude enzyme solution (1:4 diluted with K-P buffer) and 50 mM K-P buffer (pH 7.6) containing 0.1 mM Na-EDTA was finalized to 1 ml total volume. For each sample, a sample blank was prepared by adding 100 μl of H_2O_2 and 900 μl of K-P buffer. After addition of H_2O_2 the reaction was started, and the decrease in absorbance was recorded in 30 sec intervals for 2 min at 25°C [Feierabend et al., 1992].

2.3.10. Chlorophyll and Carotenoid Determination

The second fully expanded leaves were freshly used from each pot to calculate total chlorophyll and carotenoids according to Wellburn's method [Lichtenthaler, 1987; Abadi and Sepehri, 2016]. After a fine chopping, portions weighing approximately 0.5 g were measured off on an analytical balance. Fresh leaf specimens were frozen by using liquid nitrogen and then kept at -80°C. Homogenization of frozen leaf specimens was performed by using a ball mill (MM200; Retsch, Haan, Germany), liquid nitrogen and 5 ml of 80% acetone. In this way, a primary acetone extract including all chloroplast pigments was provided. The extract was then centrifuged at 2 times (7500 and 12000 rpm for 15 min at 4°C, respectively). The supernatant was diluted by adding 5 ml of 80% acetone per ml of extract since concentration of pigments was too great for reading to be applied on a spectrophotometer in the most cases. The measurements were carried out by using a UV-Visible spectrophotometer (C-7100S, Peak Instruments Inc., USA). The optical density of the supernatants was read at 470, 663 and 646 nm for Carotenoids, chlorophyll *a* and chlorophyll *b*, respectively. Calculations were performed according to the formula below, where

“Ca” indicates chlorophyll *a*, “Cb” chlorophyll *b* and “Cx+c” carotenoid concentrations (nmol g⁻¹).

$$C_a = (12.25 \cdot A_{663.2} - 2.79 \cdot A_{646.8} \cdot 100) / fw.$$

$$C_b = (21.5 \cdot A_{646.8} - 5.1 \cdot A_{663.2} \cdot 100) / fw.$$

$$C_{x+c} = [(1000A_{470} - 1.82 \cdot C_a - 85.02 \cdot C_b) / 198 \times (100)] / fw.$$

2.3.11. Estimation of Lipid Peroxides

Lipid peroxidation was determined spectrophotometrically using a modified thiobarbituric acid-melondialdehyde (TBA-MDA) assay according to Heath and Packer. To do so, the third and fourth youngest leaves were cut into small pieces and pooled thoroughly. Fresh leaf samples (ca. 0.5 g) were removed from liquid N₂ and quickly ground using a liquid N₂-cooled ball mill (MM200; Retsch, Haan, Germany) at a speed of 30 oscillations per second for 30 second. The homogenization was repeated after adding of 12.5 ml of 80% ethyl alcohol. The leaf homogenates then were clarified by centrifugation (at 4°C for 15 min and at 15,000×g), and aliquots of the supernatant were stored at -80°C. The assay was carried out in two stages.

At the first stage, after adding 1 ml of 20% trichloroacetic acid (TCA) containing 1 ml of 0.01% butylated hydroxytoluene (BHT) to 1 ml aliquot of the supernatant, the mixture was vortexed vigorously and heated at 95°C for 30 min. Afterwards, the mixture was quickly cooled in an ice-bath and centrifuged at 10,000 x g for 10 min.

At the second stage, after adding 1 ml of 20% trichloroacetic acid (TCA) containing 0.65% thiobarbituric acid (TBA) to 1 ml aliquot of the supernatant, remaining steps were carried out to the mixture as explained in the first stage.

Absorbances were read at 440 nm, 532 nm, and 600 nm and corrections were made for interfering compounds. MDA equivalents were calculated in the following manner [Heath and Packer, 1968]:

$$1. [(ABS_{532} + TBA) - (ABS_{600} + TBA) - (ABS_{532} - TBA) - (ABS_{600} - TBA)] = A$$

$$2. [(ABS_{440} + TBA - ABS_{600} + TBA) \times 0.0571] = B$$

$$3. \text{nmol MDA / ml} = (A - B / 157,000) \times 106$$

2.3.12. Digestion and Mineral Analyses

The mineral concentrations in the samples were determined by using inductively coupled plasma optical emission spectrometry (ICP-OES) (Agilent 5800, USA). Prior to acid-digestion (ca. 0.2 g sample in 2 ml 30 % H₂O₂ and 5 ml 65% HNO₃) using a closed vessel microwave system (MarsExpressCEM Corp.; Matthews, NC, USA), dried shoot samples were milled to fine powder with a grinder (MKM6000; Bosch; Germany). Subsequent to acid-digestion step, the final sample volumes were topped up to 20 ml by ultrapure water (EC: 10-15 $\mu\text{S}.\text{cm}^{-1}$) and filtered through quantitative filter paper. To assess the accuracy and precision of the method, the certified standard reference materials obtained from the National Institute of Standards and Technology (Gaithersburg, MD, USA) were used. For instrument calibration, all the standard solutions (1000 mg/L) for B, P, K, Mg, S, Ca, Fe, Zn, Mn, and Cu were spectroscopic grade and obtained from Merck (TraceCERT®, Sigma-Aldrich, Germany).

2.3.13. Glycine-Betaine (GB) Assay

GB determination was executed in dried and finely milled leaf powder [Greive and Grattan, 1983]. Plant samples (ca. 0.25 g) were shaken mechanically with 10 ml of ultra-deionized water for 48 h at 24-27°C. The samples were centrifuged at 8,000 x g for 20 min at 25°C, and then 2 ml of upper phase was stored in freezer until analysis. Dilution of thawed extracts was done with 2 N sulfuric acid at a 1:1 ratio. 0.5 ml aliquot was added into test tube and kept in dry ice for 1 h. 0.2 ml of potassium iodide reagent (cold) was added to the aliquot and then the mixture was gently vortexed. The samples were preserved at 0-4°C for 16 h and transferred to centrifuge tubes to centrifuge at 10,000 x g for 15 min at 0°C for clarification. Upper phase was diligently taken with 1 ml micropipette and 9 ml of 1,2-dichloro ethane was added to dissolve periodide crystals and a robust vortex was carried out. After 2-2.5 h, the absorbance was measured at 365 nm with UV-Visible spectrophotometer. Reference standards preparation of GB (50-200 $\mu\text{g ml}^{-1}$) was carried out in 2 N sulfuric acid, the procedure for sample estimation was followed.

2.3.14. Activity of Phenylalanine Ammonia-Lyase (PAL)

The procedures described by Cheng and Breen and Christopoulos and Tsantili were applied for the extraction and assay of phenylalanine ammonia-lyase (PAL) activity with some alternations. Approximately 0.5 g of pulverized (in liquid N₂) fresh leaf samples from the first fully expanded leaves were homogenized with 5 ml of a chilled acetone using a Retsch MM 400 shaker-type ball mill at 30 Hertz for 30 sec. 25 mM borate buffer (pH: 8.8) was prepared using sodium tetraborate decahydrate and boric acid. The leaf homogenates were kept in a freezer (-18°C) for 15 min and then centrifuged at 4°C for 15 min at 15,000×g. The pellet containing proteins and enzymes was dried and extracted at 4°C by diligent stirring with 25 mM borate buffer (2.5 ml.g⁻¹ fresh weight) containing 2 mM EDTA, and 5 mM β-mercaptoethanol and 1% (w/v) polyvinylpyrrolidone (PVPP). After 1 h, the homogenates were centrifuged as above. 0.2 to 0.5 ml of the buffer supernatant, depending on the PAL activity, was incubated with 1 ml of 10 mM L-phenylalanine solution and 1.8 ml of a 25 mM borate buffer in a total volume of 3 ml at 37°C for 60 min under darkness. The reaction was stopped with 0.5 ml of 6 M HCl. PAL activity was determined by the production of trans-cinnamic during incubation, and the absorbance was read at 290 nm using a spectrophotometer versus a blank. Trans-cinnamic was used as a standard. A unit of enzyme activity was described as the amount of enzyme required for the formation of 1 ml of product in 1 sec under the procedure conditions [Zucker, 1965; Cheng and Breen, 1991; Christopoulos and Tsantili, 2015].

2.3.15. Trolox Equivalent Antioxidative Capacity (TEAC) Assay

Leaf samples (ca. 0.5 g) belonging to the second fully expanded youngest leaves were removed from liquid N₂ and quickly ground using a liquid N₂-cooled ball mill (MM200; Retsch, Haan, Germany) at a speed of 30 oscillations per second for 30 second. The homogenization was repeated in the presence of 5 ml of absolute ethanol. The leaf homogenates then were centrifuged to clarify at 4°C for 15 min and at 15,000×g, and upper phase of the samples were immediately stored at -80°C.

TEAC assay was carried out spectrophotometrically according to the method described by Re et al. with some modifications. The assay is based on the

decolorization of the ABTS (2,2-azinobis-[3-ethylbenzothiazoline-6-sulphonic acid]) radical cation (conversion from blue to clear).

ABTS was dissolved in dH₂O to a 7 mM concentration. ABTS radical cation (ABTS^{•+}) was obtained by allowing the reaction of 7 mM ABTS stock solution with 2.45 mM potassium persulfate (v/v) for 12–16 h in the dark and 24–25°C before use. ABTS^{•+} solution was diluted with absolute ethanol (1:50 ratio) until its absorbance reached of 0.70 (±0.02) at 734 nm. Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid; Aldrich Chemical Co., Gillingham, Dorset, UK), being a vitamin E analogue, was used as an antioxidant standard. Trolox stock solution was dissolved in the ethanol to a 15 mM concentration. A Trolox standard curve was prepared with a series of dilutions of 15 mM stock solution (0.2, 0.4, 0.8, 1.2, 1.5 mM). A sample blank was prepared, to which 10 µl of Trolox standards (0–1.50 mM) and 990 µl of diluted ABTS^{•+} solution was added. The reaction mixture was composed of 10 µl of sample extract (1:10 diluted with the ethanol) and 990 µl of diluted ABTS^{•+} solution and the absorbance of each reaction mixture was then read in 60 sec intervals for 6 min right after sample addition and recorded. The TEAC values of the samples were calculated, using a Trolox standard curve, on the basis of the percentage inhibition of absorbance at 734 nm and denoted in µmol Trolox/g sample fw. The range of concentration of Trolox used for calibration curve was 0–15 µM [Re et al., 1999; Fares et al., 2010].

2.3.16. Free Proline Assay

Free proline was estimated by the method of Bates et al. from material of leaf tissue. In this sense, the third and fourth youngest leaves were cut into small pieces and pooled thoroughly. Frozen plant material (ca. 0.5 g) was homogenized in a ball mill (MM200; Retsch, Haan, Germany) with 10 ml of 3% aqueous sulfosalicylic acid. The homogenate was then centrifuged at 8,000 x g for 20 min at 25°C. Reaction of 2 ml supernatant with 2 ml acid ninhydrin and 2 ml of glacial acetic acid was carried out in a test tube for 1 hour at 100°C, and the reaction was finalized in an ice bath. The reaction mixture was treated with 4 ml toluene, stirred strongly with a test tube stirrer for 15–25 sec. The chromophore containing toluene phase was removed by aspiration from the aqueous phase, then kept in an ice bath to warm up to 24–25°C. Reference standards of proline (0.1, 0.2, 0.3, 0.4, 0.5, 0.6 mM) were prepared in 3%

aqueous sulfosalicylic acid, and the sample estimation procedure was executed. Absorbances of standards and samples were spectrophotometrically determined at 520 nm and toluene was used for a blank. Finally, the proline concentration was appointed from a standard curve and calculated on a fresh weight basis as follows: $[(\mu\text{g proline/ml} \times \text{ml toluene}) / 115.5 \mu\text{g}/\mu\text{mole}] / [(\text{g sample})/5] = \mu\text{moles proline/g of fresh weight material}$ [Bates et al., 1973].

2.3.17. Histochemical Analysis

Root staining procedure was executed according to the method by Kumar et al. with some modifications [Kumar et al., 2009]. In order to demonstrate colonization, 10 root samples were selected randomly from colonized and non-colonized wheat roots. The root samples were harvested randomly from corresponding treatments (10 root samples from each pot, 320 samples in total) washed thoroughly under running tap water and cut into 3-cm segments from the tips. The root segments were immersed in 10% KOH (w/v) for 15 min (or until become transparent) to soften, acidified with 1 M HCl solution for 10 min, and finally stained with 0.02% Trypan blue solution overnight [Dickson et al., 1998; Phillips & Hayman, 1970]. The following day, the segments were rinsed with dH₂O to remove excess dye and destained with 50% lactoglycerol (lactic acid: glycerol: dH₂O, 1: 1: 1, v/v/v) for 1-2 h prior to observation using a fluorescent-light microscope (Olympus BX-51). All root segments were photographed with KAMERAM software, assisted by a KAMERAM fluorescent camera.

2.3.18. Genomic DNA Isolation from Plant Roots

In this study, *P. indica* DNA from wheat roots was successfully extracted with two different extraction methods: commercial extraction kit (Plant DNeasy kit) and modified CTAB (cetyltrimethylammonium bromide) method. Fungus DNA extraction with kit was carried out according to the manufacturer's instructions (Plant DNeasy kit, Qiagen). Besides that, fungus DNA extraction with CTAB method was executed according to the classical method with certain modifications due to low cost and high efficiency of the method [Bousquet et al., 1990; Lipp et al., 1999; Sahu et al., 2012].

Plant roots were cut 5 cm-length from tips and carefully washed with sterile distilled water to remove the attached debris. The root samples were taken from one representative pot of each treatment (128/4=32 in total). The samples were kept preserving in 70% ethanol until storing in -80°C. The samples (ca. 200 ng) were frozen in liquid N₂ and transferred to a 2-ml sterile polypropylene tubes including small balls. The tubes were placed in N₂-cooled jars of a mill (MM200; Retsch, Haan, Germany) and pulverized. 400 µl AP1 buffer (Plant DNeasy kit; Qiagen, Hilden, Germany) and 4 µl of RNase (supplied with the Plant DNeasy kit) were added to each tube and incubated for 15 min at 65°C. The following isolation steps were executed according to the manufacturer's instructions.

As a second fungus DNA extraction method from wheat root, CTAB β-mercaptoethanol method was used as follows: Before treating the root samples with CTAB extraction buffer (20 g/L CTAB; 1.4 mol/L NaCl; 0.1 mol/L Tris-HCl; 20 mmol/L EDTA; 0,02% β-mercaptoethanol), pulverization step was carried out as described above. The buffer (pH:8) was incubated at 65°C for 15 min and added up to 500 µl to each tube, subsequently. The mixture was vortexed and incubated at 65°C for 20 min and allowed cooling to room temperature. Further, 500 µL chloroform-octanol (24:1) were added and overturned at 20-30 times, then centrifuged at 15,000 rpm for 15 min at room temperature. The supernatant (1000 µL) was transferred to a new 2 mL sterile reaction tube. 250 µL of 5 M NaCl (pre-chilled at +4°C) were added and overturned at 20-30 times. 95% ethanol was pre-chilled at -20°C, added to the mixture (v/v) and vortexed, vigourously. The tubes including the mixtures were kept at +4°C for 24 hours or at -20°C for 1 hour. Afterwards, the tubes were centrifuged at 15,000 rpm for 15 min at +4°C and the supernatants were discarded. The pellet was washed with 1000 µL of 70% ethanol. After centrifugation at 15,000 rpm for 15 min at +4°C, the supernatant was discarded, and the pellet was dried at 37°C for 60 min. The dry pellet was dissolved in 30 µL of deionized sterile water and stored at -20°C.

2.3.19. PCR Analysis

PCR reactions were executed in a final volume of 50 µl, consisting of 10 µl of 5x MyTaq reaction buffer (Bioline), 1 µl of each primer (20 µM), 1 µl of MyTaq DNA polymerase (500U, Bioline MyTaq™ DNA Polymerase), and 2 µl of genomic DNA

(ca. 100 ng) made up to 50 µl with sterile ultra-pure water. To quantify the presence of *P. indica* in wheat roots, EF-1-alpha (*tef*) gene (accession no: AJ249912, 227 bp) was amplified by using *PITEF* primer pairs [Pitef-F (5'-TCGTCGCTGTCAACAAGATG-3') and Pitef-R (5'-GAGGGCTCGAGCATGTTGT-3')] which were synthesized according to the sequence available in GenBank. PCR reactions were executed in a T-Professional Thermocycler (Biometra) set to the following reaction conditions: an initial denaturation at 94°C for 5 minutes, followed by denaturation at 94°C for 40 seconds for 35 cycles; annealing at 59°C for 40 seconds; and extension at 72°C for 30 seconds. For a negative control, PCR tubes without DNA template were added to each experiment. To avoid false-positive results by cross-reaction with plant DNA, genomic DNA extracted from non-inoculated roots were tested with using the fungus specific primers. No samples created amplified fragments in non-inoculated roots for the fungus specific primers.

2.3.20. DNA Concentration, Purity, and Fragmentation State

Determination of the DNA concentration was performed spectrophotometrically by measuring the absorbance at 260 nm. The purity of DNA extracts was evaluated by the ratio of the absorbance at 260 nm and 280 nm. Each sample was measured 3 times and average values are calculated. For this purpose, a NanoDrop Lite spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) was employed for spectrophotometry assay. After PCR analysis, the integrity of extracted fungus DNA was assessed by electrophoresis in 0.8% agarose gel (GeneON Bioscience, Ludwigshafen, Germany) with 1x TAE buffer (ClearBand TAE 50x Electrophoresis Buffer) at 90 V for 40 min. The approximate size of DNA fragments run on gel electrophoresis was determined using 100 bp DNA ladder (GeneRuler 100 bp DNA Ladder, Thermo Fischer Scientific). Agarose gel was stained with a nucleic acid staining solution (RedSafe™) prior to observation under UV illuminator with the Azure c600 Gel Imaging System (Azure Biosystems, Dublin, CA, USA).

2.3.21. Calculations and Statistical Analysis

To calculate the mineral contents for a stated plant portion (e.g., shoot or straw) the mineral concentrations were multiplied by the dry weight of that plant portion.

Accordingly, grain yield was multiplied by grain mineral concentrations to find out the total amounts of minerals of interest deposited in the grain samples. Statistical analyses were carried out using a statistical software (JMP, version 16.0.0). All reported values represent means of 4 replicates. The significance of the effects of the treatments and their interactions on the stated traits was evaluated by analysis of variance (ANOVA). Additionally, determination of significant differences between means was performed by Tukey's protected honestly significant difference (HSD) test at 95% confidence ($p \leq 0.05$) where ANOVA reported a significant effect.



3. RESULTS

3.1. First Soil Experiment

As shown by three-way ANOVA, the triple interaction of B supply, soil sterilization, and *P. indica* inoculation significantly affected the shoot dry matter of bread wheat but not that of durum wheat. On the other hand, the main effects of all three factors on the shoot dry weight were significant in both tested cultivars (**Table 3.1**). In terms of B toxicity, increasing B supply to high levels resulted in decreased shoot dry weights in both cultivars (**Table 3.2**). The experimental plants inoculated with *P. indica* displayed elevated shoot dry weights compared to those grown in the absence of endophytic fungus in both bread and durum wheat cultivars. Besides that, plants grown in autoclaved soil had higher shoot dry weights than those grown in non-autoclaved soils for both Nusrat and Saricanak-98. On the whole, *P. indica* and soil sterilization positively affected the shoot dry matter production (**Table 3.2**).

The general condition and visual appearance of 41-day-old plants differed greatly depending on the treatments at this stage. Under non-autoclaved soil conditions, increasing the B supply to toxic levels impaired plant vigour, and caused leaf symptoms of increasing severity, including chlorosis and necrosis for both cultivars whilst inoculated plants displayed enhanced vigour (**Figure 3.1**).

Histological studies and characterization of fungal colonization were executed by identifying inter and intra-cellular pear-shaped spore structures (**Figure 3.2B, C**) in roots colonized with *P. indica* at medium B level as in comparison with corresponding control (non-colonized) plants (**Figure 3.2A**). The chlamydospores were scattered from one another or grouped in chains with two-to-many spores. Not every spore had the typical structure; some also were monitored to have a round, oval, or rectangular form (**Figure 3.2B, C**).

Table 3.1: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot DW (g), REL (%), MDA (nmol g⁻¹), and total chlorophyll (nmol g⁻¹) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions at vegetative stage (41 days after sowing, DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Shoot DW (g)		REL (%)		MDA (nmol g ⁻¹)		Total Chlorophyll (nmol g ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	<0.001	0.036	<0.001	4.1	0.006	3.2	0.697	n.s.
	Soil Sterilization (S)	1	<0.001	0.036	0.213	n.s.	<0.001	3.2	0.009	83
	Boron Supply (B)	3	<0.001	0.066	<0.001	7.7	0.020	5.8	0.075	n.s.
	I X S	1	0.123	n.s.	0.060	n.s.	0.463	n.s.	0.004	156
	I X B	3	0.652	n.s.	0.005	12.9	0.436	n.s.	0.156	n.s.
	S X B	3	0.001	0.114	0.023	12.9	0.678	n.s.	0.023	262
	I X S X B	3	0.018	0.184	0.234	n.s.	0.925	n.s.	0.349	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Shoot DW (g)		REL (%)		MDA (nmol g ⁻¹)		Total Chlorophyll (nmol g ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	0.024	<0.001	2.5	0.008	3.8	0.014	76
	Soil Sterilization (S)	1	<0.001	0.024	<0.001	2.5	0.163	n.s.	0.467	n.s.
	Boron Supply (B)	3	<0.001	0.045	<0.001	4.6	<0.001	7.2	0.030	143
	I X S	1	0.037	0.045	0.006	4.6	0.451	n.s.	0.405	n.s.
	I X B	3	0.361	n.s.	0.001	7.8	0.487	n.s.	0.005	241
	S X B	3	0.497	n.s.	<0.001	7.8	0.608	n.s.	0.371	n.s.
	I X S X B	3	0.869	n.s.	0.201	n.s.	0.519	n.s.	0.437	n.s.

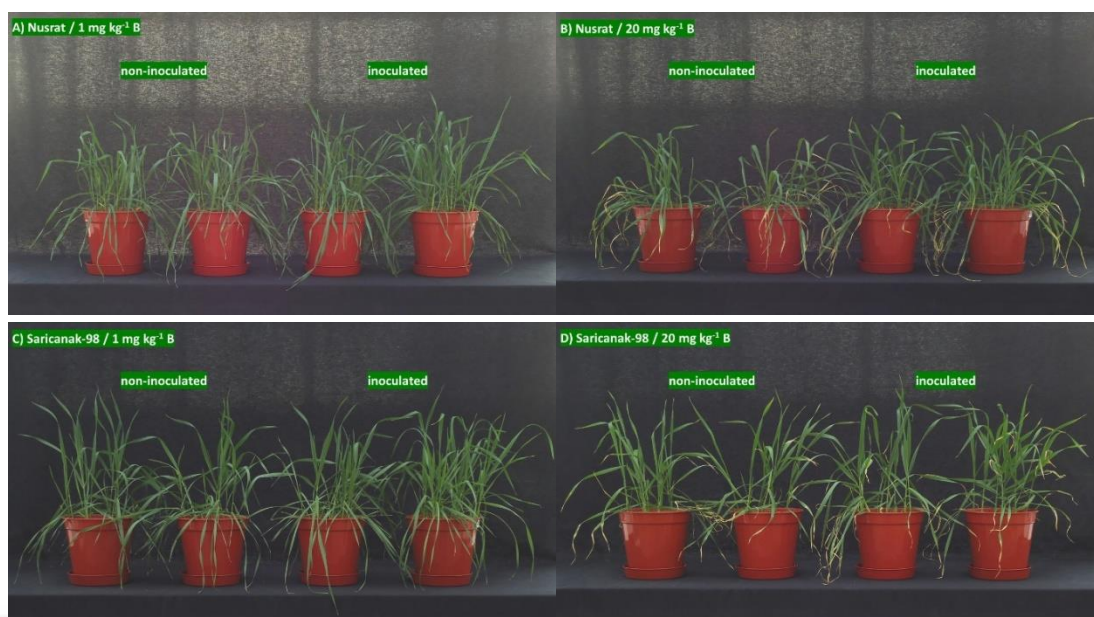


Figure 3.1: Effects of *P. indica* on 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) (A-B) and durum wheat (*Triticum durum* cv. Saricanak-98) (C-D) grown in non-autoclaved soil at control B (1 mg kg⁻¹) and medium B toxicity (20 mg kg⁻¹)

Table 3.2: Shoot dry weight (g plant⁻¹) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent the means and standard deviations of four replicates.

Cultivar	Soil Sterilization	<i>P. indica</i>	Shoot DW (g plant ⁻¹)											
			B Supply (mg kg ⁻¹)											
			1			10			20			30		
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved	(-)	0.58	±	0.08	0.53	±	0.17	0.56	±	0.11	0.47	±	0.06
		(+)	0.73	±	0.08	0.82	±	0.07	0.68	±	0.02	0.58	±	0.03
	Non-autoclaved	(-)	0.62	±	0.10	0.50	±	0.03	0.33	±	0.03	0.27	±	0.03
		(+)	0.71	±	0.03	0.54	±	0.08	0.52	±	0.04	0.38	±	0.02
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved	(-)	0.68	±	0.05	0.67	±	0.03	0.56	±	0.04	0.50	±	0.05
		(+)	0.84	±	0.03	0.76	±	0.10	0.63	±	0.04	0.58	±	0.03
	Non-autoclaved	(-)	0.53	±	0.02	0.52	±	0.08	0.35	±	0.02	0.34	±	0.03
		(+)	0.60	±	0.06	0.57	±	0.06	0.39	±	0.06	0.36	±	0.04

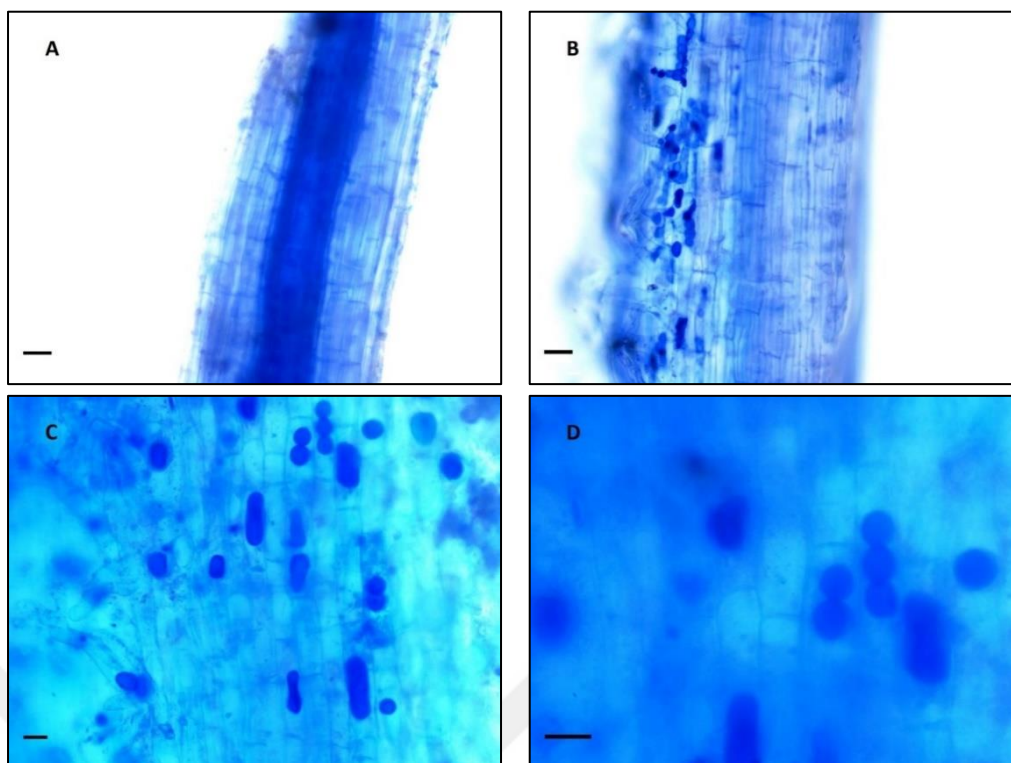


Figure 3.2: Trypan blue staining of inoculated (B, C) and non-inoculated (A) durum wheat (*Triticum durum* cv. Saricanak-98) roots at medium B levels ($20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) under greenhouse conditions showing intracellular *P. indica* spores observed at day 41 DAS. Bars represent 50 μM .

In addition to the histological works, PCR was utilized to confirm the presence of *P. indica* in wheat roots by amplifying the *tef* gene (**Figure 3.3**). At 41 DAS, *tef* DNA fragment of *P. indica* was detectable in the roots of inoculated plants. Besides that, non-inoculated wheat roots did not display any band formation as expected. All things considered, experimental plants of both cultivars were successfully infected and colonized by *P. indica*, and the endophytic fungus persisted in host tissues of all B treatment groups without showing any specific pattern in terms of colonization caused by B supply.

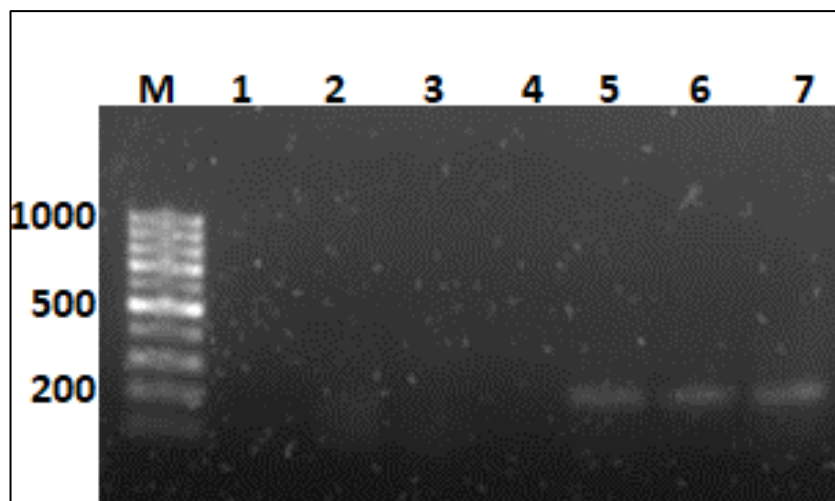


Figure 3.3: Polymerase chain reaction (PCR) detection of EF-1 α (*tef*) gene of *P. indica* in DNA of wheat roots. The plants were inoculated *P. indica* into pot culture in soil. PCR fragments originate from plants harvested at day 41 DAS. The lanes represent non-inoculated (lanes 2, 3 and 4) and inoculated (lanes 5, 6 and 7) pots (M=100 bp size marker; lane 1= negative control; lane 2= durum wheat roots at high B; lane 3 and 4 = bread wheat roots at high and medium B levels, respectively; lane 5 and 6= bread wheat roots at medium B and high B levels, respectively; lane 7= durum wheat roots at high B).

It was revealed that *P. indica* inoculated plants displayed decreased REL in both Nusrat and Saricanak-98 (**Table 3.1; Figure 3.4A**). Soil sterilization was associated with elevated REL in durum wheat, (**Figure 3.4B**). For both cultivars, B toxicity significantly affected REL at medium and high B levels whereas the low level of B toxicity did not cause significant increases in REL (**Figure 3.4C**).

The shoot MDA concentration was significantly affected by all single treatments in bread wheat whereas it was affected by only inoculation and B supply in durum wheat (**Table 3.1**). For both cultivars, inoculated plants exhibited significantly lower shoot MDA concentrations (**Figure 3.5A**). Although soil sterilization caused lower shoot MDA concentrations in Nusrat, it did not have any effect in Saricanak-98 (**Figure 3.5B**). Increasing levels of B toxicity generally led to significant elevations in MDA concentrations; nevertheless, the lowest level of toxic B supply was not associated with a considerable elevation compared to the control B supply (**Figure 3.5C**).

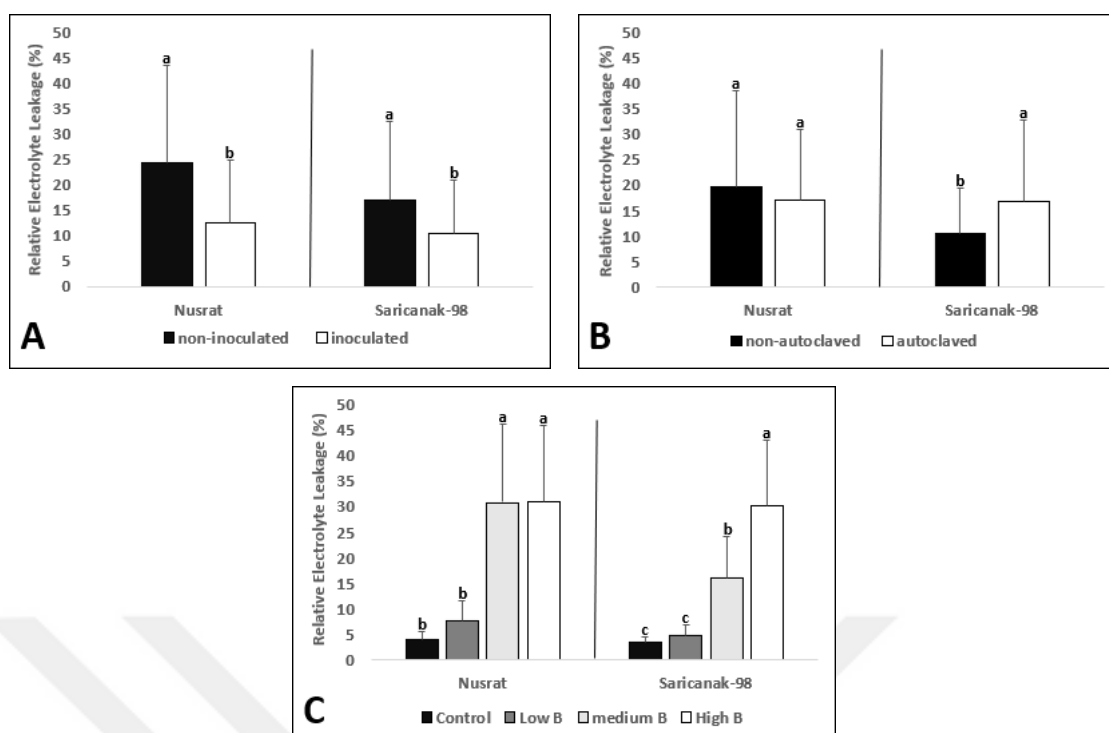


Figure 3.4: Relative electrolyte leakage (%) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica* grown in autoclaved and non-autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions (A-C). Different letters indicate significant differences between means according to three-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

The total chlorophyll concentration was affected by inoculation and B supply, as well as their double interaction in Saricanak-98; nonetheless, only soil sterilization and its double interactions with inoculation and B supply considerably had an effect on the leaf chlorophyll level in Nusrat (**Table 3.1**). As shown in **Table 3.3**, elevated chlorophyll levels were evaluated in the group inoculated with *P. indica* in durum wheat. Even though soil sterilization improved the total chlorophyll in bread wheat, it did not in durum wheat. As regards to B toxicity, increasing the B supply did not cause any considerable change in the leaf chlorophyll biosynthesis in Nusrat whilst no consistent response to increasing B levels was monitored in Saricanak-98 (**Table 3.3**).

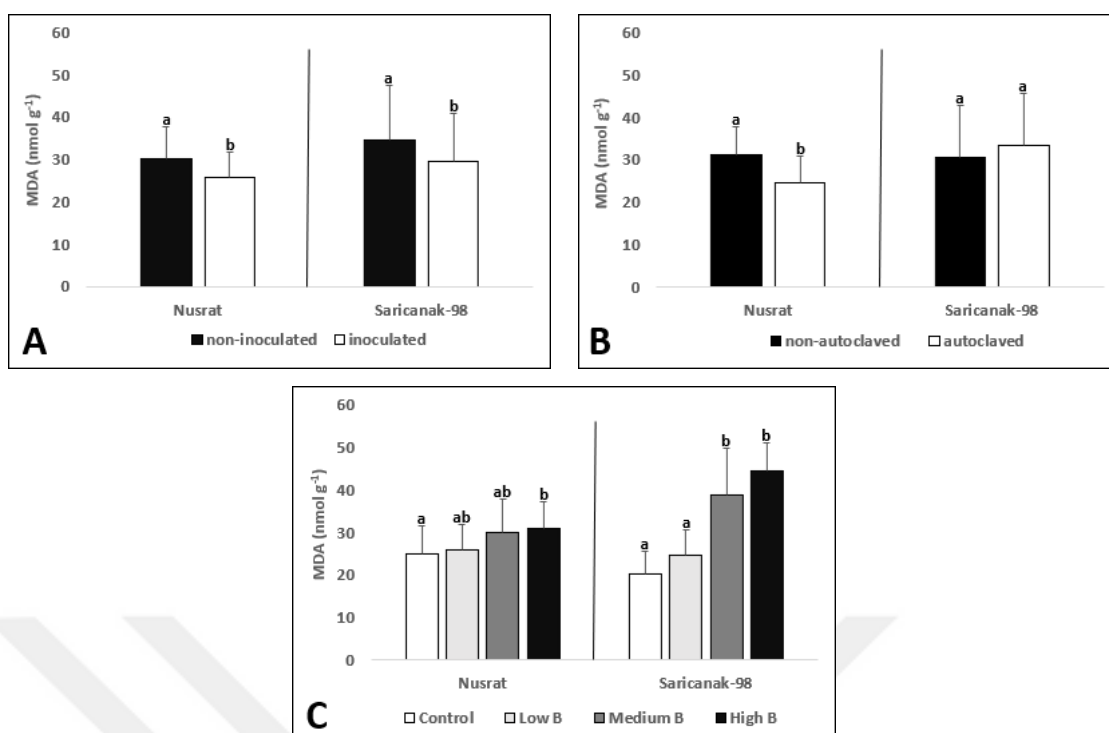


Figure 3.5: Shoot MDA concentration (nmol g⁻¹) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions (A-C). Different letters indicate significant differences between means according to three-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

Table 3.3: Effects of *P. indica* on the total chlorophyll (nmol g⁻¹) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved or non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Cultivar	Soil Sterilization	<i>P. indica</i>	Total Chlorophyll (nmol g ⁻¹)											
			B Supply (mg kg ⁻¹)											
			1			10			20			30		
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved	(-)	1067	±	104	794	±	147	658	±	72	529	±	50
		(+)	1155	±	127	879	±	40	734	±	150	778	±	46
	Non-autoclaved	(-)	873	±	213	897	±	227	616	±	118	507	±	179
		(+)	700	±	169	643	±	44	927	±	253	727	±	86
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved	(-)	723	±	162	789	±	169	842	±	126	778	±	167
		(+)	1092	±	97	808	±	40	878	±	230	679	±	92
	Non-autoclaved	(-)	822	±	120	832	±	327	659	±	72	786	±	301
		(+)	795	±	245	853	±	126	828	±	129	604	±	70

According to ANOVA, the shoot B concentration, in Nusrat, was substantially affected by the single effects of soil sterilization and B supply as well as their double interaction. In Saricanak-98, however, only the main effects of soil sterilization and B supply on the shoot B concentrations were considerable (**Table 3.4**). Neither a positive nor negative effect on shoot B concentrations was observed by the presence of *P. indica* in both cultivars (**Figure 3.6A**). By contrast, the experimental plants grown in autoclaved soil showed lower shoot B concentrations compared to those grown in native soil in both the bread and durum cultivars (**Figure 3.6B**). Dose-dependent increments were observed in both cultivars with increasing the B supply from the control to the high level (**Figure 3.6C**).

Table 3.4: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot concentrations of B (mg kg⁻¹), Mn (mg kg⁻¹), P(%) and straw Zn (mg kg⁻¹), of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

Triticum aestivum cv. Nusrat	Source of Variation	DF	Shoot B Conc. (mg kg ⁻¹)		Shoot Mn Conc. (mg kg ⁻¹)		Shoot P Conc. (%)		Shoot Zn Conc. (mg kg ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.357	n.s.	0.004	5.26	<0.001	0.014	0.105	n.s.
	Soil Sterilization (S)	1	0.015	26	<0.001	5.26	<0.001	0.014	<0.001	1.45
	Boron Supply (B)	3	<0.001	48	<0.001	9.75	<0.001	0.026	0.004	2.70
	I X S	1	0.885	n.s.	0.266	n.s.	0.478	n.s.	0.553	n.s.
	I X B	3	0.512	n.s.	0.885	n.s.	0.447	n.s.	0.615	n.s.
	S X B	3	0.040	80	0.002	16.42	<0.001	0.047	0.001	4.54
	I X S X B	3	0.424	n.s.	0.985	n.s.	0.914	n.s.	0.567	n.s.
Triticum durum cv. Saricanak-98	Source of Variation	DF	Shoot B Conc. (mg kg ⁻¹)		Shoot Mn Conc. (mg kg ⁻¹)		Shoot P Conc. (%)		Shoot Zn Conc. (mg kg ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.826	n.s.	0.132	n.s.	0.051	n.s.	0.004	1.33
	Soil Sterilization (S)	1	<0.001	27	<0.001	3.31	<0.001	0.016	<0.001	1.33
	Boron Supply (B)	3	<0.001	51	0.735	n.s.	<0.001	0.029	0.005	2.46
	I X S	1	0.373	n.s.	0.002	6.2	0.733	n.s.	0.020	2.46
	I X B	3	0.997	n.s.	0.176	n.s.	0.134	n.s.	0.101	n.s.
	S X B	3	0.123	n.s.	0.005	10.44	<0.001	0.05	<0.001	4.07
	I X S X B	3	0.908	n.s.	0.531	n.s.	0.774	n.s.	0.053	n.s.

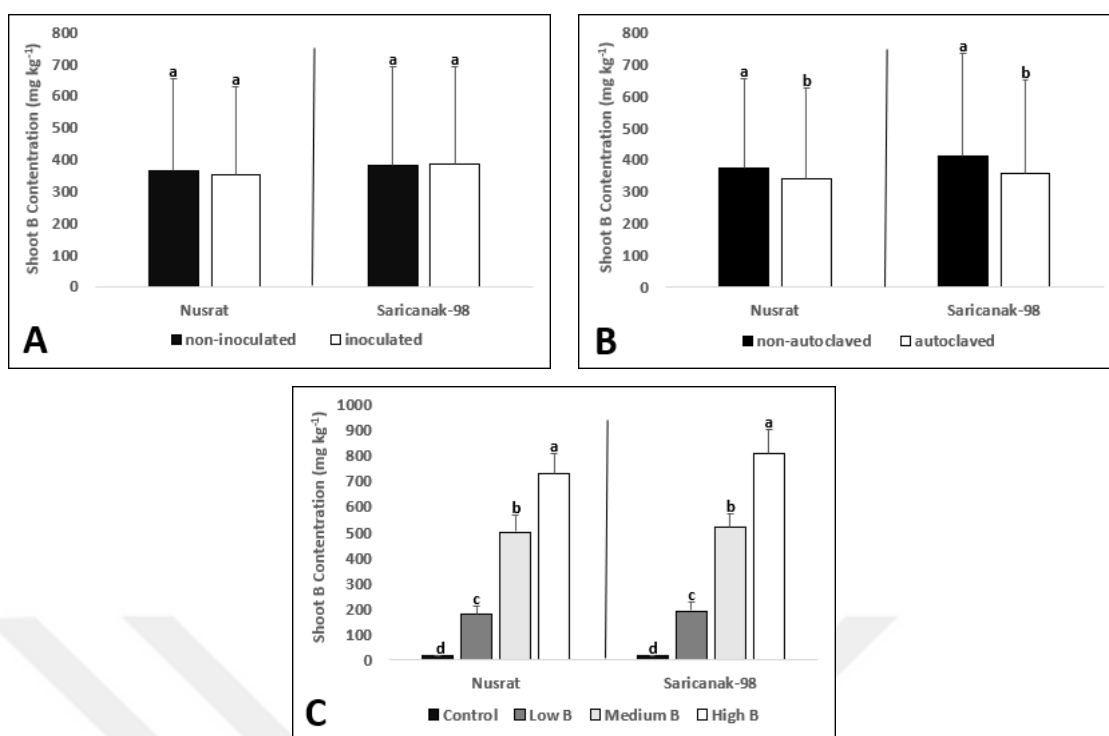


Figure 3.6: Shoot B concentration (mg kg^{-1}) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; low B: $10 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; medium B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; high B: $30 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) under greenhouse conditions (A-C). Different letters indicate significant differences between means according to three-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

Whilst the shoot Mn concentration was considerably affected by all treatments and the double interaction of soil sterilization and B supply in bread wheat, it was significantly affected by just soil sterilization and its double interactions with inoculation and B supply in durum wheat (**Table 3.4**). Despite the fact that the shoot Mn accumulation was reduced by *P. indica* in Nusrat, was not affected by the endophytic fungus in Saricanak-98 (**Figure 3.7A**). Compared to plants grown in native soil, the plants grown in sterilized soil showed greatly high (almost 3X) shoot Mn concentrations (**Figure 3.7B**). The presence or absence of significant effects of B supply on the shoot Mn concentrations depended on the cultivar (**Figure 3.7C**).

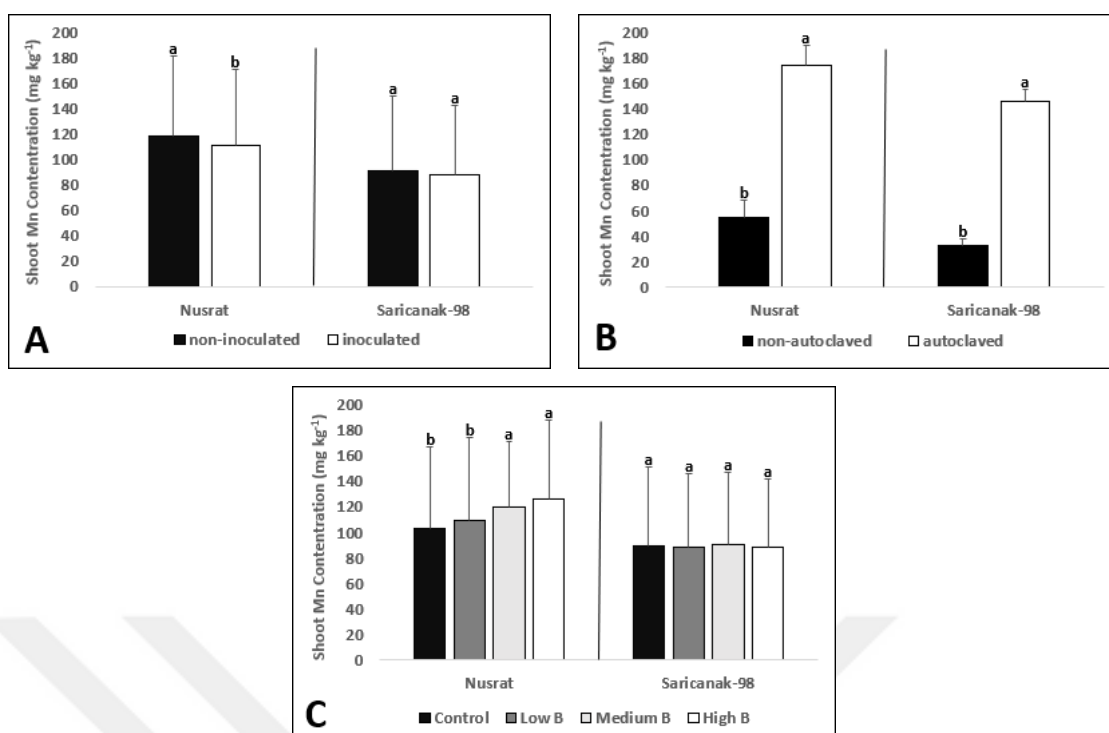


Figure 3.7: Shoot Mn concentration (mg kg^{-1}) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; low B: $10 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; medium B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; high B: $30 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) under greenhouse conditions (A-C). Different letters indicate significant differences between means according to three-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

The shoot P concentrations were considerably affected by all treatments and the double interaction of soil sterilization and B supply (**Table 3.4**). Even though *P. indica* significantly reduced the shoot P concentration in Nusrat, this trend was not observed in that of Saricanak-98 (**Figure 3.8A**). Besides that, soil sterilization resulted in elevated shoot P concentrations in both bread and durum wheat cultivars (**Figure 3.8B**). For both cultivars, experimental plants belonging to the high B treatment displayed the highest shoot P concentrations whilst those belonging to the control B group exhibited the lowest P concentrations (**Figure 3.8C**).

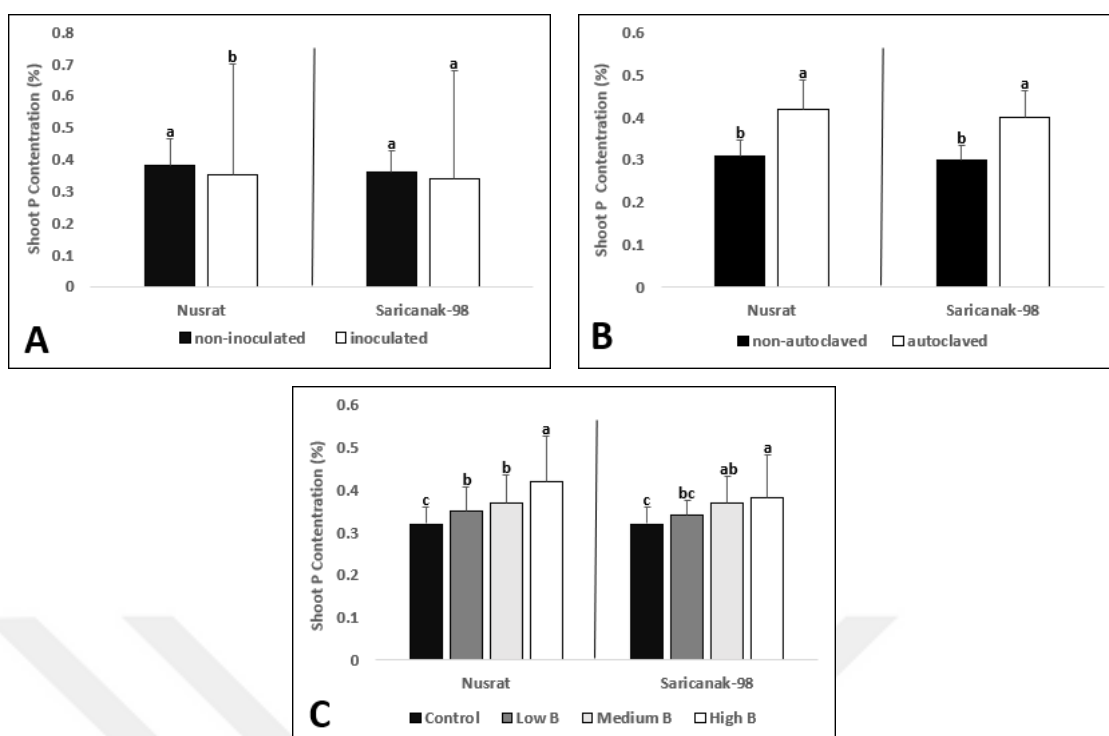


Figure 3.8: Shoot P concentration (%) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions (A-C). Different letters indicate significant differences between means according to three-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

P. indica did not lead to any considerable effect on the shoot Zn concentration in bread wheat; however, the shoot Zn concentration was reduced by *P. indica* in durum wheat (Table 3.4 and Figure 3.9A). The experimental plants grown in sterilized soil had higher shoot Zn concentrations than those grown in native soil in both bread and durum wheat cultivars (Figure 3.9B). Increasing the B level from the control to the high caused inconsistent shoot Zn concentrations in either cultivar (Figure 3.9C).

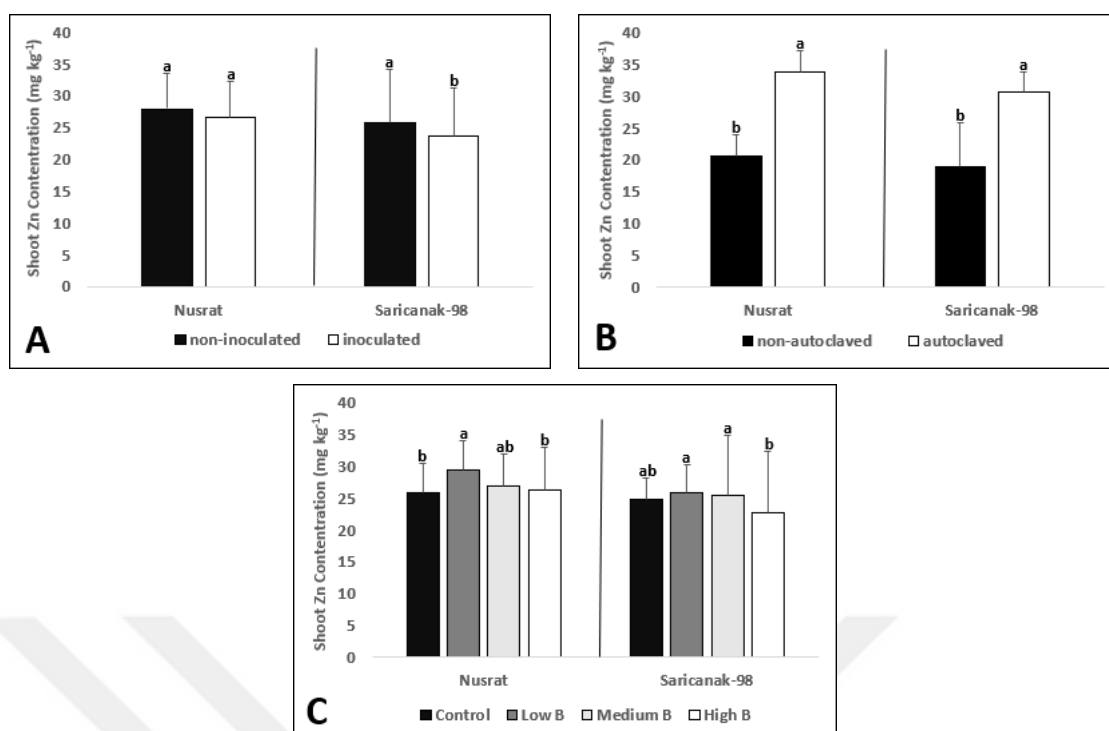


Figure 3.9: Shoot Zn concentration (mg kg⁻¹) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions (A-C). Different letters indicate significant differences between means according to three-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

According to the collective data in **Tables 3.5** and **3.6**, shoot Ca concentration was significantly affected by all single treatments in Nusrat whereas it was affected by none of which in Saricanak-98. In Nusrat, both *P. indica* inoculation and soil sterilization significantly reduced the shoot Ca concentration. Increasing B supply from control to medium, caused no effect on Ca concentrations but high level led to increase it considerably in Nusrat cultivar.

P. indica inoculated plants displayed reduced shoot K concentrations in both cultivars. On the other hand, shoot K concentrations were significantly increased by soil sterilization in both cultivars. In Nusrat, increasing B from the control to the high level increased the shoot K concentrations although transition from the low to the medium level is not significant. In Saricanak-98, increasing B supply from the

control to the high level of B increased the shoot K concentrations in overall; however, transition from medium to high level is not significant (**Tables 3.5 and 3.6**).

Even though *P. indica* inoculation did not resulted in any significant change in Nusrat, it caused higher shoot Mg concentration in Saricanak-98 compared to corresponding control (non-inoculated) plants. Soil sterilization caused no significant difference in Nusrat; conversely, it reduced the shoot Mg concentration in Saricanak-98. In Nusrat, increasing B supply from the control to the high level reduced the shoot Mg concentrations overall although the high level caused the lowest shoot Mg concentration, and the shoot Mg concentrations belonging to medium and high level B treatments were not differentiated from one another significantly. In Saricanak-98, medium level of B caused the highest shoot Mg concentrations and the plants belonging to control, low and high levels of B treatment groups did not show any significant effect on the shoot Mg concentrations (**Tables 3.5 and 3.6**).

Inoculated plants did not show any considerable change in the shoot S concentration compared to non-inoculated plants in Nusrat. On the other hand, the presence of *P. indica* in the substrate reduced the shoot S concentration in Saricanak-98. In bread wheat, soil sterilization did not lead to any significant change in the shoot S concentration; however, it considerably reduced that of durum wheat. In terms of B toxicity, increasing the B level from the control to the medium level did not cause any significant difference in the shoot S concentration and the high level increased it in bread wheat. In durum wheat, increasing the B level from the control to the medium B level did not cause any significant change in the shoot S concentration, but the high B level reduced it (**Tables 3.5 and 3.6**).

Table 3.5: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot selected macronutrients of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Ca (%)		K (%)		Mg (%)		S (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	<0.001	0.03	<0.001	0.12	0.9524	n.s.	0.460	n.s.
	Soil Sterilization (S)	1	<0.001	0.03	<0.001	0.12	0.696	n.s.	0.325	n.s.
	Boron Supply (B)	3	0.023	0.05	<0.001	0.21	<0.001	0.007	<0.001	0.02
	I X S	1	0.382	n.s.	0.577	n.s.	<0.001	0.007	0.825	n.s.
	I X B	3	0.114	n.s.	0.917	n.s.	0.0468	0.012	0.118	n.s.
	S X B	3	0.002	0.09	0.159	n.s.	<0.001	0.012	<0.001	0.03
	I X S X B	3	0.022	0.18	0.062	n.s.	0.701	n.s.	0.462	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Ca (%)		K (%)		Mg (%)		S (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.666	n.s.	<0.001	0.08	0.0393	0.002	0.028	0.01
	Soil Sterilization (S)	1	0.545	n.s.	<0.001	0.08	<0.001	0.002	<0.001	0.01
	Boron Supply (B)	3	0.626	n.s.	<0.001	0.15	0.022	0.005	<0.001	0.02
	I X S	1	0.144	n.s.	0.018	0.15	0.0032	0.005	0.790	n.s.
	I X B	3	0.325	n.s.	0.004	0.28	0.3415	n.s.	0.507	n.s.
	S X B	3	0.133	n.s.	<0.001	0.28	<0.001	0.009	<0.001	0.03
	I X S X B	3	0.252	n.s.	0.627	n.s.	0.8545	n.s.	0.008	0.03

Table 3.6: Selected macronutrient concentrations of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Ca (%)		K (%)		Mg (%)		S (%)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	0.76	± 0.01	4.60	± 0.26	0.20	± 0.01	0.31	± 0.01
			(+)	0.68	± 0.05	4.19	± 0.09	0.19	± 0.01	0.31	± 0.03
		Non-autoclaved	(-)	0.77	± 0.06	4.05	± 0.14	0.20	± 0.01	0.34	± 0.02
			(+)	0.69	± 0.01	3.39	± 0.14	0.21	± 0.00	0.34	± 0.02
	Low	Autoclaved	(-)	0.80	± 0.11	4.93	± 0.10	0.20	± 0.01	0.34	± 0.01
			(+)	0.60	± 0.05	4.68	± 0.20	0.17	± 0.01	0.33	± 0.01
		Non-autoclaved	(-)	0.72	± 0.08	4.61	± 0.16	0.17	± 0.00	0.31	± 0.01
			(+)	0.67	± 0.02	3.87	± 0.20	0.18	± 0.01	0.32	± 0.02
	Medium	Autoclaved	(-)	0.67	± 0.03	5.18	± 0.29	0.16	± 0.00	0.32	± 0.02
			(+)	0.65	± 0.06	4.77	± 0.11	0.16	± 0.01	0.34	± 0.02
		Non-autoclaved	(-)	0.92	± 0.07	4.54	± 0.38	0.17	± 0.01	0.34	± 0.02
			(+)	0.74	± 0.10	4.12	± 0.22	0.19	± 0.01	0.35	± 0.02
	High	Autoclaved	(-)	0.78	± 0.10	5.84	± 0.59	0.17	± 0.02	0.40	± 0.06
			(+)	0.63	± 0.04	5.09	± 0.20	0.16	± 0.00	0.38	± 0.02
		Non-autoclaved	(-)	0.97	± 0.15	4.65	± 0.23	0.16	± 0.01	0.37	± 0.02
			(+)	0.71	± 0.05	4.37	± 0.19	0.16	± 0.00	0.33	± 0.01
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	0.95	± 0.02	4.12	± 0.10	0.13	± 0.01	0.40	± 0.02
			(+)	0.91	± 0.05	3.56	± 0.04	0.13	± 0.00	0.38	± 0.01
		Non-autoclaved	(-)	0.85	± 0.06	3.53	± 0.16	0.12	± 0.00	0.38	± 0.01
			(+)	0.92	± 0.08	3.34	± 0.11	0.13	± 0.01	0.40	± 0.04
	Low	Autoclaved	(-)	0.92	± 0.04	4.14	± 0.08	0.13	± 0.00	0.37	± 0.02
			(+)	0.82	± 0.05	3.96	± 0.23	0.13	± 0.01	0.33	± 0.03
		Non-autoclaved	(-)	0.90	± 0.05	3.69	± 0.18	0.14	± 0.01	0.40	± 0.02
			(+)	0.94	± 0.03	3.65	± 0.28	0.14	± 0.00	0.41	± 0.03
	Medium	Autoclaved	(-)	0.89	± 0.02	4.81	± 0.12	0.13	± 0.01	0.33	± 0.02
			(+)	0.87	± 0.02	4.17	± 0.24	0.13	± 0.01	0.33	± 0.03
		Non-autoclaved	(-)	1.00	± 0.07	4.16	± 0.35	0.14	± 0.00	0.43	± 0.03
			(+)	0.90	± 0.06	3.87	± 0.22	0.15	± 0.01	0.38	± 0.03
	High	Autoclaved	(-)	0.89	± 0.13	4.77	± 0.13	0.13	± 0.00	0.37	± 0.01
			(+)	0.90	± 0.04	4.71	± 0.15	0.13	± 0.01	0.37	± 0.02
		Non-autoclaved	(-)	0.83	± 0.10	3.68	± 0.23	0.13	± 0.01	0.35	± 0.04
			(+)	0.90	± 0.21	3.69	± 0.12	0.14	± 0.01	0.33	± 0.02

It was observed that *P. indica* inoculated plants belonging to Nusrat showed reduced shoot Cu concentrations but did not cause any change in Saricanak-98. Soil sterilization was associated with increased shoot Cu concentration in Nusrat whereas did not cause any difference in Saricanak-98. Increasing the B level from the low level to the high gradually increased the shoot Cu concentration in Nusrat. In Saricanak-98, increasing the B level from the low level to the medium increased the shoot Cu concentration but, the high level did not cause any further significant elevation (**Tables 3.7 and 3.8**).

The inoculated plants showed decreased shoot Fe concentrations compared to those non-inoculated in both cultivars. Soil sterilization resulted in elevated shoot Fe concentrations in both Nusrat and Saricanak-98. Increasing the B supply caused inconsistent shoot Fe concentrations in Nusrat. Namely, only the medium B level reduced the shoot Fe concentration compared to other B treatments. On the other hand, in Saricanak-98, B toxicity did not result in any significant difference (**Tables 3.7 and 3.9**).

As shown by three-way ANOVA, the triple interaction of B supply, soil sterilization, and *P. indica* inoculation significantly affected the total carotenoid of durum wheat, but not that of bread wheat. On the other hand, the main effects of inoculation and B supply on the total carotenoid were significant in both tested cultivars. Additionally, the shoot total carotenoid of durum wheat was affected by double interactions of soil sterilization with both *P. indica* and B supply. The shoot total carotenoid contents of both cultivars were enhanced by *P. indica*. Soil sterilization, however, was not associated with the total carotenoid contents in both Nusrat and Saricanak-98. Increasing B supply resulted in a dose-dependent reduction in Nusrat. In Saricanak-98, only the high B level significantly reduced the total carotenoid content (**Tables 3.7 and 3.10**).

Table 3.7: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot selected micronutrients and total carotenoids (nmol g⁻¹) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Total Caretonoids (nmol g ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.002	0.60	0.003	5.46	<0.001	19
	Soil Sterilization (S)	1	<0.001	0.60	<0.001	5.46	0.079	n.s.
	Boron Supply (B)	3	<0.001	1.11	<0.001	10.24	<0.001	35
	I X S	1	0.003	1.11	0.014	10.24	0.126	n.s.
	I X B	3	0.023	1.90	0.043	16.92	0.498	n.s.
	S X B	3	<0.001	1.90	0.612	n.s.	0.082	n.s.
	I X S X B	3	0.336	n.s.	0.447	n.s.	0.061	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Total Caretonoids (nmol g ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.056	n.s.	0.013	5.82	<0.001	21
	Soil Sterilization (S)	1	0.793	n.s.	0.004	5.82	0.3103	n.s.
	Boron Supply (B)	3	<0.001	1.43	0.101	n.s.	<0.001	39
	I X S	1	0.003	1.43	0.771	n.s.	0.0469	39
	I X B	3	0.177	n.s.	0.484	n.s.	0.0969	n.s.
	S X B	3	<0.001	2.43	0.817	n.s.	0.006	67
	I X S X B	3	0.662	n.s.	0.997	n.s.	0.0154	108

Table 3.8: Effects of *P. indica* on the shoot Cu concentrations (mg kg⁻¹) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved or non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Shoot Cu Concentration (mg kg ⁻¹)										
Cultivar	Soil Sterilization	<i>P. indica</i>	B Supply (mg kg ⁻¹)							
			1		10		20		30	
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved	(-)	23.5	± 0.7	24.1	± 1.5	24.5	± 1.4	29.2	± 3.0
		(+)	21.9	± 0.7	21.4	± 0.6	23.5	± 1.5	26.5	± 0.7
	Non-autoclaved	(-)	21.4	± 1.0	19.1	± 0.2	22.2	± 1.1	24.5	± 1.1
		(+)	20.7	± 0.8	19.8	± 0.5	24.0	± 0.8	22.5	± 1.2
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved	(-)	23.6	± 1.1	22.8	± 0.6	24.4	± 1.3	25.2	± 1.0
		(+)	22.2	± 0.8	20.3	± 1.5	21.3	± 1.5	24.4	± 2.0
	Non-autoclaved	(-)	18.6	± 0.5	21.1	± 1.6	26.6	± 2.1	24.5	± 2.2
		(+)	20.5	± 1.9	22.0	± 1.0	25.4	± 2.2	24.7	± 2.0

Table 3.9: Effects of *P. indica* on the shoot Fe concentrations (mg kg⁻¹) of 41-day-old bread wheat (*Triticum durum* cv. Saricanak-98) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved or non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Cultivar	Soil Sterilization	<i>P. indica</i>	Shoot Fe Concentration (mg kg ⁻¹)											
			B Supply (mg kg ⁻¹)											
			1			10			20			30		
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved	(-)	109.7	±	15.6	105.3	±	11.9	84.6	±	6.6	115.1	±	8.6
		(+)	89.6	±	10.6	95.6	±	9.3	82.9	±	6.0	84.4	±	6.4
	Non-autoclaved	(-)	94.5	±	8.7	80.5	±	6.9	67.0	±	8.4	80.0	±	11.0
		(+)	81.7	±	11.5	82.2	±	19.0	73.3	±	10.8	78.7	±	11.8
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved	(-)	88.2	±	15.8	87.0	±	7.0	85.0	±	15.5	84.5	±	7.2
		(+)	84.1	±	15.5	83.1	±	12.6	68.7	±	10.1	75.3	±	12.0
	Non-autoclaved	(-)	78.0	±	12.8	79.3	±	8.5	78.3	±	13.7	70.0	±	8.3
		(+)	74.9	±	10.3	75.4	±	12.0	64.8	±	9.4	63.8	±	5.8

Table 3.10: Effects of *P. indica* on the total carotenoids (nmol g⁻¹) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved or non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Cultivar	Soil Sterilization	<i>P. indica</i>	Total Carotenoids (nmol g ⁻¹)											
			B Supply (mg kg ⁻¹)											
			1			10			20			30		
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved	(-)	543	±	39	472	±	35	419	±	20	378	±	20
		(+)	659	±	21	501	±	28	500	±	42	475	±	48
	Non-autoclaved	(-)	507	±	49	414	±	34	348	±	63	341	±	52
		(+)	606	±	46	544	±	49	507	±	28	487	±	56
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved	(-)	481	±	43	427	±	30	448	±	30	270	±	17
		(+)	570	±	33	466	±	56	454	±	46	445	±	41
	Non-autoclaved	(-)	458	±	48	417	±	14	404	±	33	379	±	39
		(+)	520	±	36	516	±	9	529	±	30	481	±	68

P. indica inoculation as well as soil sterilization resulted in higher shoot B contents in both Nusrat and Saricanak-98 cultivars compared to corresponding control groups (non-inoculated and native soil, respectively). In terms of B toxicity, the shoot B

contents of plants belonging to either cultivar increased dose-dependently by increasing B levels (**Tables 3.11** and **3.12**).

The experimental plants inoculated with *P. indica* had significantly higher shoot P contents than the control (non-inoculated) groups in both bread and durum wheat cultivars. The experimental plants grown in sterilized soil exhibited higher shoot P contents compared to the plants grown in native soil for both cultivars. For both cultivars, increasing the B levels from the control to the low did not result in any change; however, the medium B level decreased P contents and the high level of B did not cause any further reductions (**Tables 3.11** and **3.13**).

Table 3.11: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot selected micronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	B ($\mu\text{g plant}^{-1}$)		P (mg plant^{-1})	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	16.43	<0.001	0.014
	Soil Sterilization (S)	1	<0.001	16.43	<0.001	0.014
	Boron Supply (B)	2	<0.001	30.78	0.002	0.026
	I X S	1	0.379	n.s.	0.242	n.s.
	I X B	2	0.006	52.87	0.738	n.s.
	S X B	2	<0.001	50.79	<0.001	0.046
	I X S X B	2	0.024	82.00	0.100	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	B ($\mu\text{g plant}^{-1}$)		P (mg plant^{-1})	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.009	16.03	0.016	0.012
	Soil Sterilization (S)	1	<0.001	16.03	<0.001	0.012
	Boron Supply (B)	2	<0.001	30.02	<0.001	0.023
	I X S	1	0.778	n.s.	0.184	n.s.
	I X B	2	0.383	n.s.	0.460	n.s.
	S X B	2	<0.001	50.55	<0.001	0.042
	I X S X B	2	0.528	n.s.	0.585	n.s.

Table 3.12: Effects of *P. indica* on the shoot B contents ($\mu\text{g plant}^{-1}$) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved or non-autoclaved soils at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; low B: $10 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; medium B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; high B: $30 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Shoot B Content ($\mu\text{g plant}^{-1}$)												
Cultivar	Soil Sterilization	<i>P. indica</i>	B Supply (mg kg^{-1})									
			1		10		20		30			
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved	(-)	7.6	$\pm$ 2.0	89.3	$\pm$ 19.9	282.2	$\pm$ 20.7	346.4	$\pm$ 80.1		
		(+)	9.5	$\pm$ 1.6	121.2	$\pm$ 18.1	301.2	$\pm$ 11.1	423.4	$\pm$ 51.7		
	Non-autoclaved	(-)	9.6	$\pm$ 1.0	103.9	$\pm$ 13.4	175.4	$\pm$ 33.7	203.8	$\pm$ 35.5		
		(+)	12.5	$\pm$ 1.9	105.2	$\pm$ 14.4	298.7	$\pm$ 33.9	264.3	$\pm$ 39.4		
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved	(-)	11.0	$\pm$ 0.8	111.5	$\pm$ 6.1	276.9	$\pm$ 32.7	379.6	$\pm$ 85.7		
		(+)	13.6	$\pm$ 1.8	120.9	$\pm$ 27.0	298.7	$\pm$ 24.9	441.2	$\pm$ 34.9		
	Non-autoclaved	(-)	7.8	$\pm$ 0.6	111.9	$\pm$ 27.9	186.5	$\pm$ 20.9	287.8	$\pm$ 39.4		
		(+)	10.7	$\pm$ 2.7	130.9	$\pm$ 5.6	225.2	$\pm$ 45.8	304.7	$\pm$ 19.2		

Table 3.13: Effects of *P. indica* on the shoot P contents (mg plant^{-1}) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved or non-autoclaved soils at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; low B: $10 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; medium B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$; high B: $30 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Shoot P Content (mg plant^{-1})												
Cultivar	Sterilization Status	<i>P. indica</i>	B Supply (mg kg^{-1})									
			1		10		20		30			
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved Soil	(-)	0.22	$\pm$ 0.02	0.26	$\pm$ 0.01	0.25	$\pm$ 0.06	0.25	$\pm$ 0.03		
		(+)	0.25	$\pm$ 0.03	0.33	$\pm$ 0.03	0.27	$\pm$ 0.02	0.28	$\pm$ 0.03		
	Non-autoclaved Soil	(-)	0.19	$\pm$ 0.03	0.16	$\pm$ 0.02	0.11	$\pm$ 0.02	0.09	$\pm$ 0.03		
		(+)	0.19	$\pm$ 0.01	0.16	$\pm$ 0.03	0.16	$\pm$ 0.01	0.11	$\pm$ 0.01		
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved Soil	(-)	0.25	$\pm$ 0.04	0.25	$\pm$ 0.02	0.25	$\pm$ 0.02	0.23	$\pm$ 0.02		
		(+)	0.28	$\pm$ 0.02	0.27	$\pm$ 0.03	0.25	$\pm$ 0.04	0.29	$\pm$ 0.04		
	Non-autoclaved Soil	(-)	0.16	$\pm$ 0.01	0.18	$\pm$ 0.04	0.12	$\pm$ 0.01	0.10	$\pm$ 0.02		
		(+)	0.17	$\pm$ 0.03	0.18	$\pm$ 0.04	0.12	$\pm$ 0.02	0.11	$\pm$ 0.01		

As shown in **Tables 3.14** and **3.15**, the experimental plants inoculated with the fungal inoculum displayed higher shoot Ca contents than those non-inoculated in both Nusrat and Saricanak-98 cultivars. Similar to the effect caused by endophytic

fungus, soil sterilization resulted in elevated shoot Ca contents in both cultivars. As regards to B toxicity, increasing the B levels led to dose-dependent reductions in either cultivar in general.

P. indica inoculation resulted in higher shoot K contents compared to the plants grown without the fungal inoculum in both cultivars. Similarly, the shoot K contents were significantly increased by soil sterilization in Nusrat as well as Saricanak-98. On the whole, the shoot K contents of both cultivars were reduced by increasing B levels in a dose-dependent manner (**Tables 3.14** and **3.15**).

The shoot Mg contents were increased by *P. indica* as well as soil sterilization in both bread and durum wheat cultivars. Overall, increasing the B levels from the control to the high level resulted in reduced shoot Mg contents in a dose-dependent manner in both cultivars (**Tables 3.14** and **3.15**).

In terms of the shoot S content, compared to corresponding control groups belonging to each treatment, *P. indica* and soil sterilization caused considerably higher contents in both bread and durum wheat cultivars. In bread wheat, increasing B levels from the control to the low level did not result in any difference; however, the medium B level lowered S contents and, the high B level did not cause any further reduction. In durum wheat, increasing B levels from the control to the medium reduced the S content; however, high level of B did not cause any further change (**Tables 3.14** and **3.15**).

Table 3.14: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot selected macronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	0.028	<0.001	0.156	<0.001	0.006	<0.001	0.012
<i>Triticum durum</i> cv. Saricanak-98	Soil Sterilization (S)	1	0.011	0.028	<0.001	0.156	<0.001	0.006	<0.001	0.012
	Boron Supply (B)	2	<0.001	0.053	<0.001	0.292	<0.001	0.01	<0.001	0.021
	I X S	1	0.426	n.s.	0.119	n.s.	0.374	n.s.	0.3514	n.s.
	I X B	2	0.032	n.s.	0.513	n.s.	0.341	n.s.	0.4219	n.s.
	S X B	2	0.442	0.088	<0.001	0.513	<0.001	0.019	<0.001	0.038
	I X S X B	2	0.603	n.s.	0.013	n.s.	0.337	n.s.	0.4576	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	0.0241	0.007	0.102	<0.001	0.004	<0.001	0.010
<i>Triticum durum</i> cv. Saricanak-98	Soil Sterilization (S)	1	<0.001	0.0241	<0.001	0.102	<0.001	0.004	<0.001	0.010
	Boron Supply (B)	2	<0.001	0.045	<0.001	0.194	<0.001	0.005	<0.001	0.018
	I X S	1	0.763	n.s.	0.530	n.s.	0.793	n.s.	0.335	n.s.
	I X B	2	0.125	n.s.	0.490	n.s.	0.184	n.s.	0.298	n.s.
	S X B	2	0.167	n.s.	0.040	n.s.	0.152	n.s.	0.003	0.031
	I X S X B	2	0.467	n.s.	0.568	n.s.	0.939	n.s.	0.239	n.s.

Table 3.15: Selected macronutrient contents of 41-day-old old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	0.44	± 0.07	2.66	± 0.24	0.12	± 0.02	0.18	± 0.03
			(+)	0.49	± 0.06	3.04	± 0.29	0.14	± 0.02	0.22	± 0.04
		Non-autoclaved	(-)	0.47	± 0.05	2.50	± 0.44	0.12	± 0.02	0.21	± 0.04
			(+)	0.49	± 0.01	2.40	± 0.08	0.15	± 0.01	0.24	± 0.02
	Low	Autoclaved	(-)	0.41	± 0.12	3.01	± 0.05	0.12	± 0.01	0.21	± 0.00
			(+)	0.49	± 0.06	3.85	± 0.44	0.14	± 0.01	0.27	± 0.03
		Non-autoclaved	(-)	0.36	± 0.04	2.30	± 0.13	0.09	± 0.01	0.15	± 0.01
			(+)	0.36	± 0.05	2.09	± 0.31	0.10	± 0.01	0.17	± 0.02
	Medium	Autoclaved	(-)	0.37	± 0.07	2.90	± 0.67	0.09	± 0.02	0.18	± 0.04
			(+)	0.44	± 0.05	3.22	± 0.11	0.11	± 0.01	0.23	± 0.02
		Non-autoclaved	(-)	0.31	± 0.02	1.51	± 0.23	0.06	± 0.01	0.11	± 0.02
			(+)	0.38	± 0.02	2.15	± 0.11	0.10	± 0.01	0.18	± 0.01
	High	Autoclaved	(-)	0.37	± 0.07	2.73	± 0.49	0.08	± 0.02	0.19	± 0.03
			(+)	0.37	± 0.03	2.95	± 0.25	0.10	± 0.01	0.22	± 0.02
		Non-autoclaved	(-)	0.26	± 0.06	1.24	± 0.20	0.04	± 0.01	0.10	± 0.02
			(+)	0.27	± 0.03	1.66	± 0.11	0.06	± 0.00	0.13	± 0.01
<i>Triticum durum</i> cv. Saricanak-98	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
	Control	Autoclaved	(-)	0.65	± 0.05	2.82	± 0.22	0.09	± 0.01	0.28	± 0.03
			(+)	0.76	± 0.02	2.98	± 0.14	0.11	± 0.01	0.32	± 0.01
		Non-autoclaved	(-)	0.45	± 0.03	1.87	± 0.09	0.06	± 0.00	0.20	± 0.01
			(+)	0.55	± 0.07	2.01	± 0.23	0.08	± 0.01	0.24	± 0.03
	Low	Autoclaved	(-)	0.62	± 0.05	2.78	± 0.17	0.09	± 0.00	0.25	± 0.02
			(+)	0.62	± 0.07	3.00	± 0.22	0.10	± 0.01	0.25	± 0.02
		Non-autoclaved	(-)	0.47	± 0.08	1.91	± 0.34	0.07	± 0.02	0.21	± 0.04
			(+)	0.53	± 0.05	2.08	± 0.33	0.08	± 0.01	0.24	± 0.03
	Medium	Autoclaved	(-)	0.50	± 0.04	2.68	± 0.15	0.07	± 0.01	0.19	± 0.02
			(+)	0.55	± 0.03	2.64	± 0.32	0.08	± 0.01	0.21	± 0.03
		Non-autoclaved	(-)	0.35	± 0.03	1.44	± 0.08	0.05	± 0.00	0.15	± 0.01
			(+)	0.35	± 0.04	1.51	± 0.16	0.06	± 0.01	0.15	± 0.01
	High	Autoclaved	(-)	0.44	± 0.05	2.36	± 0.27	0.06	± 0.01	0.18	± 0.02
			(+)	0.52	± 0.04	2.74	± 0.06	0.07	± 0.01	0.21	± 0.01
		Non-autoclaved	(-)	0.27	± 0.01	1.25	± 0.06	0.04	± 0.01	0.12	± 0.02
			(+)	0.32	± 0.08	1.32	± 0.13	0.05	± 0.00	0.12	± 0.01

As tackled in **Tables 3.16** and **3.17**, the shoot Cu contents were increased by not only *P. indica* but also by soil sterilization in both cultivars. Overall, increasing B levels resulted in dose-dependent decreases in the shoot B contents for either cultivar.

Compared to corresponding control groups for each cultivar, the presence of *P. indica* caused higher shoot Fe content in Nusrat whereas did not result in any significant difference in Saricanak-98. Experimental plants grown in autoclaved soil exhibited higher (almost doubled) shoot Fe contents for both cultivars compared to plants grown in native soil. Even though increasing B level from the control to the low level did not cause any significant change, at the medium level plants showed reduced shoot Fe contents and the high level did not cause any further change compared to medium level in both cultivars (**Tables 3.16** and **3.17**).

The shoot Mn contents were increased by *P. indica* as well as soil sterilization compared to corresponding control groups of both cultivars. Taken together, increasing B supply resulted in dose-dependent decreases in the shoot Mn contents in both Nusrat and Saricanak-98 cultivars (**Tables 3.16** and **3.17**).

Inoculated plants had higher shoot Zn content than non-inoculated ones in Nusrat whereas were not associated with any significant change in Saricanak-98. The experimental plants grown in autoclaved soil displayed dramatically (doubled) higher shoot Zn contents compared to plants grown in non-autoclaved soil in both Nusrat and Saricanak-98 cultivars. Overall, increasing B levels from the control to the high level resulted in reduced shoot Zn contents in a dose-dependent manner in either cultivar (**Tables 3.16** and **3.17**).

Table 3.16: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot selected micronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Cu ($\mu\text{g kg}^{-1}$)		Fe ($\mu\text{g kg}^{-1}$)		Mn ($\mu\text{g kg}^{-1}$)		Zn ($\mu\text{g kg}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	0.828	0.003	3.716	<0.001	6.16	<0.001	1.47
	Soil Sterilization (S)	1	<0.001	0.828	<0.001	3.716	<0.001	6.16	<0.001	1.47
	Boron Supply (B)	2	<0.001	1.533	<0.001	6.819	0.017	11.65	<0.001	2.73
	I X S	1	0.823	n.s.	0.656	n.s.	0.024	11.65	0.006	2.73
	I X B	2	0.344	n.s.	0.075	11.486	0.952	n.s.	0.7834	n.s.
	S X B	2	<0.001	2.584	<0.001	n.s.	0.019	20.00	0.006	4.60
	I X S X B	2	0.191	n.s.	0.141	n.s.	0.381	n.s.	0.031	7.41
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Cu ($\mu\text{g kg}^{-1}$)		Fe ($\mu\text{g kg}^{-1}$)		Mn ($\mu\text{g kg}^{-1}$)		Zn ($\mu\text{g kg}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	0.657	0.305	n.s.	<0.001	3.01	0.235	n.s.
	Soil Sterilization (S)	1	<0.001	0.657	<0.001	4.10	<0.001	3.01	<0.001	0.946
	Boron Supply (B)	2	<0.001	1.232	<0.001	7.59	<0.001	5.64	<0.001	1.79
	I X S	1	0.655	n.s.	0.578	n.s.	0.035	5.64	0.824	n.s.
	I X B	2	0.129	n.s.	0.376	n.s.	0.111	n.s.	0.160	n.s.
	S X B	2	0.029	2.075	0.912	n.s.	<0.001	9.49	0.039	2.90
	I X S X B	2	0.542	n.s.	0.911	n.s.	0.226	n.s.	0.030	5.05

Table 3.17: Selected micronutrient contents of 41-day-old old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Cu (µg kg ⁻¹)		Fe (µg kg ⁻¹)		Mn (µg kg ⁻¹)		Zn (µg kg ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	13.6	± 1.6	63	± 8	100	± 16	17.4	± 1.6
			(+)	15.9	± 1.9	65	± 4	117	± 15	22.6	± 3.3
		Non-autoclaved	(-)	13.2	± 2.6	58	± 12	26	± 6	13.7	± 2.6
			(+)	14.7	± 0.8	58	± 7	27	± 3	14.3	± 1.6
	Low	Autoclaved	(-)	14.7	± 0.4	65	± 9	111	± 2	18.4	± 4.8
			(+)	17.6	± 1.6	79	± 12	139	± 23	28.7	± 5.4
		Non-autoclaved	(-)	9.6	± 0.5	40	± 4	25	± 2	12.8	± 1.9
			(+)	10.7	± 1.5	44	± 5	22	± 3	11.7	± 1.7
	Medium	Autoclaved	(-)	13.8	± 3.3	47	± 11	99	± 25	19.6	± 5.1
			(+)	15.9	± 1.2	56	± 5	112	± 7	22.3	± 1.7
		Non-autoclaved	(-)	7.4	± 1.0	22	± 5	23	± 2	7.0	± 1.4
			(+)	12.6	± 0.8	38	± 5	36	± 4	10.0	± 1.5
	High	Autoclaved	(-)	13.7	± 2.6	54	± 7	90	± 19	16.7	± 3.1
			(+)	15.3	± 1.1	49	± 5	105	± 11	20.0	± 2.7
		Non-autoclaved	(-)	6.5	± 0.9	22	± 5	18	± 3	4.6	± 0.6
			(+)	8.6	± 0.6	30	± 3	24	± 2	6.8	± 0.7
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	16.2	± 1.6	60	± 11	105	± 12	19.8	± 1.5
			(+)	18.5	± 1.2	71	± 15	124	± 9	22.5	± 1.4
		Non-autoclaved	(-)	9.8	± 0.5	41	± 6	14	± 1	12.5	± 0.6
			(+)	12.3	± 1.8	45	± 10	18	± 2	12.0	± 2.2
	Low	Autoclaved	(-)	15.3	± 0.9	58	± 4	103	± 4	24.5	± 4.4
			(+)	15.4	± 1.5	63	± 10	104	± 9	21.1	± 0.8
		Non-autoclaved	(-)	11.0	± 2.2	41	± 8	16	± 2	10.2	± 2.6
			(+)	12.5	± 1.5	43	± 9	19	± 2	11.1	± 1.7
	Medium	Autoclaved	(-)	13.6	± 1.3	47	± 6	84	± 8	19.2	± 1.1
			(+)	13.4	± 1.5	43	± 8	89	± 6	19.3	± 1.7
		Non-autoclaved	(-)	9.2	± 0.3	27	± 5	13	± 1	6.0	± 0.9
			(+)	9.9	± 1.0	26	± 6	13	± 1	7.7	± 1.3
	High	Autoclaved	(-)	12.5	± 1.5	42	± 6	70	± 7	14.8	± 2.3
			(+)	14.2	± 0.9	44	± 6	82	± 7	17.2	± 2.3
		Non-autoclaved	(-)	8.3	± 1.2	24	± 4	11	± 3	5.2	± 0.7
			(+)	8.8	± 0.6	23	± 2	14	± 1	5.7	± 0.7

The shoot CAT activities of both cultivars were not affected by the endophytic fungus. Soil sterilization, in both cultivars, reduced the shoot CAT activities. On the other hand, B toxicity did not cause any significant effects on the shoot CAT activities (**Tables 3.18 and 3.19**).

Even though the experimental plants inoculated with *P. indica* displayed higher shoot SOD activity than non-inoculated plants in Nusrat, the fungus did not cause any significant difference in Saricanak-98. Soil sterilization did not cause any significant effects on the shoot SOD activities compared to the plants grown in native soil in both cultivars. For Nusrat, increasing B level from the control to the low level did not change the shoot SOD activity, the activity increased at medium B level, and increasing B level from the medium level to the high did not cause any further change. For Saricanak-98, on the other hand, increasing B level resulted in inconsistent shoot SOD activity (**Tables 3.18 and 3.19**).

As showed in **Tables 3.18 and 3.19**, the shoot GRX activity was increased by *P. indica* in durum wheat but was not associated with the fungal inoculum in bread wheat. Soil sterilization did not have any significant effects on the shoot GRX activities in both cultivars. In Nusrat, increasing B level from the control to the low level increased the shoot GRX activity; nevertheless, the medium and high levels did not cause any further change. In Saricanak-98, increasing B level did not cause any significant effect on the shoot GRX activity.

Although *P. indica* increased the shoot APX activity in Nusrat, it had no effect on that of Saricanak-98. Similar to the fungal inoculum, soil sterilization resulted in elevated shoot APX activity in Nusrat, but it had no effect on that of Saricanak-98. Increasing B toxicity did not cause consistent shoot APX activities in both Nusrat and Saricanak-98 (**Tables 3.18 and 3.19**).

Table 3.18: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot selected antioxidant enzyme activities of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	CAT Act. (-mmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)		SOD Act. (U g ⁻¹ FW)		GR Act. (μmol NADPH g ⁻¹ FW min ⁻¹)		APX Act. (μmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.088	n.s.	0.043	12.94	0.060	n.s.	0.040	4.15
	Soil Sterilization (S)	1	0.010	0.52	0.900	n.s.	0.519	n.s.	0.003	4.15
	Boron Supply (B)	3	0.271	n.s.	<0.001	24.53	0.012	0.67	0.010	7.91
	I X S	1	0.117	n.s.	0.008	24.53	0.020	0.67	0.141	n.s.
	I X B	3	0.266	n.s.	0.003	40.57	0.010	1.20	0.015	13.38
	S X B	3	0.376	n.s.	0.010	40.57	0.433	n.s.	<0.001	12.89
	I X S X B	3	0.237	n.s.	0.014	62.94	<0.001	1.87	0.146	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	CAT Act. (-mmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)		SOD Act. (U g ⁻¹ FW)		GR Act. (μmol NADPH g ⁻¹ FW min ⁻¹)		APX Act. (μmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.160	n.s.	0.112	n.s.	0.003	0.37	0.342	n.s.
	Soil Sterilization (S)	1	<0.001	0.82	0.107	n.s.	0.164	n.s.	0.070	n.s.
	Boron Supply (B)	3	0.337	n.s.	<0.001	24.69	0.329	n.s.	0.022	8.57
	I X S	1	0.367	n.s.	0.055	n.s.	0.024	0.68	0.030	8.89
	I X B	3	0.019	1.62	<0.001	43.31	0.005	1.18	0.480	n.s.
	S X B	3	0.054	n.s.	0.011	41.59	<0.001	1.18	0.002	14.43
	I X S X B	3	0.436	n.s.	<0.001	72.36	0.003	1.84	0.002	24.17

Table 3.19: Selected antioxidant enzyme activities of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	CAT Act.		SOD Act.		GR Act.		APX Act.	
				(-mmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)		(U g ⁻¹ FW)		(μmol NADPH g ⁻¹ FW min ⁻¹)		(μmol H ₂ O ₂ g ⁻¹ FW min ⁻¹)	
Triticum aestivum cv. Nusrat	Control	Autoclaved	(-)	2.35	± 0.44	151	± 12	2.2	± 0.4	45.4	± 7.8
			(+)	3.44	± 0.16	219	± 7	3.4	± 1.0	50.8	± 6.4
		Non-autoclaved	(-)	3.94	± 1.29	153	± 47	3.1	± 0.6	20.5	± 4.0
			(+)	4.78	± 1.18	156	± 35	2.6	± 0.3	34.2	± 3.3
	Low	Autoclaved	(-)	3.43	± 1.52	116	± 15	2.8	± 0.2	35.2	± 6.5
			(+)	3.05	± 0.64	195	± 16	2.5	± 0.8	36.3	± 7.7
		Non-autoclaved	(-)	3.74	± 1.13	159	± 28	1.5	± 0.4	30.9	± 10.0
			(+)	3.39	± 1.29	168	± 14	2.7	± 1.2	38.5	± 9.8
	Medium	Autoclaved	(-)	2.27	± 0.23	135	± 13	2.2	± 0.3	31.7	± 4.0
			(+)	2.88	± 0.51	95	± 21	1.3	± 0.0	33.0	± 5.5
		Non-autoclaved	(-)	3.12	± 1.50	153	± 37	2.5	± 0.3	13.2	± 4.7
			(+)	3.32	± 0.61	143	± 16	2.2	± 0.4	33.0	± 2.1
	High	Autoclaved	(-)	1.96	± 0.38	128	± 32	3.4	± 0.4	31.0	± 8.4
			(+)	4.14	± 1.65	145	± 37	0.8	± 0.2	28.4	± 9.4
		Non-autoclaved	(-)	3.70	± 0.41	124	± 21	2.4	± 0.5	40.4	± 15.8
			(+)	3.16	± 1.18	115	± 12	2.1	± 0.5	28.9	± 4.6
Triticum durum cv. Saricanak-98	Control	Autoclaved	(-)	5.92	± 2.18	85	± 3	1.7	± 0.4	23.6	± 5.0
			(+)	5.79	± 0.94	210	± 15	2.9	± 1.2	41.1	± 13.5
		Non-autoclaved	(-)	6.71	± 1.51	159	± 32	2.7	± 0.4	52.9	± 6.5
			(+)	5.26	± 1.43	204	± 36	4.8	± 1.3	40.9	± 5.2
	Low	Autoclaved	(-)	6.90	± 1.67	195	± 16	3.0	± 1.2	47.1	± 10.3
			(+)	3.53	± 0.51	157	± 13	3.1	± 1.1	35.3	± 12.1
		Non-autoclaved	(-)	8.68	± 2.86	182	± 18	3.5	± 0.4	41.5	± 1.8
			(+)	6.90	± 1.57	178	± 22	3.9	± 0.5	45.3	± 6.8
	Medium	Autoclaved	(-)	4.62	± 0.81	151	± 37	2.3	± 0.3	24.9	± 4.4
			(+)	5.21	± 0.55	95	± 31	3.1	± 0.4	30.1	± 9.8
		Non-autoclaved	(-)	8.39	± 1.37	158	± 13	3.7	± 0.7	36.4	± 12.1
			(+)	7.49	± 0.48	153	± 19	2.4	± 0.3	41.6	± 12.4
	High	Autoclaved	(-)	4.49	± 1.06	153	± 17	2.9	± 0.5	38.3	± 10.6
			(+)	6.54	± 0.28	241	± 34	4.7	± 0.7	57.1	± 8.5
		Non-autoclaved	(-)	8.35	± 3.04	184	± 10	2.7	± 0.4	41.0	± 4.4
			(+)	8.65	± 2.12	138	± 26	2.0	± 0.6	32.3	± 1.1

The shoot TEAC levels were reduced by *P. indica* in both bread and durum wheat cultivars. The experimental plants grown in sterilized soil had a decreased shoot

TEAC level than those grown in native soil in bread wheat; however, soil sterilization was not associated with the shoot TEAC level in durum wheat. B toxicity did not have any effect on the shoot TEAC levels of both cultivars (**Tables 3.20 and 3.21**).

The presence of *P. indica* caused lower GB levels compared to the plants grown in the absence of fungal inoculum in both Nusrat and Saricanak-98. The shoot GB level was significantly reduced by soil sterilization in Nusrat whereas increased in Saricanak-98. Increasing B levels from the control to the medium level did not cause any significant change and only the high level resulted in increased shoot GB levels in both cultivars (**Tables 3.20 and 3.21**).

Although the proline concentration was reduced by *P. indica* in bread wheat, increased in durum wheat. The experimental plants grown in sterilized soil had significantly lower proline concentration than those grown in non-sterilized soil in bread wheat; however, soil sterilization was not associated with the proline concentration in durum wheat. In terms of B toxicity, the proline concentration of Nusrat cultivar was increased by increasing the B levels. For Saricanak-98, increasing the B levels from the control to the low significantly increased the proline concentration; however, the medium B level did not cause any significant change compared to the low level and the high level resulted in the highest proline concentration (**Tables 3.20 and 3.21**).

P. indica inoculation did not have any significant effects on the total protein concentrations in both Nusrat and Saricanak-98 cultivars. Even though the shoot protein concentration was reduced by soil sterilization in Nusrat, was not significantly affected in Saricanak-98. The shoot protein concentration of Nusrat was dose-dependently reduced by B toxicity; however, that of Saricanak-98 was not associated with B supply (**Tables 3.20 and 3.21**).

Table 3.20: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on total antioxidant capacity (mmol TE /100g FW), GB (%), proline (%) and protein concentrations (%) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Total Antiox. Cap. (mmol TE /100g FW)		GB Conc. (%)		Proline Conc. (%)		Protein Conc. (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	2.19	<0.001	0.036	<0.001	2.09	0.482	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Soil Sterilization (S)	1	<0.001	2.19	0.003	0.036	<0.001	2.09	0.013	0.200
	Boron Supply (B)	3	0.532	n.s.	0.001	0.069	<0.001	3.84	<0.001	0.370
	I X S	1	0.188	n.s.	0.042	0.069	<0.001	3.92	0.563	n.s.
	I X B	3	0.158	n.s.	0.729	n.s.	<0.001	6.73	0.112	n.s.
	S X B	3	0.012	7.39	0.008	0.117	<0.001	6.73	0.697	n.s.
	I X S X B	3	0.308	n.s.	0.231	n.s.	<0.001	11.27	0.405	n.s.
	Source of Variation	DF	Total Antiox. Cap. (mmol TE /100g FW)		GB Conc. (%)		Proline Conc. (%)		Protein Conc. (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.002	1.88	0.0326	0.038	0.022	1.7	0.1304	n.s.
	Soil Sterilization (S)	1	0.425	n.s.	<0.001	0.038	0.066	n.s.	0.565	n.s.
	Boron Supply (B)	3	0.091	n.s.	<0.001	0.074	<0.001	3.18	0.352	n.s.
	I X S	1	0.081	n.s.	0.890	n.s.	0.927	n.s.	0.271	n.s.
	I X B	3	0.211	n.s.	0.392	n.s.	0.075	n.s.	<0.001	0.91
	S X B	3	0.008	6.08	0.003	0.123	0.101	n.s.	<0.001	0.91
	I X S X B	3	0.731	n.s.	0.215	n.s.	0.012	n.s.	0.986	n.s.

Table 3.21: Total antioxidant capacity (mmol TE /100g FW), GB (%), proline (%) and protein concentrations (%) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Total Antiox. Cap.		GB Conc.		Proline Conc.		Protein Conc.	
				(mmol TE /100g FW)		(%)		(%)		(%)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	17.3	± 3.5	0.86	± 0.15	19.9	± 1.4	1.24	± 0.44
			(+)	12.8	± 3.5	0.79	± 0.08	22.0	± 1.1	1.93	± 0.56
		Non-autoclaved	(-)	17.9	± 1.9	1.01	± 0.04	20.3	± 0.8	1.65	± 0.11
			(+)	16.1	± 3.8	0.97	± 0.08	19.3	± 0.8	1.86	± 0.13
	Low	Autoclaved	(-)	14.6	± 3.6	0.97	± 0.06	20.0	± 1.7	1.15	± 0.36
			(+)	15.8	± 3.5	0.92	± 0.10	22.1	± 0.7	1.09	± 0.52
		Non-autoclaved	(-)	20.3	± 1.3	0.98	± 0.03	22.3	± 1.4	1.28	± 0.61
			(+)	18.6	± 2.3	0.89	± 0.06	22.5	± 1.0	1.61	± 0.78
	Medium	Autoclaved	(-)	17.8	± 1.3	0.94	± 0.10	22.3	± 1.3	0.91	± 0.29
			(+)	13.6	± 2.2	0.96	± 0.06	22.9	± 4.2	0.57	± 0.21
		Non-autoclaved	(-)	23.5	± 6.2	1.02	± 0.06	29.8	± 5.9	1.21	± 0.38
			(+)	17.6	± 3.9	0.91	± 0.07	22.3	± 1.3	1.21	± 0.38
	High	Autoclaved	(-)	12.7	± 2.6	1.00	± 0.04	21.9	± 3.1	0.82	± 0.19
			(+)	10.3	± 1.1	0.98	± 0.02	20.8	± 0.7	0.58	± 0.31
		Non-autoclaved	(-)	30.3	± 5.4	1.13	± 0.08	58.3	± 14.6	0.78	± 0.22
			(+)	18.2	± 5.8	0.95	± 0.07	26.9	± 6.1	1.11	± 0.34
<i>Triticum durum</i> cv. Saricanak-98	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Total Antiox. Cap.		GB Conc.		Proline Conc.		Protein Conc.	
				(mmol TE /100g FW)		(%)		(%)		(%)	
	Control	Autoclaved	(-)	11.4	± 1.8	0.79	± 0.05	20.0	± 0.6	0.82	± 0.28
			(+)	11.5	± 1.4	0.75	± 0.04	19.9	± 1.4	1.94	± 0.95
		Non-autoclaved	(-)	17.4	± 3.8	0.86	± 0.11	20.3	± 1.8	1.61	± 0.21
			(+)	16.1	± 3.2	0.73	± 0.06	22.3	± 0.9	3.14	± 0.90
	Low	Autoclaved	(-)	16.2	± 4.1	0.90	± 0.05	22.3	± 3.3	2.70	± 0.48
			(+)	12.1	± 2.5	0.85	± 0.03	23.6	± 1.0	1.62	± 0.14
		Non-autoclaved	(-)	17.6	± 6.0	0.69	± 0.14	25.0	± 1.9	2.60	± 0.39
			(+)	10.7	± 0.7	0.70	± 0.06	25.1	± 2.2	1.93	± 0.40
	Medium	Autoclaved	(-)	16.3	± 3.8	0.92	± 0.04	25.4	± 1.2	1.73	± 0.37
			(+)	15.9	± 2.8	0.80	± 0.07	30.0	± 2.9	2.09	± 0.55
		Non-autoclaved	(-)	19.4	± 2.2	0.71	± 0.08	27.9	± 2.8	1.90	± 0.44
			(+)	16.9	± 16.9	0.69	± 0.09	24.2	± 1.0	2.40	± 0.87
	High	Autoclaved	(-)	18.0	± 2.3	0.98	± 0.07	32.8	± 3.4	2.46	± 0.37
			(+)	17.1	± 2.8	1.02	± 0.10	34.8	± 4.6	2.31	± 0.92
		Non-autoclaved	(-)	17.2	± 1.8	0.89	± 0.08	33.3	± 5.4	1.28	± 0.17
			(+)	9.3	± 1.2	0.85	± 0.09	43.2	± 8.7	1.50	± 0.96

As regards to POX activity, the presence of endophytic fungus resulted in higher shoot activity in Nusrat but did not have any significant effect on that of Saricanak-98. The shoot POX activity of Saricanak-98 was enhanced by soil sterilization compared to those grown in native soil whereas that of Nusrat was not associated with soil sterilization. In terms of B toxicity, the shoot POX activity of Nusrat was only increased at the high level. B toxicity did not have any significant effect on the shoot POX activity in Saricanak-98 (**Tables 3.22** and **3.23**).

According to the collective data in **Tables 3.22** and **3.24**, although the shoot PAL level was not associated with *P. indica* in Nusrat, reduced by the fungal inoculum in Saricanak-98. Soil sterilization did not have any effect on the shoot PAL levels in either cultivar. Increasing B levels did not cause consistent shoot PAL activities. Control, low and high B levels did not cause any considerable difference; however, the medium levels of both cultivars caused elevated shoot PAL activities.

Table 3.22: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on shoot selected activities belonging to POX (mmol Tetraguaiacol g⁻¹ FW min⁻¹) and PAL (µg g⁻¹ FW hour⁻¹) activities of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 41 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	POX. Act. (mmol Tetraguaiacol g ⁻¹ FW min ⁻¹)		Transsinnamic Acid (µg g ⁻¹ FW hour ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.016	0.86	0.091	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Soil Sterilization (S)	1	0.182	n.s.	0.079	n.s.
	Boron Supply (B)	3	0.004	1.64	<0.001	3.61
	I X S	1	0.122	n.s.	0.805	n.s.
	I X B	3	0.115	n.s.	0.211	n.s.
	S X B	3	<0.001	2.74	0.603	n.s.
	I X S X B	3	0.013	4.47	0.866	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of variation	DF	POX. Act. (mmol Tetraguaiacol g ⁻¹ FW min ⁻¹)		Transsinnamic Acid (µg g ⁻¹ FW hour ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.432	n.s.	0.029	2.80
	Soil Sterilization (S)	1	<0.001	0.84	0.222	n.s.
	Boron Supply (B)	3	0.779	n.s.	<0.001	5.20
	I X S	1	0.607	n.s.	0.476	n.s.
	I X B	3	0.834	n.s.	0.501	n.s.
	S X B	3	<0.001	2.68	0.130	n.s.
	I X S X B	3	0.571	n.s.	0.617	n.s.

Table 3.23: Effects of *P. indica* on peroxidase activity (mmol Tetraguaiacol g⁻¹ FW min⁻¹) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved or non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

POX. Act. (mmol Tetraguaiacol g ⁻¹ FW min ⁻¹)														
Cultivar	Soil Sterilization	<i>P. indica</i>	B Supply (mg kg ⁻¹)											
			1			10			20			30		
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved	(-)	9.9	±	0.7	9.6	±	2.5	9.7	±	0.8	12.3	±	3.5
		(+)	10.0	±	1.1	9.9	±	1.1	10.3	±	1.4	18.5	±	3.1
	Non-autoclaved	(-)	11.1	±	0.8	10.6	±	1.1	10.8	±	1.9	9.5	±	1.4
		(+)	13.0	±	1.4	10.7	±	1.0	10.8	±	2.2	9.2	±	1.0
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved	(-)	14.0	±	1.6	14.0	±	1.5	13.3	±	3.2	12.1	±	0.5
		(+)	15.3	±	2.8	14.5	±	2.6	12.2	±	1.2	11.8	±	1.8
	Non-autoclaved	(-)	7.7	±	1.0	7.9	±	0.9	10.0	±	1.5	11.2	±	1.2
		(+)	8.2	±	0.9	8.2	±	2.1	11.4	±	0.8	11.1	±	1.3

Table 3.24: Effects of *P. indica* on the phenylalanine ammonia-lyase (PAL) activity (µg g⁻¹ FW hour⁻¹) of 41-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown in autoclaved or non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; medium B: 20 mg kg⁻¹ H₃BO₃; high B: 30 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Transsinnamic Acid (µg g ⁻¹ FW hour ⁻¹)														
Cultivar	Soil Sterilization	<i>P. indica</i>	B Supply (mg kg ⁻¹)											
			1			10			20			30		
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved	(-)	23.2	±	4.5	28.4	±	3.0	30.0	±	1.8	25.8	±	4.9
		(+)	23.9	±	5.1	27.8	±	1.8	25.4	±	3.5	24.6	±	3.8
	Non-autoclaved	(-)	25.5	±	2.7	28.3	±	0.7	34.3	±	8.3	27.3	±	3.0
		(+)	24.2	±	2.1	29.9	±	1.7	29.1	±	1.9	24.5	±	5.2
<i>Triticum durum</i> cv. Saricanak-98	Autoclaved	(-)	26.5	±	3.3	29.5	±	5.1	35.5	±	7.7	27.6	±	5.2
		(+)	22.4	±	4.9	25.6	±	6.8	26.9	±	5.0	27.6	±	1.8
	Non-autoclaved	(-)	21.5	±	2.1	21.1	±	2.8	32.3	±	6.5	33.2	±	11.4
		(+)	21.9	±	4.2	21.7	±	4.4	27.2	±	4.3	28.8	±	5.6

3.2. Second Soil Experiment

The experimental plants belonging to the second experiment in which the plants were grown to full maturity showed in **Figure 3.10**. As displayed in **Figure 3.10**, the general condition and visual appearance of the plants altered according to the treatments at 50 DAS. Inoculation with *P. indica* enhanced the plant vigour evidently in both cultivars. For both cultivars, in non-autoclaved soil, increasing the B level from control to medium resulted in typical B toxicity symptoms, encompassing chlorosis and necrosis which embarked from the tips of mature leaves. Moreover, in Saricanak-98, which was developmentally ahead of Nusrat at 50 DAS, B toxicity also impaired stem elongation (**Figure 3.10C, D**).



Figure 3.10: Effects of *P. indica* on 50-day-old bread wheat (*Triticum aestivum* cv. Nusrat) (A-B) and durum wheat (*Triticum durum* cv. Saricanak-98) (C-D) grown in non-autoclaved soil at control B (1 mg kg⁻¹) and severe B toxicity (20 mg kg⁻¹) levels under greenhouse conditions.

As revealed by four-way ANOVA, all single effect of treatments and double interaction of soil sterilization and B supply affected the grain B concentration in Nusrat. In the case of Saricanak-98, the single effect of soil sterilization and B supply

affected the grain B concentration (**Table 3.25**). In more detail, *P. indica* inoculation reduced the grain B concentration in bread wheat; however, did not significantly affect that of durum wheat. For both cultivars, soil sterilization led to reduced grain B concentrations. As regards to B toxicity, increasing B levels from control to high caused dose-dependent elevations in both cultivars (**Table 3.25** and **Figure 3.11**).

Table 3.25: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on grain B concentrations (mg kg⁻¹), straw DW (g), grain number (plant⁻¹) and grain yield (g) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

Triticum aestivum cv. Nusrat	Source of Variation	DF	Grain B Conc. (mg kg ⁻¹)		Straw DW (g)		Grain Number (plant ⁻¹)		Grain Yield plant ⁻¹ (g)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.220	n.s.	0.001	0.117	0.003	3.463	0.595	n.s.
Triticum durum cv. Saricanak-98	Soil Sterilization (S)	1	0.066	n.s.	<0.001	0.117	0.004	3.463	<0.001	0.125
	Boron Supply (B)	2	<0.001	0.212	0.567	n.s.	0.006	5.110	<0.001	0.185
	I X S	1	0.163	n.s.	0.120	n.s.	0.363	n.s.	0.145	n.s.
	I X B	2	0.244	n.s.	0.207	n.s.	0.033	8.897	0.342	n.s.
	S X B	2	0.013	0.372	0.523	n.s.	0.379	n.s.	0.250	n.s.
	I X S X B	2	0.340	n.s.	0.977	n.s.	0.181	n.s.	0.970	n.s.
	Source of Variation	DF	Grain B Conc. (mg kg ⁻¹)		Straw DW (g)		Grain Number (plant ⁻¹)		Grain Yield plant ⁻¹ (g)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
Triticum durum cv. Saricanak-98	Inoculation (I)	1	0.022	0.412	0.041	0.231	0.011	3.463	0.385	n.s.
	Soil Sterilization (S)	1	0.001	0.412	<0.001	0.231	<0.001	3.463	<0.001	0.150
	Boron Supply (B)	2	<0.001	0.611	0.608	n.s.	<0.001	5.112	<0.001	0.219
	I X S	1	0.643	n.s.	0.410	n.s.	0.719	n.s.	0.889	n.s.
	I X B	2	0.162	n.s.	0.462	n.s.	0.341	n.s.	0.402	n.s.
	S X B	2	0.021	1.045	0.602	n.s.	0.124	n.s.	0.648	n.s.
	I X S X B	2	0.840	n.s.	0.308	n.s.	0.497	n.s.	0.682	n.s.

According to ANOVA, the straw dry weights were significantly affected by the single effects of *P. indica* inoculation and soil sterilization in both cultivars (**Table 3.25**). In this study, the experimental plants inoculated with *P. indica* displayed

elevated straw dry weights compared to those non-inoculated with *P. indica* in both cultivars. Similarly, the plants grown in sterilized soils showed increased straw dry matters compared to those grown in native soils for both cultivars. In response to B supply, the straw dry weights of the tested cultivars did not change (**Tables 3.25 and Figure 3.12**).

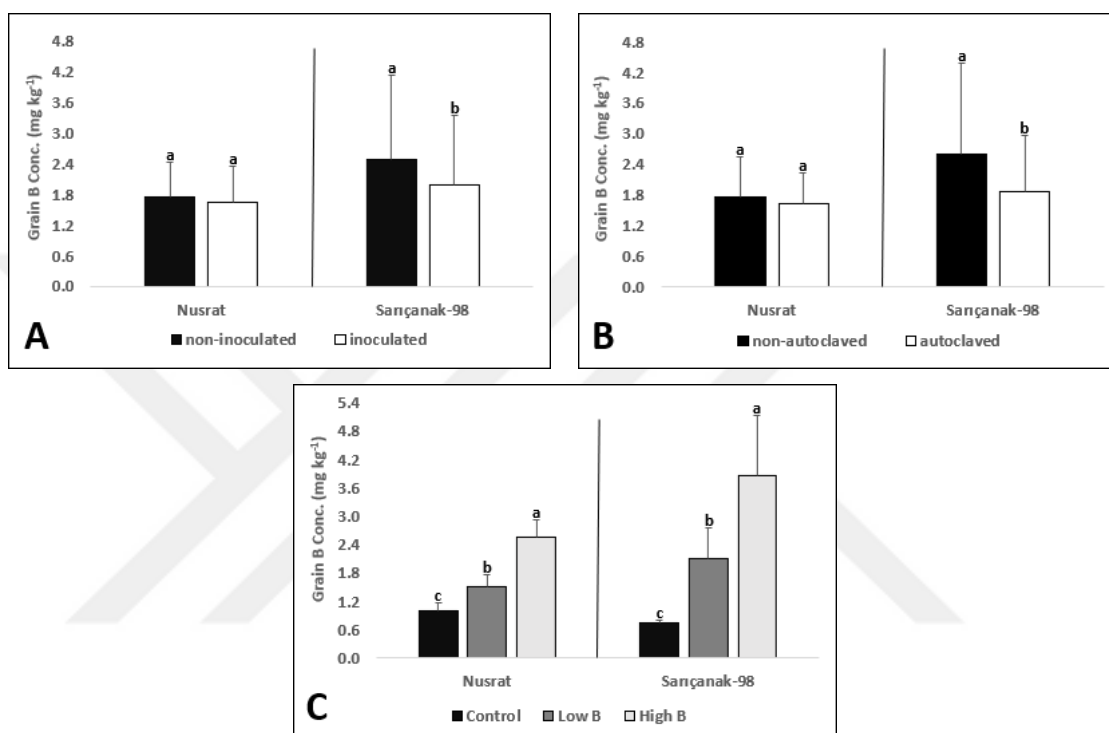


Figure 3.11: The grain B concentrations (mg kg⁻¹) of mature bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Sarıçanak-98) plants as affected by *P. indica* (A), soil sterilization (B) and B toxicity (C) (control: 1 mg kg⁻¹; low B toxicity: 10 mg kg⁻¹; high B toxicity: 20 mg kg⁻¹) under greenhouse conditions. Different letters indicate significant differences between means according to Tukey's HSD test ($p \leq 0.05$). Statistical analysis was performed separately for Nusrat and Sarıçanak-98. Statistical analysis was performed separately for Nusrat and Sarıçanak-98.

According to ANOVA shown in **Table 3.25**, all single effects of treatments and only double interaction of *P. indica* and B supply affected the grain number in Nusrat. In Sarıçanak-98, however, all single effects of treatments affected the grain number (**Table 3.25**). In terms of fungal inoculation, *P. indica* significantly increased the grain numbers in both cultivars. Similar to fungal inoculation, soil sterilization resulted in elevated grain numbers in both cultivars. For both cultivars, increasing B

levels significantly decreased the grain numbers in a dose-dependent manner (**Table 3.25** and **Figure 3.13**).

As revealed by four-way ANOVA, for both cultivars, the single effects of soil sterilization and B toxicity significantly affected the grain yield (**Table 3.25**). In this study, the grain yield of neither bread nor durum wheat was affected by *P. indica* inoculation. On the other hand, for both cultivars, the experimental plants grown in sterile soil displayed elevated grain yields compared to those grown in native soil. In terms of B toxicity, increasing B level from control to high decreased the grain yield dose-dependently in Nusrat. In Saricanak-98, however, only high level of B caused a reduction compared to control group (**Table 3.25** and **Figure 3.14**).

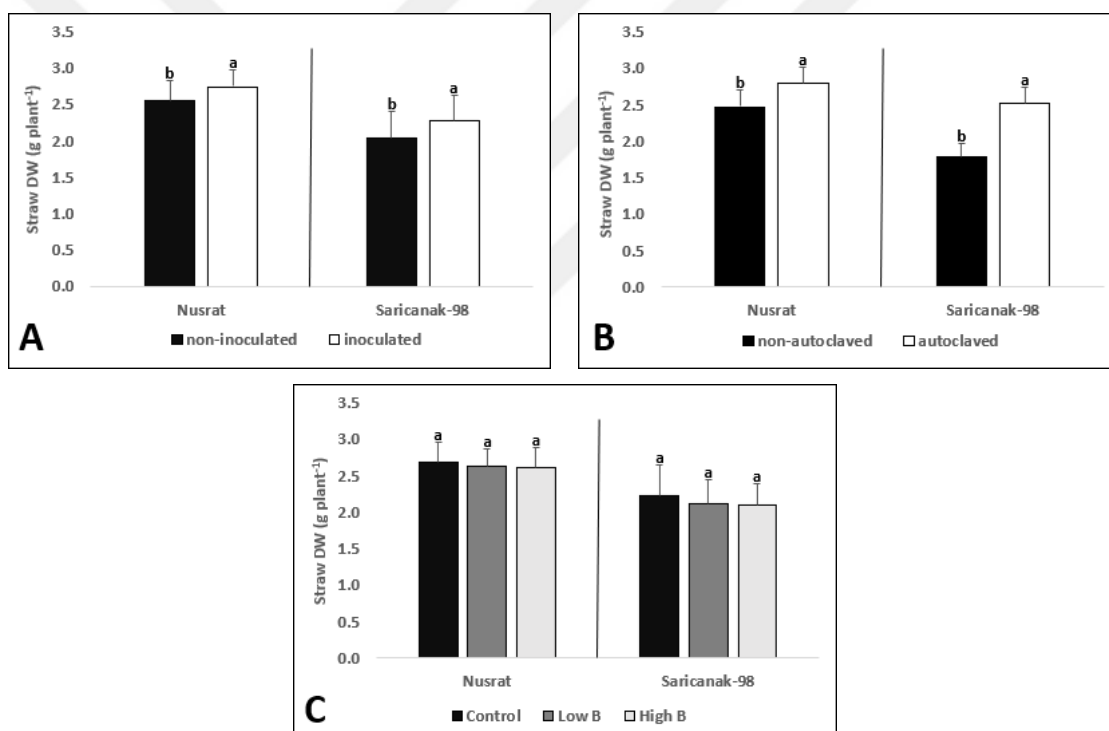


Figure 3.12: Straw dry weight (g plant⁻¹) of mature bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants as affected by *P. indica* (A), soil sterilization (B) and B toxicity (C) (control: 1 mg kg⁻¹; low B toxicity: 10 mg kg⁻¹; high B toxicity: 20 mg kg⁻¹) under greenhouse conditions. Different letters indicate significant differences between means according to Tukey's HSD test ($p \leq 0.05$). Statistical analysis was performed separately for Nusrat and Saricanak-98.

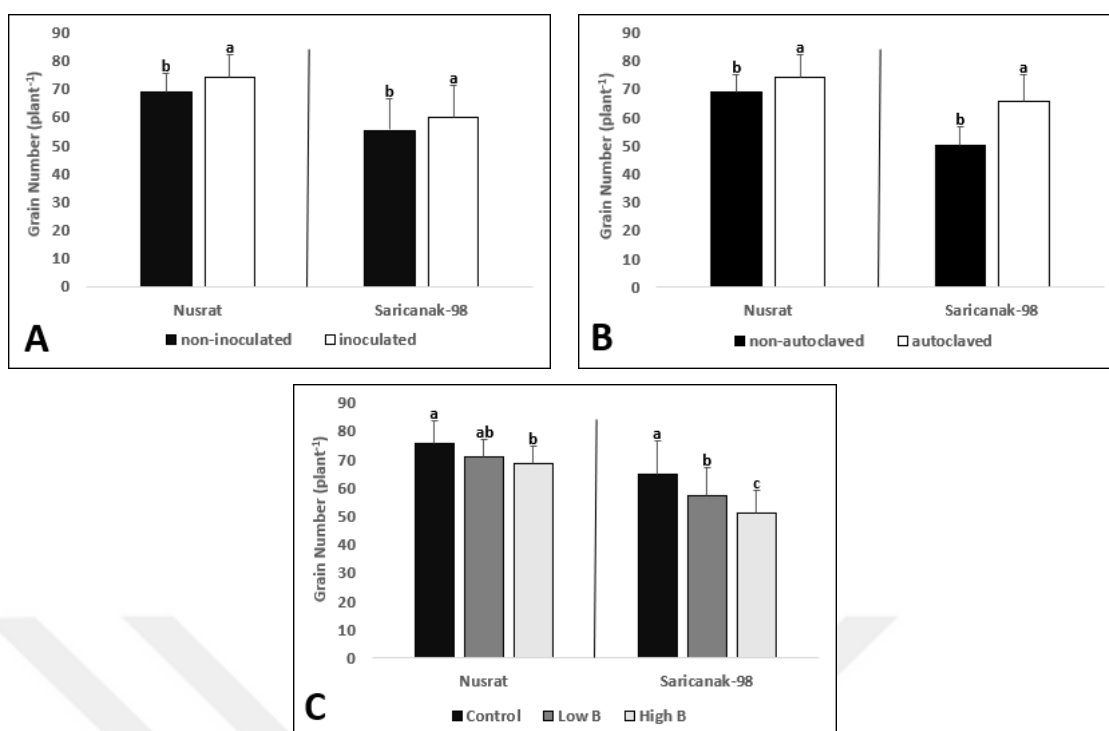


Figure 3.13: Grain number (plant⁻¹) of mature bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants as affected by *P. indica* (A), soil sterilization (B) and B toxicity (C) (control: 1 mg kg⁻¹; low B toxicity: 10 mg kg⁻¹; high B toxicity: 20 mg kg⁻¹) under greenhouse conditions. Different letters indicate significant differences between means according to Tukey's HSD test ($p \leq 0.05$). Statistical analysis was performed separately for Nusrat and Saricanak-98.

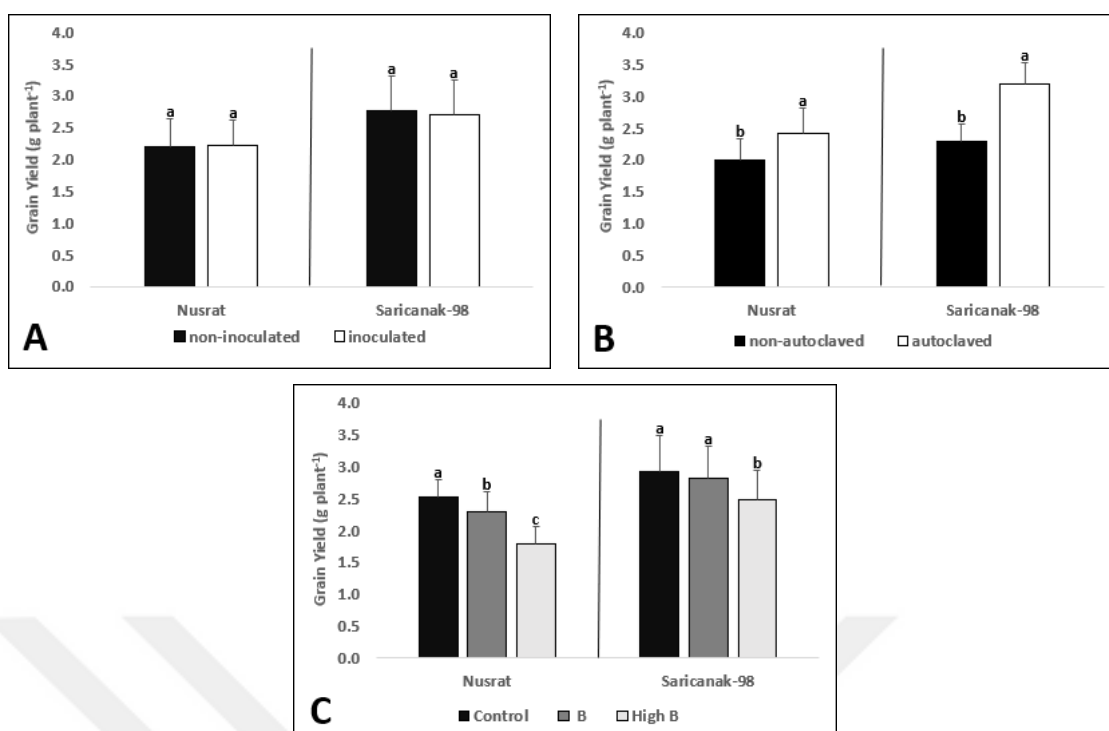


Figure 3.14: Grain yield (g plant⁻¹) of mature bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants as affected by *P. indica* (A), soil sterilization (B) and B toxicity (C) (control: 1 mg kg⁻¹; low B toxicity: 10 mg kg⁻¹; high B toxicity: 20 mg kg⁻¹) under greenhouse conditions. Different letters indicate significant differences between means according to Tukey's HSD test ($p \leq 0.05$). Statistical analysis was performed separately for Nusrat and Saricanak-98.

According to ANOVA, all single effects of treatments and only double interaction of soil sterilization and B supply affected the straw B concentration in bread wheat. In durum wheat, single effects of the soil sterilization and B supply affected the straw B concentration (**Table 3.26**). The presence of *P. indica* reduced the straw B concentration in Nusrat and did not affect that of Saricanak-98. For both cultivars, soil sterilization resulted in reduced straw B concentrations. Regarding B toxicity, increasing B increased the straw B concentrations dose-dependently in both cultivars (**Table 3.26** and **Figure 3.15**).

This study revealed that the straw P concentration of both cultivars was not considerably affected by the endophytic fungus (**Tables 3.26** and **3.27**).

Table 3.26: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected straw and grain concentrations and 1000 Kernel Weight (mg) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores

Triticum aestivum cv. Nusrat	Straw Mineral Concentrations						Grain Mineral Concentration			
	Source of Variation	DF	B (mg kg ⁻¹)		P (%)		P (%)		1000 Kernel Weight (mg)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.001	19	0.592	n.s.	0.543	n.s.	0.080	n.s.
	Soil Sterilization (S)	1	<0.001	19	<0.001	0.003	<0.001	0.018	<0.001	1.90
	Boron Supply (B)	2	<0.001	28	<0.001	0.002	<0.001	0.028	<0.001	2.81
	I X S	1	0.535	n.s.	0.015	0.005	0.022	0.04	0.522	n.s.
	I X B	2	0.188	n.s.	0.025	0.006	0.770	n.s.	0.615	n.s.
	S X B	2	<0.001	49	0.029	0.006	0.401	n.s.	0.741	n.s.
	I X S X B	2	0.251	n.s.	0.868	n.s.	0.213	n.s.	0.243	n.s.
Triticum durum cv. Saricanak-98	Source of Variation	DF	B (mg kg ⁻¹)		P (%)		P (%)		1000 Kernel Weight (mg)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.250	n.s.	0.072	n.s.	0.169	n.s.	0.004	1.73
	Soil Sterilization (S)	1	0.002	32	0.009	0.003	<0.001	0.02	0.002	1.73
	Boron Supply (B)	2	<0.001	48	<0.001	0.005	0.255	n.s.	0.002	2.56
	I X S	1	0.568	n.s.	0.016	0.007	0.026	0.04	0.946	n.s.
	I X B	2	0.075	n.s.	0.647	n.s.	0.545	n.s.	0.602	n.s.
	S X B	2	0.154	n.s.	<0.001	0.009	0.659	n.s.	0.157	n.s.
	I X S X B	2	0.747	n.s.	0.007	0.015	0.564	n.s.	0.395	n.s.

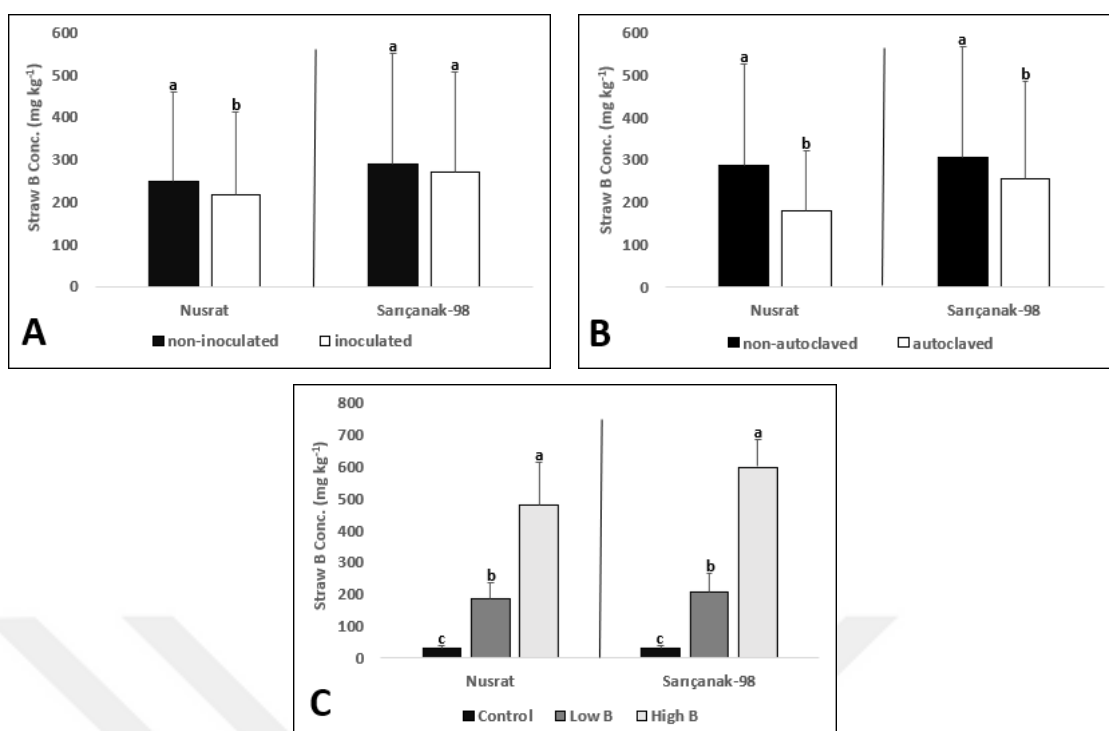


Figure 3.15: The straw B concentrations (mg kg⁻¹) of mature bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Sarıcanak-98) plants as affected by *P. indica* (A), soil sterilization (B) and B toxicity (C) (control: 1 mg kg⁻¹; low B toxicity: 10 mg kg⁻¹; high B toxicity: 20 mg kg⁻¹) under greenhouse conditions. Different letters indicate significant differences between means according to Tukey's HSD test ($p \leq 0.05$). Statistical analysis was performed separately for Nusrat and Sarıcanak-98. Statistical analysis was performed separately for Nusrat and Sarıcanak-98.

As displayed by four-way ANOVA, single effect of soil sterilization and B supply treatments and double interaction of *P. indica* inoculation and soil sterilization affected the grain P concentration in Nusrat. In Sarıcanak-98, only single effect of the soil sterilization and double interaction of *P. indica* inoculation and soil sterilization affected the grain P concentration (**Table 3.26**). The present study revealed that *P. indica* inoculated plants did not display significant effects in terms of grain P concentrations in both cultivars. The experimental plants grown in sterilized soil showed reduced grain P concentrations in both cultivars compared to the plants grown in native soil. Increasing the B supply increased the grain P concentration dose-dependently in Nusrat cultivar, but it did not have any significant change in Sarıcanak-98 (**Tables 3.26 and 3.28**).

Table 3.27: Straw P concentration of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Straw P Concentration (%)									
Cultivar	Sterilization Status	<i>P. indica</i>	B Supply (mg/ kg ⁻¹)						
			1			10			20
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved Soil	(-)	0.02	±	0.00	0.03	±	0.00	0.05 ± 0.01
		(+)	0.03	±	0.00	0.04	±	0.01	0.05 ± 0.01
	Non-autoclaved Soil	(-)	0.03	±	0.00	0.04	±	0.00	0.07 ± 0.00
		(+)	0.03	±	0.00	0.04	±	0.00	0.06 ± 0.00
<i>Triticum durum</i> cv. Saricanak	Autoclaved Soil	(-)	0.02	±	0.00	0.03	±	0.00	0.06 ± 0.01
		(+)	0.03	±	0.00	0.04	±	0.01	0.06 ± 0.01
	Non-autoclaved Soil	(-)	0.03	±	0.00	0.04	±	0.01	0.04 ± 0.01
		(+)	0.03	±	0.00	0.03	±	0.00	0.05 ± 0.01

Table 3.28: Grain P concentration of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Grain P Concentration (%)									
Cultivar	Sterilization Status	<i>P. indica</i>	B Supply (mg kg ⁻¹)						
			1			10			20
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved Soil	(-)	0.27	±	0.01	0.28	±	0.02	0.35 ± 0.05
		(+)	0.30	±	0.02	0.34	±	0.02	0.35 ± 0.04
	Non-autoclaved Soil	(-)	0.36	±	0.03	0.42	±	0.03	0.45 ± 0.03
		(+)	0.35	±	0.01	0.39	±	0.02	0.45 ± 0.03
<i>Triticum durum</i> cv. Saricanak	Autoclaved Soil	(-)	0.24	±	0.01	0.26	±	0.02	0.28 ± 0.02
		(+)	0.25	±	0.00	0.28	±	0.01	0.28 ± 0.02
	Non-autoclaved Soil	(-)	0.38	±	0.06	0.35	±	0.05	0.36 ± 0.04
		(+)	0.30	±	0.03	0.32	±	0.05	0.34 ± 0.05

1000-kernel weight (TKW) of bread wheat was not affected by *P. indica*; however, compared to those non-inoculated, the plants inoculated with *P. indica* showed a reduction in that of durum wheat. For both cultivars, the plants grown in sterile soil

displayed increased TKWs compared to those grown in native soil. B toxicity caused inconsistent TKWs of tested cultivars (**Tables 3.26 and 3.29**).

Table 3.29: 1000 Kernel weight (mg) of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

1000 Kernel Weight (mg)											
Cultivar	Sterilization Status	<i>P. indica</i>	B Supply (mg kg ⁻¹)								
			1		10		20				
<i>Triticum aestivum</i> cv. Nusrat	Autoclaved Soil	(-)	38.31	±	0.55	34.53	±	0.86	28.52	±	5.90
		(+)	31.70	±	2.83	36.36	±	4.84	26.83	±	2.81
	Non-autoclaved Soil	(-)	34.37	±	2.71	30.72	±	1.58	26.74	±	2.01
		(+)	32.78	±	0.98	30.62	±	1.59	22.61	±	1.73
<i>Triticum durum</i> cv. Saricanak	Autoclaved Soil	(-)	47.60	±	0.55	51.98	±	0.15	50.59	±	2.42
		(+)	43.35	±	2.37	48.84	±	0.56	51.49	±	2.24
	Non-autoclaved Soil	(-)	47.91	±	3.01	47.59	±	1.31	47.60	±	1.23
		(+)	46.19	±	4.76	44.98	±	1.87	41.98	±	4.85

The grain Cu concentrations of both cultivars were not affected by the endophytic fungus. The experimental plants grown in sterile soil displayed reduced grain Cu concentration in Nusrat; however, in Saricanak-98, soil sterilization caused elevated grain Cu concentration. In terms of B toxicity, the grain Cu concentration was increased only at the high level of B in bread wheat. However, for durum wheat, increasing B levels led to dose-dependent increments (**Tables 3.30 and 3.31**).

The grain Fe concentrations of tested cultivars were not affected by the presence of *P. indica*. For both cultivars, the plants grown in sterile soil showed elevated grain Fe concentrations compared to those grown in native soil. B toxicity did not have any significant effect on the grain Fe concentration in Nusrat; however, in Saricanak-98, increasing B level from control to low significantly increased the grain Fe concentration, but high level B did not cause any further change (**Tables 3.30 and 3.31**).

In terms of grain Zn concentration, *P. indica* inoculum did not cause any significant difference compared to those grown without the inoculum in Nusrat. In Saricanak-

98, the presence of endophytic fungus caused a reduction in the grain Zn concentration. Even though the plants grown in sterilized soil displayed a reduction in the grain Zn concentration in Nusrat, did not show any significant change in Saricanak-98 compared to native soils. In Nusrat, increasing B levels increased the grain Zn concentration in a dose-dependent manner; however, B toxicity did not have any effect on that of Saricanak-98 (Tables 3.30 and 3.31).

Table 3.30: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected grain micronutrient concentration of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Mn (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.137	n.s.	0.568	n.s.	0.433	n.s.	0.443	n.s.
	Soil Sterilization (S)	1	<0.001	0.19	0.002	1.67	<0.001	2.48	<0.001	1.21
	Boron Supply (B)	2	<0.001	0.28	0.092	n.s.	<0.001	3.66	<0.001	1.78
	I X S	1	0.493	n.s.	0.566	n.s.	0.963	n.s.	0.010	2.28
	I X B	2	0.998	n.s.	0.568	n.s.	0.048	6.39	0.1812	n.s.
	S X B	2	0.036	0.49	0.839	n.s.	0.033	6.39	0.234	n.s.
	I X S X B	2	0.554	n.s.	0.766	n.s.	0.037	10.50	0.414	n.s.
<i>Triticum durum</i> cv. Saricanak-98										

Table 3.31: Selected grain micronutrient concentrations of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Mn (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	6.62	± 0.33	35.0	± 1.4	64.2	± 2.8	20.8	± 0.5
			(+)	6.45	± 0.28	34.5	± 1.4	63.0	± 2.5	21.7	± 0.9
		Non-autoclaved	(-)	7.01	± 0.20	32.9	± 2.9	33.1	± 5.7	22.9	± 0.9
			(+)	6.91	± 0.42	31.9	± 1.8	30.3	± 1.1	22.7	± 2.2
	Low	Autoclaved	(-)	6.74	± 0.31	34.9	± 0.9	63.9	± 3.9	21.7	± 0.6
			(+)	6.81	± 0.38	35.1	± 1.9	70.7	± 1.9	24.5	± 1.3
		Non-autoclaved	(-)	7.24	± 0.18	31.6	± 3.0	30.9	± 1.9	25.8	± 2.0
			(+)	6.89	± 0.31	32.8	± 2.3	30.7	± 2.0	23.6	± 2.0
	High	Autoclaved	(-)	7.32	± 0.05	38.7	± 3.1	79.3	± 6.4	25.9	± 2.6
			(+)	7.19	± 0.34	36.0	± 1.2	70.5	± 3.8	25.8	± 2.0
		Non-autoclaved	(-)	8.23	± 0.22	33.9	± 5.4	34.6	± 5.4	31.6	± 2.2
			(+)	8.05	± 0.22	33.7	± 1.6	34.8	± 2.1	27.6	± 2.5
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	8.35	± 0.23	31.2	± 1.8	46.2	± 2.5	20.1	± 1.3
			(+)	8.30	± 0.32	30.5	± 2.4	50.8	± 3.0	18.7	± 0.7
		Non-autoclaved	(-)	8.10	± 0.93	25.2	± 2.9	27.0	± 5.2	25.1	± 4.2
			(+)	6.71	± 0.49	23.7	± 4.0	20.1	± 3.1	17.6	± 1.2
	Low	Autoclaved	(-)	8.58	± 0.30	33.7	± 3.1	51.1	± 6.9	22.3	± 2.4
			(+)	8.94	± 0.15	33.4	± 1.9	57.2	± 3.9	22.5	± 1.0
		Non-autoclaved	(-)	7.94	± 0.44	26.4	± 1.4	24.6	± 2.7	25.2	± 5.1
			(+)	8.86	± 0.38	28.0	± 4.0	24.8	± 2.0	19.6	± 2.9
	High	Autoclaved	(-)	9.44	± 0.26	37.0	± 1.9	51.3	± 5.5	23.7	± 2.0
			(+)	8.91	± 0.74	30.2	± 1.4	50.6	± 4.2	20.9	± 0.9
		Non-autoclaved	(-)	8.89	± 0.14	27.7	± 2.8	28.1	± 1.0	25.6	± 2.9
			(+)	9.17	± 0.67	27.5	± 1.9	24.3	± 2.6	20.3	± 3.3

This study revealed that even though the presence of *P. indica* did not affect the grain Ca concentration in Nusrat, it significantly reduced that of Saricanak-98. For both cultivars, compared to the plants grown in native soil, grain Ca concentrations were reduced by soil sterilization. In terms of B toxicity, increasing B levels from control to medium did not affect the grain Ca concentrations; however, high levels of B significantly increased it in both cultivars (Tables 3.32 and 3.33).

According to collective data shown in **Tables 3.32** and **3.33**, the grain K concentrations of both cultivars were not significantly affected by the endophytic fungus. For both cultivars, soil sterilization considerably reduced the grain K concentrations. Increasing B level from control to medium did not cause any significant change; however, the high level of B increased the grain K concentration in bread wheat. On the other hand, B toxicity had no significant effect on that of durum wheat.

Although the experimental plants belonging to Nusrat inoculated with *P. indica* did not display any significant effect on the grain Mg concentration compared to non-inoculated plants, the presence of fungal inoculum reduced the grain Mg concentration in the case of Saricanak-98. For both cultivars, soil sterilization caused reductions in the grain Mg concentrations. In terms of B toxicity, only the plants grown in the soil containing the high B level increased the grain Mg concentration in Nusrat; however, B toxicity caused no effect on that of Saricanak-98 (**Tables 3.32** and **3.33**).

As shown in the **Tables 3.32** and **3.33**, the presence of *P. indica* reduced the grain S concentrations of both cultivars. Similarly, soil sterilization resulted in reduced grain S concentrations in both cultivars. Increasing the B supply increased the grain S concentrations dose-dependently in both tested cultivars.

Table 3.32: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected grain macronutrient concentrations of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Ca (%)		K (%)		Mg (%)		S (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.687	n.s.	0.631	n.s.	0.809	n.s.	<0.001	0.004
	Soil Sterilization (S)	1	<0.001	0.002	<0.001	0.034	<0.001	0.002	<0.001	0.004
	Boron Supply (B)	2	<0.001	0.002	<0.001	0.051	<0.001	0.004	<0.001	0.006
	I X S	1	0.432	n.s.	0.585	n.s.	0.009	0.005	0.465	n.s.
	I X B	2	0.871	n.s.	0.971	n.s.	0.598	n.s.	0.874	n.s.
	S X B	2	0.303	n.s.	0.842	n.s.	0.116	n.s.	0.056	n.s.
	I X S X B	2	0.018	0.006	0.669	n.s.	0.031	0.013	0.099	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Ca (%)		K (%)		Mg (%)		S (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.044	0.002	0.573	n.s.	0.017	0.004	0.0012	0.004
	Soil Sterilization (S)	1	<0.001	0.002	<0.001	0.028	<0.001	0.004	<0.001	0.004
	Boron Supply (B)	2	0.006	0.002	0.469	n.s.	0.312	n.s.	<0.001	0.007
	I X S	1	0.170	n.s.	0.841	n.s.	0.047	0.008	0.265	n.s.
	I X B	2	0.380	n.s.	0.536	n.s.	0.123	n.s.	0.161	n.s.
	S X B	2	0.956	n.s.	0.821	n.s.	0.806	n.s.	0.953	n.s.
	I X S X B	2	0.097	n.s.	0.329	n.s.	0.460	n.s.	0.018	0.02

Table 3.33: Selected grain macronutrient concentrations of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

		B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Ca (%)		K (%)		Mg (%)		S (%)	
Triticum aestivum cv. Nusrat	Control		Autoclaved	(-)	0.03	± 0.00	0.37	± 0.01	0.11	± 0.01	0.17	± 0.00
				(+)	0.03	± 0.00	0.35	± 0.01	0.12	± 0.00	0.16	± 0.01
			Non-autoclaved	(-)	0.04	± 0.01	0.43	± 0.04	0.13	± 0.01	0.19	± 0.00
				(+)	0.04	± 0.00	0.44	± 0.05	0.13	± 0.01	0.18	± 0.01
	Low		Autoclaved	(-)	0.03	± 0.00	0.36	± 0.02	0.11	± 0.00	0.18	± 0.00
				(+)	0.04	± 0.00	0.37	± 0.02	0.12	± 0.00	0.18	± 0.00
			Non-autoclaved	(-)	0.04	± 0.00	0.49	± 0.05	0.13	± 0.01	0.20	± 0.00
				(+)	0.04	± 0.00	0.45	± 0.05	0.13	± 0.00	0.19	± 0.01
	High		Autoclaved	(-)	0.04	± 0.00	0.43	± 0.10	0.12	± 0.01	0.21	± 0.01
				(+)	0.04	± 0.00	0.44	± 0.08	0.12	± 0.01	0.19	± 0.00
			Non-autoclaved	(-)	0.05	± 0.00	0.55	± 0.03	0.14	± 0.01	0.21	± 0.01
				(+)	0.05	± 0.01	0.52	± 0.08	0.14	± 0.00	0.20	± 0.00
Triticum durum cv. Saricanak-98	Control		Autoclaved	(-)	0.04	± 0.00	0.34	± 0.02	0.10	± 0.00	0.18	± 0.01
				(+)	0.04	± 0.00	0.35	± 0.01	0.10	± 0.00	0.16	± 0.01
			Non-autoclaved	(-)	0.05	± 0.00	0.49	± 0.08	0.12	± 0.01	0.18	± 0.00
				(+)	0.04	± 0.00	0.46	± 0.05	0.10	± 0.01	0.18	± 0.00
	Low		Autoclaved	(-)	0.04	± 0.00	0.34	± 0.02	0.10	± 0.01	0.18	± 0.01
				(+)	0.04	± 0.00	0.37	± 0.03	0.11	± 0.01	0.19	± 0.01
			Non-autoclaved	(-)	0.05	± 0.00	0.46	± 0.06	0.11	± 0.01	0.21	± 0.01
				(+)	0.05	± 0.01	0.47	± 0.05	0.11	± 0.01	0.19	± 0.01
	High		Autoclaved	(-)	0.05	± 0.00	0.37	± 0.03	0.11	± 0.01	0.20	± 0.01
				(+)	0.04	± 0.01	0.36	± 0.01	0.10	± 0.00	0.18	± 0.01
			Non-autoclaved	(-)	0.05	± 0.01	0.47	± 0.07	0.12	± 0.01	0.21	± 0.01
				(+)	0.05	± 0.00	0.52	± 0.04	0.11	± 0.01	0.20	± 0.00

The grain Cu contents of both cultivars were not affected by the presence of *P. indica*. For both cultivars, the plants grown in autoclaved soil showed elevated grain Cu contents compared to those grown in native soil. For bread wheat, the grain Cu content was reduced only at the high B level, and increasing B levels from the control to the medium did not cause any significant effect on the content. In the case of durum wheat, B toxicity had no significant effect on the grain Cu content at any level, however (Tables 3.34 and 3.35).

The presence of *P. indica* did not result in any significant change in grain Fe contents for both cultivars. Similar to grain Fe concentrations, soil sterilization significantly increased the grain Fe contents in both cultivars. In Nusrat, increasing B level reduced grain Fe content in a dose-dependent manner; however, in Saricanak-98, increasing B level did not affect the grain Fe content consistently and only medium B level increased the content (**Tables 3.34 and 3.35**).

Similar to Mn concentration, the grain Mn contents of both cultivars were not affected by the presence of endophytic fungus. The grain Mn contents of both cultivars were increased dramatically (almost tripled) by soil sterilization. For both cultivars, only high level of B significantly decreased the grain Mn contents compared to control group (**Tables 3.34 and 3.35**).

As shown in **Tables 3.34 and 3.35**, although *P. indica* did not have any effect on the grain Zn content in Nusrat cultivar, it reduced that of Saricanak-98. For both cultivars, the grain Zn contents were increased by soil sterilization compared to native soil condition. In terms of B toxicity, in Nusrat, only high level of B significantly reduced the grain Zn content compared to control group. On the other hand, B toxicity did not have any considerable effect on the grain Zn content of Saricanak-98 cultivar.

Table 3.34: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected grain micronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS). DF: (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Cu ($\mu\text{g plant}^{-1}$)		Fe ($\mu\text{g plant}^{-1}$)		Mn ($\mu\text{g plant}^{-1}$)		Zn ($\mu\text{g plant}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.957	n.s.	0.917	n.s.	0.802	n.s.	0.684	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Soil Sterilization (S)	1	<0.001	1.028	<0.001	4.61	<0.001	8.21	0.002	3.19
	Boron Supply (B)	2	<0.001	1.517	<0.001	6.80	<0.001	12.11	0.002	4.71
	I X S	1	0.268	n.s.	0.088	n.s.	0.413	n.s.	0.459	n.s.
	I X B	2	0.487	n.s.	0.743	n.s.	0.671	n.s.	0.3027	n.s.
	S X B	2	0.151	n.s.	0.261	n.s.	0.086	n.s.	0.128	n.s.
	I X S X B	2	0.749	n.s.	0.932	n.s.	0.308	n.s.	0.422	n.s.
	Source of Variation	DF	Cu ($\mu\text{g plant}^{-1}$)		Fe ($\mu\text{g plant}^{-1}$)		Mn ($\mu\text{g plant}^{-1}$)		Zn ($\mu\text{g plant}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.487	n.s.	0.531	n.s.	0.251	n.s.	0.002	4.28
	Soil Sterilization (S)	1	<0.001	1.445	<0.001	5.04	<0.001	8.89	<0.001	4.28
	Boron Supply (B)	2	0.213	n.s.	0.010	7.33	0.012	13.11	0.055	n.s.
	I X S	1	0.942	n.s.	0.206	n.s.	0.017	16.69	0.020	8.03
	I X B	2	0.020	2.506	0.112	n.s.	0.241	n.s.	0.180	n.s.
	S X B	2	0.317	n.s.	0.506	n.s.	0.066	n.s.	0.457	n.s.
	I X S X B	2	0.163	n.s.	0.226	n.s.	0.591	n.s.	0.565	n.s.

Table 3.35: Selected grain micronutrient contents of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Cu (µg plant ⁻¹)		Fe (µg plant ⁻¹)		Mn (µg plant ⁻¹)		Zn (µg plant ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	17.9	± 1.9	94.5	± 7.7	173.6	± 14.6	56.3	± 4.2
			(+)	17.5	± 0.8	93.9	± 5.0	171.4	± 7.2	59.2	± 4.3
		Non-autoclaved	(-)	15.6	± 0.9	73.3	± 7.7	72.9	± 7.5	51.2	± 5.3
			(+)	17.2	± 1.8	79.2	± 7.1	75.1	± 6.5	56.6	± 8.4
	Low	Autoclaved	(-)	18.0	± 1.1	92.9	± 3.2	170.2	± 11.9	57.9	± 2.3
			(+)	17.0	± 2.1	87.8	± 9.4	176.8	± 18.9	60.9	± 3.3
		Non-autoclaved	(-)	14.6	± 0.7	63.6	± 4.8	62.3	± 2.6	51.9	± 2.2
			(+)	13.8	± 1.2	65.5	± 4.3	61.4	± 3.7	47.2	± 3.6
	High	Autoclaved	(-)	14.4	± 2.8	75.1	± 10.2	154.0	± 22.5	50.2	± 7.2
			(+)	14.0	± 1.9	69.6	± 7.3	136.5	± 16.8	49.8	± 6.0
		Non-autoclaved	(-)	12.8	± 0.5	52.3	± 6.9	53.5	± 8.0	48.9	± 2.8
			(+)	13.7	± 0.7	57.3	± 4.6	59.2	± 5.7	46.7	± 2.1
<i>Triticum durum</i> cv. Saricanak-98	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Cu (µg plant ⁻¹)		Fe (µg plant ⁻¹)		Mn (µg plant ⁻¹)		Zn (µg plant ⁻¹)	
	Control	Autoclaved	(-)	28.6	± 1.8	106.5	± 3.5	158.3	± 12.3	68.6	± 3.9
			(+)	28.4	± 1.0	104.6	± 6.8	174.0	± 11.9	64.1	± 2.4
		Non-autoclaved	(-)	20.2	± 1.4	63.0	± 3.9	67.0	± 9.3	62.5	± 7.3
			(+)	16.0	± 3.4	55.7	± 10.9	48.6	± 13.9	41.9	± 8.4
	Low	Autoclaved	(-)	27.7	± 1.3	108.3	± 2.5	164.0	± 14.1	71.7	± 3.7
			(+)	29.5	± 2.6	109.9	± 8.8	189.2	± 27.6	73.8	± 5.3
		Non-autoclaved	(-)	17.9	± 1.1	59.4	± 4.4	55.1	± 2.1	56.1	± 7.6
			(+)	22.0	± 0.6	69.5	± 10.0	61.3	± 2.6	48.3	± 5.3
	High	Autoclaved	(-)	26.5	± 2.8	103.8	± 9.6	143.3	± 14.0	66.1	± 5.5
			(+)	26.6	± 2.8	89.8	± 9.6	150.6	± 14.0	62.2	± 5.5
		Non-autoclaved	(-)	18.1	± 1.5	56.4	± 8.1	56.9	± 4.4	52.2	± 8.7
			(+)	19.5	± 2.5	58.4	± 6.4	51.8	± 7.9	43.4	± 9.0

P. indica inoculation did not show any significant effects on the grain Ca contents in both cultivars. Soil sterilization did not cause any significant change in bread wheat, but it resulted in elevated grain Ca content in durum. In Nusrat, increasing B level reduced the grain Ca content in a dose-dependent manner, but in Saricanak-98, B toxicity had no effect on it (Tables 3.36 and 3.37).

As displayed in **Tables 3.36** and **3.37**, neither *P. indica* inoculation nor soil sterilization significantly affected the grain K contents in both cultivars.

The grain Mg contents were not significantly affected by *P. indica* in both cultivars. Soil sterilization increased the grain Mg contents of both cultivars compared to native soil. B toxicity resulted in a dose-dependent reduction of the grain Mg content in Nusrat. In Saricanak-98, although increasing the B level from control to medium did not affect the grain Mg content, the high level of B significantly reduced it (**Tables 3.36** and **3.37**).

As regards to grain S content, the experimental plants inoculated with *P. indica* did not exhibit any significant change compared to non-inoculated ones in both cultivars. The grain S contents of both cultivars were increased by soil sterilization. For both cultivars only the high level of B significantly reduced the grain S content, increasing B level from the control to the medium level did not cause any significant effect on the contents, however (**Tables 3.36** and **3.37**).

Table 3.36: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected grain macronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS). DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.591	n.s.	0.824	n.s.	0.450	n.s.	0.3709	n.s.
	Soil Sterilization (S)	1	0.261	n.s.	0.331	n.s.	0.024	0.156	<0.001	0.208
	Boron Supply (B)	2	0.002	0.080	0.001	0.962	<0.001	0.23	<0.001	0.307
	I X S	1	0.689	n.s.	0.580	n.s.	0.984	n.s.	0.1686	n.s.
	I X B	2	0.555	n.s.	0.396	n.s.	0.376	n.s.	0.3205	n.s.
	S X B	2	0.291	n.s.	0.802	n.s.	0.099	n.s.	0.0992	n.s.
	I X S X B	2	0.039	2.334	0.317	n.s.	0.313	n.s.	0.4622	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.352	n.s.	0.299	n.s.	0.505	n.s.	0.403	n.s.
	Soil Sterilization (S)	1	0.011	0.099	0.488	n.s.	<0.001	0.21	<0.001	0.243
	Boron Supply (B)	2	0.499	n.s.	0.125	n.s.	0.014	0.31	0.020	0.359
	I X S	1	0.308	n.s.	0.928	n.s.	0.243	n.s.	0.447	n.s.
	I X B	2	0.106	n.s.	0.283	n.s.	0.085	n.s.	0.268	n.s.
	S X B	2	0.999	n.s.	0.898	n.s.	0.802	n.s.	0.924	n.s.
	I X S X B	2	0.220	n.s.	0.508	n.s.	0.529	n.s.	0.765	n.s.

Table 3.37: Selected grain macronutrient contents of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	0.85	± 0.09	9.92	± 0.99	3.01	± 0.34	4.63	± 0.25
			(+)	0.92	± 0.03	9.58	± 0.81	3.22	± 0.17	4.46	± 0.33
		Non-autoclaved	(-)	0.92	± 0.06	9.64	± 0.49	2.94	± 0.19	4.26	± 0.32
			(+)	0.97	± 0.11	11.00	± 1.50	3.14	± 0.26	4.57	± 0.44
	Low	Autoclaved	(-)	0.84	± 0.06	9.46	± 0.73	3.00	± 0.11	4.74	± 0.11
			(+)	0.90	± 0.14	9.26	± 0.61	3.08	± 0.25	4.47	± 0.36
		Non-autoclaved	(-)	0.88	± 0.08	9.84	± 1.15	2.71	± 0.11	4.10	± 0.12
			(+)	0.80	± 0.06	8.97	± 0.60	2.51	± 0.20	3.75	± 0.21
	High	Autoclaved	(-)	0.80	± 0.12	8.17	± 0.42	2.42	± 0.41	4.00	± 0.58
			(+)	0.70	± 0.06	8.39	± 1.70	2.30	± 0.28	3.73	± 0.36
		Non-autoclaved	(-)	0.76	± 0.05	8.49	± 0.44	2.22	± 0.12	3.24	± 0.07
			(+)	0.87	± 0.04	8.75	± 1.11	2.40	± 0.12	3.44	± 0.13
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	1.33	± 0.12	11.70	± 0.31	3.42	± 0.27	6.00	± 0.23
			(+)	1.26	± 0.05	11.85	± 0.25	3.33	± 0.16	5.50	± 0.24
		Non-autoclaved	(-)	1.31	± 0.06	12.33	± 1.58	3.04	± 0.09	4.50	± 0.32
			(+)	1.01	± 0.20	11.04	± 2.80	2.38	± 0.54	4.36	± 0.56
	Low	Autoclaved	(-)	1.23	± 0.15	11.01	± 1.15	3.29	± 0.32	5.94	± 0.19
			(+)	1.43	± 0.24	12.28	± 2.19	3.52	± 0.54	6.14	± 0.75
		Non-autoclaved	(-)	1.21	± 0.07	10.22	± 0.66	2.54	± 0.09	4.63	± 0.22
			(+)	1.19	± 0.22	11.75	± 1.32	2.74	± 0.16	4.76	± 0.07
	High	Autoclaved	(-)	1.32	± 0.15	10.59	± 1.84	2.98	± 0.33	5.69	± 0.43
			(+)	1.20	± 0.15	10.64	± 1.84	2.99	± 0.33	5.41	± 0.43
		Non-autoclaved	(-)	1.11	± 0.13	9.54	± 1.72	2.45	± 0.31	4.33	± 0.28
			(+)	1.14	± 0.08	11.04	± 1.45	2.34	± 0.35	4.31	± 0.31

As shown in **Tables 3.38** and **3.39**, the experimental plants inoculated with *P. indica* displayed a reduced straw B content in Nusrat; however, in Saricanak-98, no significant difference compared to those non-inoculated. Even though soil sterilization reduced the straw B content in bread wheat, had no effect on that of durum wheat. For both cultivars, increasing B levels caused dose-dependent increments in the straw B content.

Straw P content was not affected by the fungal inoculum in Nusrat; however, was increased in Saricanak-98. Even though soil sterilization had no effect on the straw P content of Nusrat cultivar, increased that of Saricanak-98. In terms of B toxicity, the straw P content increased dose-dependently in both cultivars (**Tables 3.38** and **3.39**).

According to ANOVA, the grain B content of bread wheat cultivar was affected by all single effect of treatments, double interaction of soil sterilization and B supply and triple interaction of the treatments. In durum wheat, only the single effect of B supply and double interaction of soil sterilization and B supply affected the grain B content (**Table 3.38**). As displayed in **Table 3.39**, even though the experimental plants inoculated with *P. indica* showed a reduced grain B content in Nusrat, had no effect on that of Saricanak-98 compared to corresponding control groups for both cultivars. The grain B content was decreased by soil sterilization in Nusrat; however, was not affected in Saricanak-98. Similar to the grain B concentrations, increasing B levels led to dose-dependent increments in the grain B contents for both cultivars.

The grain P contents of both cultivars were not affected by *P. indica*. Soil sterilization cultivar reduced the grain P contents in Nusrat; however, it resulted in elevated grain P content in Saricanak-98 compared to the plants grown in native soil. In Nusrat, only high level of B reduced the grain P content, and B toxicity did not have any significant effect on that of Saricanak-98 (**Tables 3.38** and **3.39**).

Table 3.38: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected straw and grain contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores

	Source of Variation	DF	Straw Mineral Contents				Grain Mineral Contents			
			B ($\mu\text{g plant}^{-1}$)		P (mg plant^{-1})		B ($\mu\text{g plant}^{-1}$)		P (mg plant^{-1})	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.002	32	0.062	n.s.	0.471	n.s.	0.055	n.s.
	Soil Sterilization (S)	1	<0.001	32	0.214	n.s.	0.022	0.375	0.010	0.38
	Boron Supply (B)	2	<0.001	47	<0.001	0.16	<0.001	0.552	<0.001	0.56
	I X S	1	0.314	n.s.	0.213	n.s.	0.902	n.s.	0.239	n.s.
	I X B	2	0.171	n.s.	0.045	0.28	0.139	n.s.	0.443	n.s.
	S X B	2	<0.001	81	0.561	n.s.	0.373	n.s.	0.434	n.s.
	I X S X B	2	0.005	133	0.923	n.s.	0.458	n.s.	0.090	n.s.
<i>Triticum durum</i> cv. Saricanak-98										

Table 3.39: Straw and grain B and P contents of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Straw Mineral Contents				Grain Mineral Contents			
				B (µg plant ⁻¹)		P (mg plant ⁻¹)		B (µg plant ⁻¹)		P (mg plant ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	69	± 1	0.61	± 0.08	3.08	± 0.54	7.18	± 0.58
			(+)	76	± 11	0.82	± 0.13	2.92	± 0.42	8.07	± 0.63
		Non-autoclaved	(-)	99	± 18	0.68	± 0.09	2.03	± 0.08	8.08	± 0.58
			(+)	72	± 14	0.79	± 0.08	2.35	± 0.29	8.60	± 0.31
	Low	Autoclaved	(-)	434	± 16	0.80	± 0.09	3.77	± 0.18	7.50	± 0.45
			(+)	390	± 54	1.13	± 0.28	3.43	± 0.43	8.38	± 0.28
		Non-autoclaved	(-)	584	± 40	0.93	± 0.11	3.80	± 0.63	8.43	± 0.51
			(+)	484	± 34	1.07	± 0.14	2.80	± 0.46	7.78	± 0.54
	High	Autoclaved	(-)	1085	± 46	1.44	± 0.22	4.57	± 0.93	6.67	± 0.67
			(+)	917	± 38	1.41	± 0.22	4.74	± 1.05	6.69	± 0.98
		Non-autoclaved	(-)	1446	± 51	1.65	± 0.20	4.34	± 0.36	7.04	± 0.51
			(+)	1463	± 116	1.50	± 0.15	4.54	± 0.47	7.62	± 0.41
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	78	± 3	0.56	± 0.01	2.55	± 0.31	8.36	± 0.59
			(+)	66	± 8	0.71	± 0.12	2.33	± 0.14	8.67	± 0.14
		Non-autoclaved	(-)	76	± 11	0.50	± 0.07	1.84	± 0.18	9.40	± 0.92
			(+)	56	± 8	0.50	± 0.09	1.90	± 0.37	7.13	± 1.66
	Low	Autoclaved	(-)	368	± 25	0.59	± 0.04	6.02	± 0.51	8.50	± 0.49
			(+)	448	± 36	1.05	± 0.14	5.44	± 1.08	9.30	± 0.94
		Non-autoclaved	(-)	401	± 36	0.72	± 0.11	6.31	± 1.09	7.86	± 0.71
			(+)	483	± 161	0.63	± 0.05	5.08	± 2.02	7.93	± 0.95
	High	Autoclaved	(-)	1283	± 75	1.29	± 0.07	9.85	± 1.42	7.86	± 0.92
			(+)	1184	± 75	1.47	± 0.07	8.05	± 1.42	8.45	± 0.92
		Non-autoclaved	(-)	1089	± 195	0.62	± 0.15	10.38	± 1.31	7.42	± 1.22
			(+)	1075	± 119	0.79	± 0.11	8.56	± 3.10	7.30	± 1.48

The straw Cu concentrations of both cultivars were not affected by the presence of *P. indica*. Even though the straw Cu concentration was reduced by soil sterilization in bread wheat, it was increased in durum wheat. In terms of B toxicity, increasing B level did not cause consistent straw Cu concentrations in bread wheat. On the other hand, in durum wheat, the straw Cu concentration significantly increased only by the high level of B compared to the control (Tables 3.40 and 3.41).

As tackled in Tables **3.40** and **3.41**, straw Fe concentrations were affected by none of the single effects significantly in both cultivars. Straw Mn concentration was only affected significantly by soil sterilization in Nusrat, and the plants grown in sterilized soil showed elevated straw Mn concentration compared to those grown in native soil. In the case of Saricanak-98, the straw Mn concentration significantly increased not only by *P. indica* inoculation but also by soil sterilization. As regards to B toxicity, even though increasing B level from the control to the low significantly increased the straw Mn concentration, the high level of B did not increase it any further (**Tables 3.40 and 3.41**).

Straw Mn concentration was only affected significantly by soil sterilization in Nusrat, and the plants grown in sterilized soil showed elevated straw Mn concentration compared to those grown in native soil. In the case of Saricanak-98, the straw Mn concentration significantly increased not only by *P. indica* inoculation but also by soil sterilization. As regards to B toxicity, even though increasing B level from the control to the low significantly increased the straw Mn concentration, high level of B did not increase it any further (**Tables 3.40 and 3.41**).

The plants inoculated with *P. indica* exhibited an elevated straw Zn concentration in bread wheat; however, *P. indica* inoculation had no significant effect on straw Zn concentration in durum wheat. Even though soil sterilization had no significant effect on the straw Zn concentration in bread wheat, caused an elevation in that of durum wheat. In terms of B toxicity, compared to control level, only high B level significantly increased the straw Zn concentrations in both cultivars (**Tables 3.40 and 3.41**).

Table 3.40: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected straw micronutrient concentrations of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Mn (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.213	n.s.	0.094	n.s.	0.093	n.s.	0.014	0.58
	Soil Sterilization (S)	1	<0.001	0.32	0.079	n.s.	<0.001	1.71	0.543	n.s.
	Boron Supply (B)	2	<0.001	0.46	0.105	n.s.	0.446	n.s.	<0.001	0.86
	I X S	1	0.490	n.s.	0.114	n.s.	0.286	n.s.	0.025	1.09
	I X B	2	0.057	n.s.	0.272	n.s.	0.748	n.s.	0.9721	n.s.
	S X B	2	0.005	0.81	0.844	n.s.	0.128	n.s.	0.981	n.s.
	I X S X B	2	0.391	n.s.	0.233	n.s.	0.590	n.s.	0.353	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Mn (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.318	n.s.	0.378	n.s.	<0.001	11.55	0.846	n.s.
	Soil Sterilization (S)	1	<0.001	0.4	0.810	n.s.	<0.001	11.55	0.030	0.61
	Boron Supply (B)	2	<0.001	0.61	0.635	n.s.	<0.001	17.10	0.003	0.90
	I X S	1	0.258	n.s.	0.158	n.s.	<0.001	21.92	0.138	n.s.
	I X B	2	0.163	n.s.	0.862	n.s.	0.036	29.25	0.078	n.s.
	S X B	2	0.073	n.s.	0.171	n.s.	<0.001	29.25	0.067	n.s.
	I X S X B	2	0.023	1.71	0.127	n.s.	0.020	47.98	0.272	n.s.

Table 3.41: Selected straw micronutrient concentrations of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Mn (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	5.05	± 0.31	51.4	± 7.9	316.3	± 35.1	3.9	± 0.3
			(+)	4.93	± 0.45	59.8	± 6.2	305.7	± 17.7	5.9	± 0.6
		Non-autoclaved	(-)	6.27	± 0.27	73.8	± 16.1	67.4	± 8.6	5.3	± 0.4
			(+)	6.55	± 0.74	55.7	± 1.8	56.3	± 2.4	5.0	± 0.3
	Low	Autoclaved	(-)	4.31	± 0.24	52.9	± 5.5	346.1	± 31.3	3.9	± 0.2
			(+)	4.60	± 0.38	58.8	± 7.2	329.8	± 35.3	5.2	± 0.6
		Non-autoclaved	(-)	4.60	± 0.25	63.5	± 14.9	61.6	± 4.6	4.8	± 0.8
			(+)	4.47	± 0.20	58.5	± 4.5	59.2	± 3.0	4.8	± 1.2
	High	Autoclaved	(-)	5.51	± 0.66	72.7	± 24.5	329.1	± 36.5	6.2	± 1.1
			(+)	5.06	± 0.54	57.4	± 4.1	284.5	± 48.8	7.1	± 0.5
		Non-autoclaved	(-)	6.88	± 0.54	75.6	± 4.9	73.3	± 11.5	6.5	± 0.1
			(+)	5.78	± 0.76	63.7	± 8.3	70.4	± 5.8	7.0	± 2.1
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	5.58	± 0.64	65.7	± 16.7	233.7	± 27.0	6.6	± 0.3
			(+)	5.00	± 0.20	60.0	± 7.6	265.5	± 29.2	6.3	± 0.4
		Non-autoclaved	(-)	4.41	± 0.24	53.9	± 8.8	20.3	± 5.6	6.7	± 0.3
			(+)	4.27	± 0.32	62.2	± 12.0	25.1	± 4.7	5.9	± 0.7
	Low	Autoclaved	(-)	4.33	± 0.45	58.9	± 6.0	264.2	± 2.8	5.3	± 0.9
			(+)	5.25	± 0.40	77.7	± 22.0	353.9	± 17.8	6.1	± 0.6
		Non-autoclaved	(-)	4.05	± 0.73	67.8	± 8.5	23.1	± 2.2	6.2	± 1.4
			(+)	4.02	± 0.44	56.8	± 9.3	24.0	± 2.6	4.8	± 0.6
	High	Autoclaved	(-)	7.17	± 0.37	43.0	± 24.7	325.3	± 36.1	7.3	± 1.0
			(+)	6.76	± 1.25	63.5	± 2.2	340.5	± 5.8	8.4	± 1.3
		Non-autoclaved	(-)	4.36	± 0.78	70.2	± 14.7	22.4	± 2.0	5.6	± 1.1
			(+)	5.86	± 0.56	64.9	± 12.1	26.2	± 2.4	6.7	± 1.1

As displayed in **Tables 3.42** and **3.43**, the straw Ca concentration was reduced by *P. indica* in Nusrat. The fungal inoculum did not cause any significant change in the straw Ca concentration of Saricanak-98, however. In terms of soil sterilization, the experimental plants grown in autoclaved soil showed a reduction in the straw Ca concentration compared to those grown in non-autoclaved soil in Nusrat. Nevertheless, the concentration was increased by soil sterilization in Saricanak-98.

Even though B toxicity had no effect on the straw Ca concentration in Nusrat, increasing B levels resulted in a dose-dependent elevation in Saricanak-98.

Although *P. indica* did not cause any significant changes in the straw K concentration in Nusrat, decreased that of Saricanak-98. In Nusrat, soil sterilization resulted in reduced straw K concentration; however, in Saricanak-98, it was increased by soil sterilization. Increasing B level from the control to the medium increased the straw K concentration, but the high level of B did not cause any significant change compared to control group in Nusrat. On the other hand, B toxicity increased the straw K concentration in a dose-dependent manner in Saricanak-98 (**Tables 3.42 and 3.43**).

The straw Mg concentrations of both cultivars were not affected by *P. indica* (**Tables 3.42 and 3.43**).

As tackled in **Tables 3.42 and 3.43**, the straw S concentration was reduced by *P. indica* in bread wheat and was unaffected by the fungal inoculum in durum wheat. For both cultivars, soil sterilization led to reductions in the straw S concentrations. In regard to B toxicity, compared to control groups, only the high levels of B caused elevated straw S concentrations in both cultivars.

Table 3.42: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected straw macronutrient concentrations of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Ca (%)		K (%)		Mg (%)		S (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.029	0.038	0.211	n.s.	0.101	n.s.	0.002	0.022
	Soil Sterilization (S)	1	<0.001	0.038	<0.001	0.06	<0.001	0.012	<0.001	0.022
	Boron Supply (B)	2	0.774	n.s.	<0.001	0.09	0.177	n.s.	0.004	0.031
	I X S	1	1.000	n.s.	0.021	0.13	0.949	n.s.	0.005	0.029
	I X B	2	0.824	n.s.	0.810	n.s.	0.704	n.s.	0.708	n.s.
	S X B	2	0.033	0.09	0.099	n.s.	0.088	n.s.	0.893	n.s.
	I X S X B	2	0.783	n.s.	0.444	n.s.	0.686	n.s.	0.820	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Ca (%)		K (%)		Mg (%)		S (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.677	n.s.	<0.001	0.08	0.057	n.s.	0.1441	n.s.
	Soil Sterilization (S)	1	<0.001	0.04	<0.001	0.08	<0.001	0.008	0.016	0.02
	Boron Supply (B)	2	<0.001	0.07	<0.001	0.012	<0.001	0.012	0.010	0.031
	I X S	1	0.166	n.s.	0.084	n.s.	0.515	n.s.	0.014	0.04
	I X B	2	0.039	0.13	0.696	n.s.	<0.001	0.021	0.076	n.s.
	S X B	2	<0.001	0.13	<0.001	0.24	<0.001	0.021	<0.001	0.054
	I X S X B	2	0.245	n.s.	0.057	n.s.	0.022	0.034	0.022	0.09

Table 3.43: Selected straw macronutrient concentrations of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Ca		K		Mg		S	
				(%)		(%)		(%)		(%)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	0.79	± 0.05	1.71	± 0.17	0.24	± 0.02	0.21	± 0.01
			(+)	0.76	± 0.01	1.68	± 0.05	0.24	± 0.02	0.22	± 0.03
		Non-autoclaved	(-)	1.10	± 0.08	2.08	± 0.17	0.33	± 0.01	0.34	± 0.02
			(+)	1.03	± 0.09	2.00	± 0.05	0.32	± 0.04	0.27	± 0.02
	Low	Autoclaved	(-)	0.87	± 0.03	1.95	± 0.03	0.25	± 0.01	0.23	± 0.02
			(+)	0.83	± 0.07	2.03	± 0.16	0.24	± 0.03	0.23	± 0.03
		Non-autoclaved	(-)	1.02	± 0.02	2.25	± 0.07	0.31	± 0.01	0.34	± 0.04
			(+)	1.01	± 0.05	2.14	± 0.08	0.29	± 0.01	0.27	± 0.04
	High	Autoclaved	(-)	0.84	± 0.05	1.94	± 0.09	0.23	± 0.01	0.28	± 0.03
			(+)	0.79	± 0.06	2.01	± 0.16	0.22	± 0.02	0.25	± 0.05
		Non-autoclaved	(-)	1.08	± 0.05	2.24	± 0.07	0.31	± 0.01	0.38	± 0.06
			(+)	1.03	± 0.07	2.02	± 0.10	0.31	± 0.02	0.30	± 0.04
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	0.84	± 0.04	1.51	± 0.13	0.17	± 0.00	0.21	± 0.01
			(+)	0.80	± 0.06	1.36	± 0.07	0.17	± 0.01	0.21	± 0.02
		Non-autoclaved	(-)	0.93	± 0.16	1.54	± 0.09	0.18	± 0.02	0.36	± 0.06
			(+)	0.83	± 0.06	1.33	± 0.07	0.15	± 0.01	0.25	± 0.03
	Low	Autoclaved	(-)	0.90	± 0.04	1.85	± 0.21	0.18	± 0.00	0.20	± 0.02
			(+)	1.10	± 0.15	1.96	± 0.16	0.22	± 0.02	0.26	± 0.02
		Non-autoclaved	(-)	0.87	± 0.07	1.78	± 0.13	0.16	± 0.01	0.28	± 0.05
			(+)	0.88	± 0.03	1.40	± 0.09	0.18	± 0.01	0.24	± 0.02
	High	Autoclaved	(-)	1.18	± 0.06	2.47	± 0.24	0.22	± 0.00	0.32	± 0.03
			(+)	1.17	± 0.10	2.21	± 0.16	0.22	± 0.01	0.30	± 0.03
		Non-autoclaved	(-)	0.91	± 0.03	1.81	± 0.13	0.15	± 0.01	0.26	± 0.03
			(+)	0.92	± 0.06	1.60	± 0.08	0.18	± 0.02	0.26	± 0.03

As shown in **Tables 3.44** and **3.45**, compared to non-inoculated plants, the experimental plants inoculated with *P. indica* showed no significant effect on the straw Cu content in Nusrat, but elevated the content in Saricanak-98. Compared to native soil, soil sterilization had no effect on the straw Cu content in Nusrat; however, increased in that of Saricanak-98. For both cultivars, increasing B levels resulted in inconsistent straw Cu contents.

The straw Fe content was not affected by any of the single effects significantly in Nusrat (**Table 3.44**). For Saricanak-98, the straw Fe content was affected by only soil sterilization and the plants grown in sterilized soil showed an increment in the content compared to plants grown in native soil (**Tables 3.44 and 3.45**).

Even though the straw Mn content was not affected by *P. indica* in Nusrat, increased by the endophytic fungus in Saricanak-98. The experimental plants grown in autoclaved soil showed increased straw Mn contents in both cultivars compared to those grown in non-autoclaved soil. B toxicity had no effects on the straw Mn contents in both cultivars (**Tables 3.44 and 3.45**).

The plants inoculated with *P. indica* exhibited elevated straw Zn content in Nusrat. On the other hand, *P. indica* inoculation had no significant effect on straw Zn content in Saricanak-98. Although soil sterilization had no considerable effect on the straw Zn content in Nusrat, led to an elevation in that of Saricanak-98. In Nusrat, compared to control level, only the high B level significantly increased the straw Zn content. On the other hand, in Saricanak-98, increasing B level resulted in inconsistent responses (**Tables 3.44 and 3.45**).

Table 3.44: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected straw micronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Cu ($\mu\text{g plant}^{-1}$)		Fe ($\mu\text{g plant}^{-1}$)		Mn ($\mu\text{g plant}^{-1}$)		Zn ($\mu\text{g plant}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.189	n.s.	0.843	n.s.	0.467	n.s.	0.002	1.87
<i>Triticum durum</i> cv. Saricanak-98	Soil Sterilization (S)	1	0.220	n.s.	0.645	n.s.	<0.001	36.55	0.145	n.s.
	Boron Supply (B)	2	<0.001	1.422	0.208	n.s.	0.181	n.s.	<0.001	2.77
	I X S	1	0.481	n.s.	0.464	n.s.	0.317	n.s.	0.143	n.s.
	I X B	2	0.012	2.475	0.166	n.s.	0.151	n.s.	0.7544	n.s.
	S X B	2	0.005	2.475	0.687	n.s.	0.018	93.89	0.798	n.s.
	I X S X B	2	0.339	n.s.	0.301	n.s.	0.139	n.s.	0.480	n.s.
<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Cu ($\mu\text{g plant}^{-1}$)		Fe ($\mu\text{g plant}^{-1}$)		Mn ($\mu\text{g plant}^{-1}$)		Zn ($\mu\text{g plant}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.018	0.84	0.102	n.s.	<0.001	31.99	0.163	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Soil Sterilization (S)	1	<0.001	0.84	0.002	23.08	<0.001	31.99	<0.001	1.398
	Boron Supply (B)	2	<0.001	1.247	0.217	n.s.	0.051	n.s.	0.014	2.08
	I X S	1	0.542	n.s.	0.192	n.s.	<0.001	60.89	0.185	n.s.
	I X B	2	0.064	n.s.	0.853	n.s.	0.089	n.s.	0.117	n.s.
	S X B	2	0.038	2.13	0.130	n.s.	0.052	n.s.	0.147	n.s.
	I X S X B	2	0.056	n.s.	0.167	n.s.	0.066	n.s.	0.510	n.s.

Table 3.45: Selected straw micronutrient contents of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Cu (µg plant ⁻¹)		Fe (µg plant ⁻¹)		Mn (µg plant ⁻¹)		Zn (µg plant ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	13.6	± 0.7	138.2	± 22.6	845.7	± 51.6	10.5	± 0.6
			(+)	14.3	± 1.3	174.6	± 28.3	887.2	± 69.5	17.3	± 2.6
		Non-autoclaved	(-)	14.8	± 1.6	173.4	± 35.8	158.5	± 19.8	12.6	± 1.2
			(+)	18.2	± 2.7	154.7	± 11.0	156.3	± 7.0	13.9	± 1.6
	Low	Autoclaved	(-)	11.8	± 0.8	145.8	± 20.7	952.2	± 106.7	10.6	± 0.7
			(+)	13.3	± 1.6	169.1	± 17.2	946.4	± 61.0	15.2	± 2.3
		Non-autoclaved	(-)	10.5	± 0.8	144.5	± 33.8	140.1	± 9.2	10.9	± 2.0
			(+)	11.6	± 0.7	152.2	± 15.1	153.8	± 11.5	12.4	± 3.6
	High	Autoclaved	(-)	15.3	± 0.7	203.5	± 78.5	914.3	± 30.4	17.5	± 3.9
			(+)	14.0	± 0.9	160.6	± 23.4	784.0	± 98.0	19.8	± 3.0
		Non-autoclaved	(-)	16.1	± 0.3	178.1	± 11.1	172.7	± 27.6	15.3	± 1.4
			(+)	14.5	± 2.5	160.1	± 26.9	176.4	± 22.6	17.7	± 5.7
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	14.3	± 1.0	165.5	± 46.3	601.6	± 56.1	17.0	± 1.1
			(+)	13.3	± 0.8	160.1	± 21.0	709.1	± 84.1	16.8	± 1.3
		Non-autoclaved	(-)	7.8	± 0.5	94.3	± 11.3	36.0	± 8.3	11.8	± 1.1
			(+)	7.9	± 1.3	120.0	± 24.3	49.3	± 13.1	11.5	± 2.5
	Low	Autoclaved	(-)	10.1	± 0.9	140.1	± 18.5	616.3	± 56.8	12.5	± 3.2
			(+)	13.0	± 1.4	196.4	± 66.0	876.3	± 92.2	14.6	± 1.5
		Non-autoclaved	(-)	7.1	± 1.2	119.5	± 15.5	40.7	± 2.7	11.0	± 2.5
			(+)	7.8	± 1.2	109.5	± 15.1	46.9	± 8.6	9.2	± 0.6
	High	Autoclaved	(-)	15.2	± 0.7	93.4	± 57.3	685.3	± 51.0	15.5	± 1.7
			(+)	15.6	± 0.7	145.2	± 57.3	786.0	± 51.0	19.2	± 1.7
		Non-autoclaved	(-)	7.2	± 1.4	116.9	± 29.6	36.8	± 2.5	9.3	± 2.5
			(+)	10.2	± 0.8	113.1	± 18.8	45.8	± 3.3	11.6	± 1.8

According to collective data displayed in **Tables 3.46** and **3.47**, even though *P. indica* did not cause any significant changes in the straw Ca content in bread wheat, increased that of durum wheat. In bread wheat, soil sterilization resulted in reduced straw Ca content; however, the content of durum was increased by soil sterilization. B toxicity did not result in any significant effects on the contents of both cultivars.

In terms of straw K content, *P. indica* increased the straw K content in Nusrat, caused no effect on the content in Saricanak-98. Even though soil sterilization caused no effect on the straw K content in Nusrat, increased in that of Saricanak-98. In terms of B toxicity, the same trend was valid for both cultivars as observed in the straw K concentrations (**Tables 3.46** and **3.47**).

As regards to Mg content, the experimental plants inoculated with *P. indica* statistically showed no differences compared to the plants non-inoculated with *P. indica* in Nusrat. On the other hand, for Saricanak-98, the fungal inoculation resulted in an elevated straw Mg content. The plants grown in sterilized soil displayed a reduction in the straw Mg content of Nusrat cultivar compared to those grown in native soil. For Saricanak-98, however, soil sterilization caused elevated content. Considering B toxicity, increasing B level gradually reduced the straw Mg content in Nusrat, but did not cause any significant changes in that of Saricanak-98 (**Tables 3.46** and **3.47**).

As tackled in **Tables 3.46** and **3.47**, the straw S contents of both cultivars were not affected by *P. indica*.

Table 3.46: Three-way analysis of variance (ANOVA) of the effects of inoculation, soil sterilization and boron applications as well as their interactions on selected straw macronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) grown under greenhouse conditions (at 111 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

<i>Triticum aestivum</i> cv. Nusrat	Source of Variation	DF	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.197	n.s.	0.004	1.922	0.130	n.s.	0.2013	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Soil Sterilization (S)	1	<0.001	1.283	0.755	n.s.	<0.001	0.397	<0.001	0.559
	Boron Supply (B)	2	0.936	n.s.	<0.001	2.839	0.045	0.586	0.0072	0.826
	I X S	1	0.071	n.s.	0.830	n.s.	0.089	n.s.	0.0784	n.s.
	I X B	2	0.202	n.s.	0.044	4.942	0.321	n.s.	0.2244	n.s.
	S X B	2	0.012	3.299	0.001	4.942	0.032	1.019	0.4594	n.s.
	I X S X B	2	0.936	n.s.	0.300	n.s.	0.929	n.s.	0.8195	n.s.
	Source of Variation	DF	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.012	1.37	0.286	n.s.	0.002	0.273	0.435	n.s.
	Soil Sterilization (S)	1	<0.001	1.37	<0.001	1.664	<0.001	0.273	<0.001	0.395
	Boron Supply (B)	2	0.074	n.s.	<0.001	2.458	0.663	n.s.	0.117	n.s.
	I X S	1	0.181	n.s.	0.065	n.s.	0.466	n.s.	0.010	0.743
	I X B	2	0.041	3.522	0.368	n.s.	0.011	0.703	0.029	1.016
	S X B	2	0.013	3.522	<0.001	4.28	0.204	n.s.	<0.001	1.016
	I X S X B	2	0.338	n.s.	0.020	7.021	0.452	n.s.	0.046	1.668

Table 3.47: Selected straw macronutrient contents of 111-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98), inoculated and non-inoculated with *P. indica*, grown in autoclaved and non-autoclaved soils at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃; low B: 10 mg kg⁻¹ H₃BO₃; high B: 20 mg kg⁻¹ H₃BO₃) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	B Supply (mg kg ⁻¹)	Soil Sterilization	<i>P. indica</i>	Ca		K		Mg		S	
				(mg plant ⁻¹)		(mg plant ⁻¹)		(mg plant ⁻¹)		(mg plant ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Control	Autoclaved	(-)	21.22	± 0.39	45.62	± 1.78	6.37	± 0.08	5.59	± 0.25
			(+)	22.06	± 1.12	48.81	± 2.14	6.82	± 0.42	6.35	± 0.80
		Non-autoclaved	(-)	25.89	± 2.26	49.07	± 5.28	7.79	± 0.63	7.91	± 0.72
			(+)	28.76	± 3.70	55.58	± 2.95	8.80	± 1.34	7.35	± 0.11
	Low	Autoclaved	(-)	24.00	± 0.90	53.63	± 2.37	6.90	± 0.38	6.20	± 0.46
			(+)	23.92	± 1.06	58.45	± 3.74	6.75	± 0.56	6.63	± 0.75
		Non-autoclaved	(-)	23.28	± 0.93	51.31	± 1.54	6.99	± 0.17	7.69	± 0.98
			(+)	26.21	± 1.31	55.58	± 1.74	7.44	± 0.14	6.95	± 1.02
	High	Autoclaved	(-)	23.56	± 1.10	54.22	± 2.49	6.42	± 0.29	7.74	± 0.24
			(+)	21.76	± 1.04	55.68	± 1.80	6.01	± 0.32	6.96	± 1.12
		Non-autoclaved	(-)	25.62	± 2.80	52.81	± 4.02	7.21	± 0.54	8.94	± 1.28
			(+)	25.85	± 2.92	50.26	± 1.55	7.70	± 0.88	7.64	± 1.15
<i>Triticum durum</i> cv. Saricanak-98	Control	Autoclaved	(-)	21.55	± 0.71	38.81	± 2.12	4.50	± 0.23	5.30	± 0.31
			(+)	21.39	± 1.88	36.37	± 1.78	4.52	± 0.35	5.58	± 0.57
		Non-autoclaved	(-)	16.37	± 2.30	27.11	± 1.31	3.21	± 0.28	6.23	± 0.89
			(+)	16.02	± 2.23	25.66	± 1.75	3.00	± 0.55	4.91	± 0.70
	Low	Autoclaved	(-)	20.87	± 1.19	42.63	± 1.38	4.13	± 0.46	4.59	± 0.21
			(+)	27.32	± 4.87	48.36	± 5.08	5.39	± 0.87	6.39	± 0.67
		Non-autoclaved	(-)	15.36	± 0.68	31.31	± 1.07	2.80	± 0.16	4.89	± 0.77
			(+)	17.12	± 1.51	27.09	± 2.26	3.46	± 0.35	4.73	± 0.52
	High	Autoclaved	(-)	25.05	± 1.47	52.20	± 4.47	4.64	± 0.24	6.85	± 0.29
			(+)	26.89	± 1.47	50.92	± 4.47	5.01	± 0.24	6.84	± 0.29
		Non-autoclaved	(-)	14.92	± 0.86	29.65	± 0.65	2.52	± 0.21	4.27	± 0.56
			(+)	16.11	± 0.77	27.96	± 1.54	3.12	± 0.26	4.61	± 0.52

3.3. Third Soil Experiment

Histological investigations and characterization of fungal colonization were executed via identifying vesicles produced by mycorrhiza (**Figure 3.16B**) in roots colonized with AM fungi at toxic B level and low P level as compared with corresponding control (non-colonized) plants (**Figure 3.16A**).

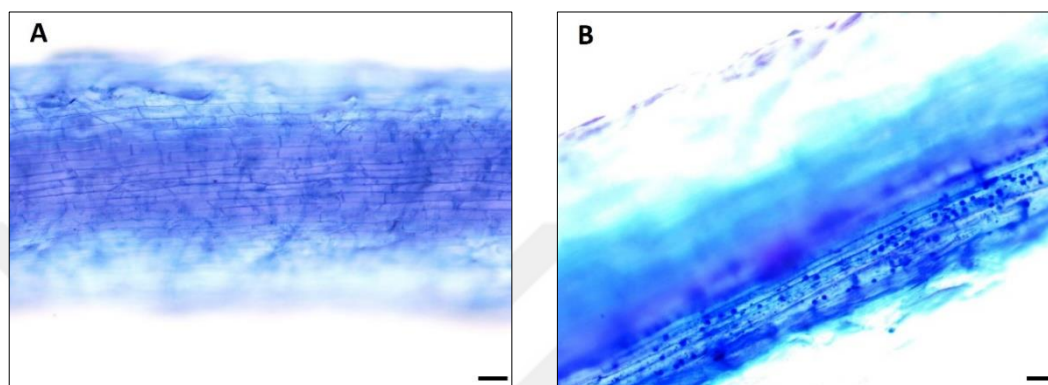


Figure 3.16: Trypan blue staining of non-inoculated (A) and inoculated (B) bread wheat (*Triticum aestivum* cv. Nusrat) plant roots at toxic B levels ($20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) and low P levels ($20 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$) under greenhouse conditions showing vesicles produced by mycorrhiza observed at day 49 DAT. Bars (black lines) represent $50 \mu\text{m}$.

As displayed by four-way ANOVA, the single effect of B and P treatments as well as double (only *P. indica* inoculation X mycorrhizal inoculation) and triple interactions (mycorrhizal inoculation X B supply X P supply) significantly affected the shoot dry matter in Nusrat cultivar. In Saricanak-98 cultivar, all single effects of the treatments except for mycorrhizal inoculation and double (*P. indica* inoculation X mycorrhizal inoculation and *P. indica* inoculation X P supply) interactions affected the shoot dry matter significantly (**Table 3.48**). In the presence of *P. indica*, the shoot dry weight was not affected significantly in Nusrat; however, for Saricanak-98, the shoot dry weight was higher than those grown without *P. indica* (**Figure 3.17A**). On the other hand, in the presence of mycorrhizal inoculum, shoot dry weights of plants were not affected significantly in both cultivars (**Figure 3.17B**). Overall, in terms of the shoot dry matter production, positive effects of *P. indica* only appeared in Saricanak-98, arbuscular mycorrhiza, however, did not cause any positive or negative effects. Increasing the B supply reduced the shoot dry weights dose-dependently in both

Nusrat and Saricanak-98 cultivars (**Figure 3.17C**). Increasing the P supply dose-dependently improved the shoot dry weights in both tested cultivars (**Figure 3.17D**).

Table 3.48: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on shoot DW (g plant⁻¹), and B (mg kg⁻¹), Mn (mg kg⁻¹) and P (%) concentrations of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Shoot DW (g plant ⁻¹)		Shoot B Conc. (mg kg ⁻¹)		Shoot Mn Conc. (mg kg ⁻¹)		Shoot P Conc. (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.142	n.s.	0.022	22	0.016	10	0.879	n.s.
	Arbuscular Mycorrhiza (AM)	1	0.551	n.s.	0.881	n.s.	0.019	10	0.146	n.s.
	Boron Supply (B)	1	0.020	0.08	<0.001	22	<0.001	10	<0.001	0.01
	Phosphorus Supply (P)	1	<0.001	0.08	0.275	n.s.	<0.001	10	<0.001	0.01
	I X AM	1	0.016	0.15	0.963	n.s.	<0.001	19	0.535	n.s.
	I X B	1	0.054	n.s.	0.023	42	0.077	n.s.	0.489	n.s.
	AM X B	1	0.291	n.s.	0.738	n.s.	0.008	19	0.1057	n.s.
	I X AM X B	1	0.884	n.s.	0.976	n.s.	0.478	n.s.	0.552	n.s.
	I X P	1	0.159	n.s.	0.508	n.s.	0.002	19	0.541	n.s.
	AM X P	1	0.542	n.s.	0.762	n.s.	0.003	19	0.395	n.s.
	I X AM x P	1	0.759	n.s.	0.621	n.s.	<0.001	33	0.083	n.s.
	B X P	1	0.446	n.s.	0.165	n.s.	0.927	n.s.	0.775	n.s.
	I X B X P	1	0.420	n.s.	0.589	n.s.	0.434	n.s.	0.744	n.s.
	AM X B X P	1	0.043	0.28	0.617	n.s.	0.963	n.s.	0.202	n.s.
	I X AM X B X P	1	0.815	n.s.	0.606	n.s.	0.337	n.s.	0.151	n.s.
<i>Triticum durum</i> cv. Saricanak-98										
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	0.10	0.703	n.s.	<0.001	8	<0.001	0.01
	Arbuscular Mycorrhiza (AM)	1	0.093	n.s.	0.180	n.s.	0.216	n.s.	0.695	n.s.
	Boron Supply (B)	1	<0.001	0.10	<0.001	19	0.005	8	<0.001	0.01
	Phosphorus Supply (P)	1	<0.001	0.10	<0.001	19	<0.001	8	<0.001	0.01
	I X AM	1	<0.001	0.18	0.574	n.s.	<0.001	16	0.890	n.s.
	I X B	1	0.548	n.s.	0.831	n.s.	0.038	16	0.0136	0.01
	AM X B	1	0.833	n.s.	0.176	n.s.	0.622	n.s.	0.083	n.s.
	I X AM X B	1	0.746	n.s.	0.761	n.s.	0.825	n.s.	0.382	n.s.
	I X P	1	0.029	0.18	<0.001	37	<0.001	16	0.190	n.s.
	AM X P	1	0.347	n.s.	0.006	36	0.204	n.s.	0.054	n.s.
	I X AM x P	1	0.530	n.s.	0.076	n.s.	0.802	n.s.	0.9961	n.s.
	B X P	1	0.661	n.s.	0.001	37	0.030	15	0.804	n.s.
	I X B X P	1	0.111	n.s.	<0.001	61	0.003	26	0.100	n.s.
	AM X B X P	1	0.979	n.s.	0.005	61	0.001	26	0.619	n.s.
	I X AM X B X P	1	0.562	n.s.	0.073	n.s.	0.928	n.s.	0.934	n.s.

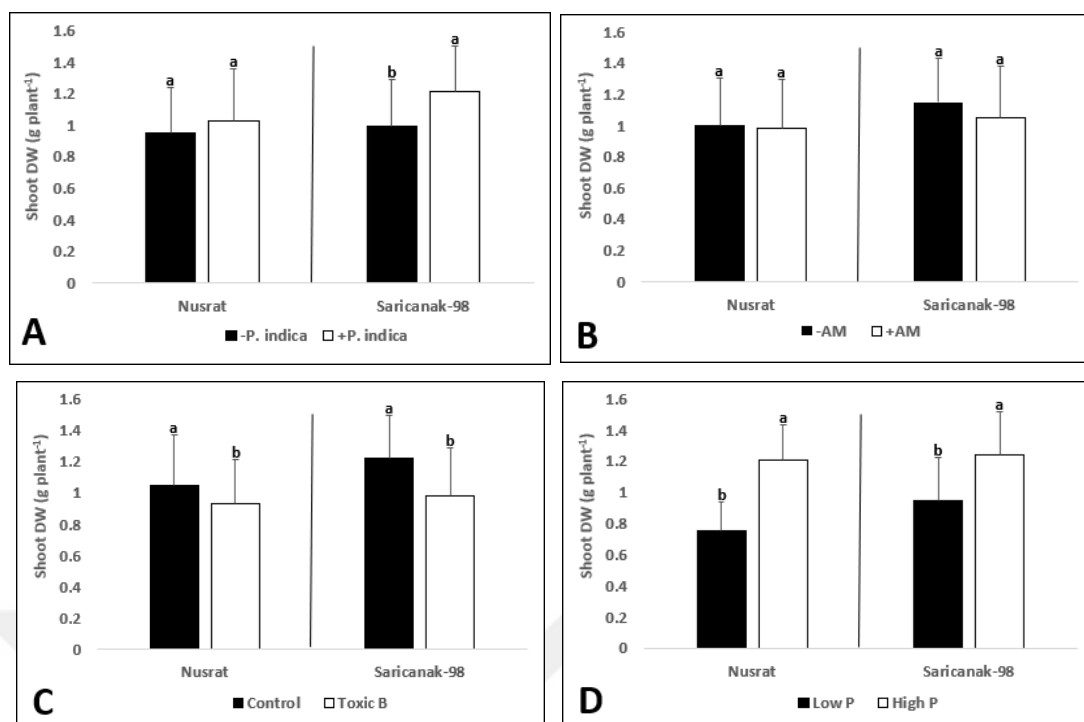


Figure 3.17: Shoot DW (g plant^{-1}) of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$ and toxic B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) and P supply (low P: $20 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$ and high P: $100 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$) under greenhouse conditions. Different letters indicate significant differences between means according to four-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

The shoot B concentration was significantly affected by the single effects of *P. indica* inoculation and B supply treatments and their double interaction in Nusrat. In Saricanak-98, single effects of B and P supply treatments, double (*P. indica* inoculation X P supply, mycorrhizal inoculation X P supply, B supply X P supply) and triple (*P. indica* inoculation X mycorrhizal inoculation X P supply, *P. indica* inoculation X B supply X P supply and mycorrhizal inoculation X B supply X P supply) interactions significantly affected the shoot B concentrations (**Table 3.48**). The presence of *P. indica* caused an increase in terms of shoot B concentrations in Nusrat; however, for Saricanak-98, it did not cause any significant effects (**Figure 3.18A**). The presence of mycorrhizal inoculation did not cause any significant effects in terms of shoot B concentrations in both cultivars, however (**Figure 3.18B**). Increasing B supply from the control to the high B level resulted in dose-dependent

elevations in both cultivars, moreover, Saricanak-98 accumulated more B compared to Nusrat overall (**Figure 3.18C**). Increasing the P supply from the control to the high P level resulted in a dose-dependent reduction of the shoot B concentrations for Saricanak-98; however, for Nusrat, it did not cause any significant effects in terms of the shoot B concentrations (**Figure 3.18D**).

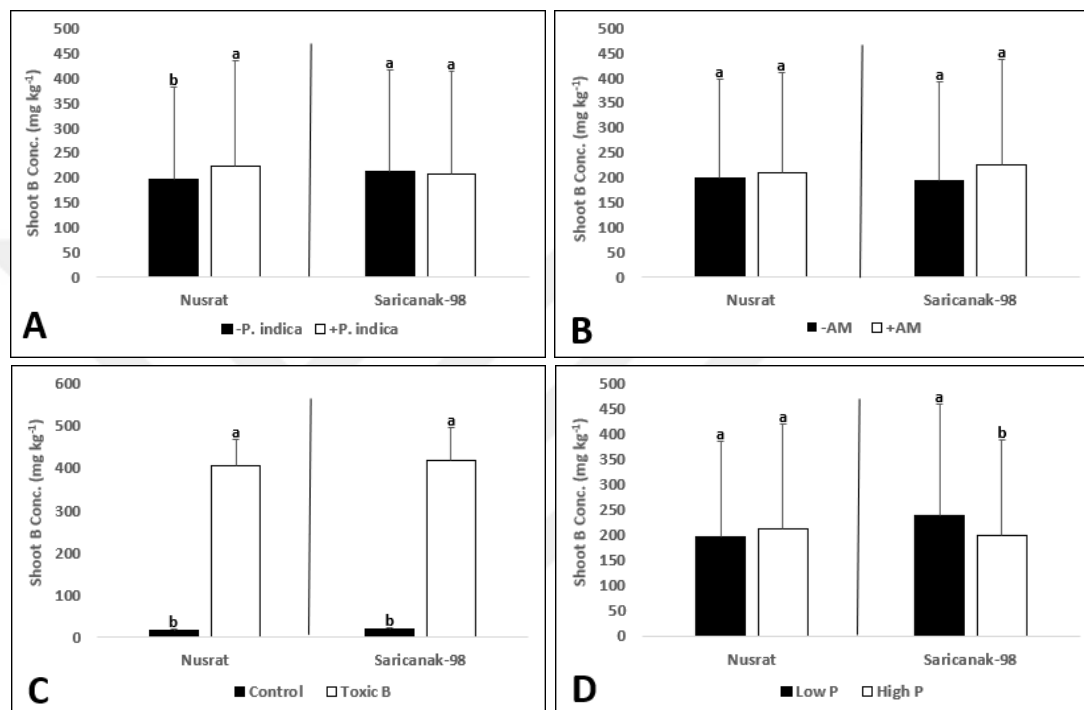


Figure 3.18: Shoot B concentrations (mg kg^{-1}) of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$ and toxic B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) and P supply (low P: $20 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$ and high P: $100 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$) under greenhouse conditions. Different letters indicate significant differences between means according to four-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

The shoot Mn concentration was significantly affected by all single effects of the treatments and double (*P. indica* inoculation X mycorrhizal inoculation, mycorrhizal inoculation X B supply, *P. indica* inoculation X P supply, mycorrhizal inoculation X P supply) and triple (*P. indica* inoculation X mycorrhizal inoculation X P supply) interactions in Nusrat. In Saricanak-98, however, it was significantly affected by all

single effects of the treatments except for mycorrhizal inoculation, double (*P. indica* inoculation X mycorrhizal inoculation, *P. indica* inoculation X B supply, *P. indica* inoculation X P supply, B supply X P supply,) and triple (*P. indica* inoculation X B supply X P supply and mycorrhizal inoculation X B supply X P supply) interactions (**Table 3.48**). In the presence of *P. indica*, the shoot Mn concentrations were reduced in Nusrat; however, for Saricanak-98, it resulted in increased shoot Mn concentrations (**Figure 3.19A**). Considering mycorrhizal inoculation, the presence of arbuscular mycorrhiza caused elevated shoot Mn concentrations; however, in Saricanak-98, it did not cause any effects (**Figure 3.19B**). Increasing B supply from the control to the high B level resulted in a dose-dependent elevation for Nusrat; however, for Saricanak-98, it resulted in a dose-dependent reduction (**Figure 3.19C**). Increasing the P supply from the control to the high P level resulted in dose-dependent reductions of the shoot Mn concentrations for both cultivars and Nusrat accumulated more Mn compared to Saricanak-98 overall (**Figure 3.19D**).

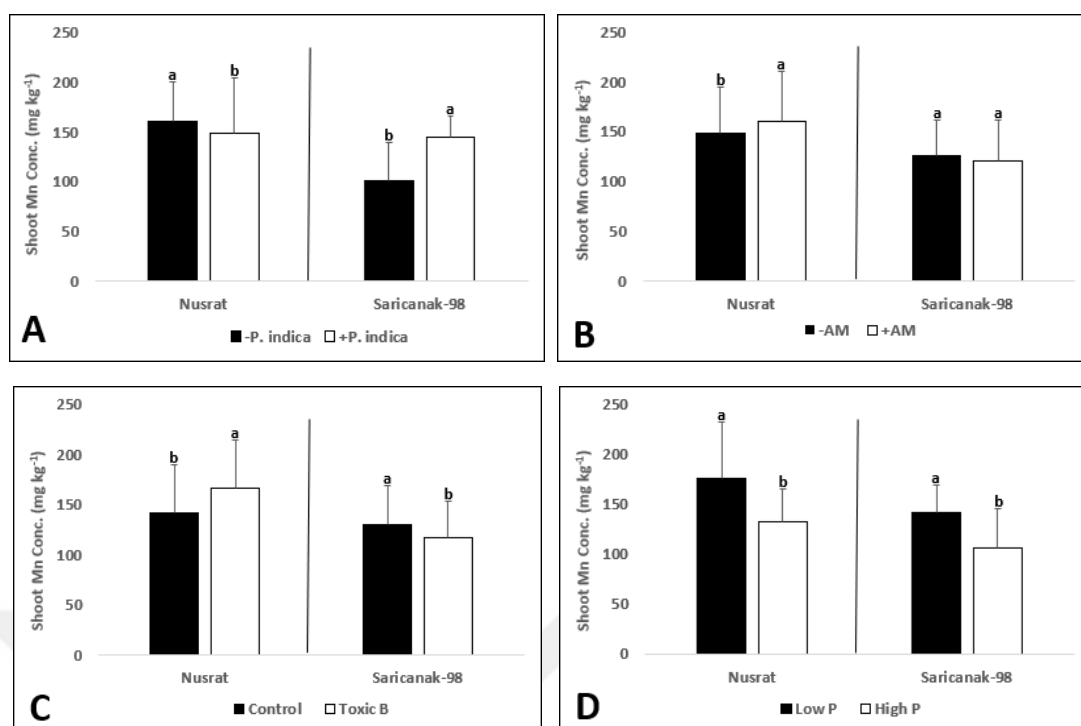


Figure 3.19: Shoot Mn concentrations (mg kg^{-1}) of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$ and toxic B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) and P supply (low P: $20 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$ and high P: $100 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$) under greenhouse conditions. Different letters indicate significant differences between means according to four-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

The shoot P concentration was significantly affected by only a single effect of B and P supplies in Nusrat. In Saricanak-98, it was significantly affected by all single effects except for mycorrhizal inoculation, moreover, double interaction of *P. indica* inoculation with B supply (**Table 3.48**). Although *P. indica* inoculated plants did not cause any change in terms of shoot P concentrations in Nusrat, it showed significantly elevated shoot P concentrations in Saricanak-98 (**Figure 3.20A**). However, the presence of arbuscular mycorrhiza did not cause any considerable change in terms of shoot P concentrations in both cultivars (**Figure 3.20B**). Increasing B supply resulted in dose-dependent elevations in both cultivars (**Figure 3.20C**). Increasing the P supply from the control to the high P level resulted in dose-dependent increases of the shoot P concentrations for both cultivars and Nusrat accumulated more P compared to Saricanak-98 overall (**Figure 3.20D**).

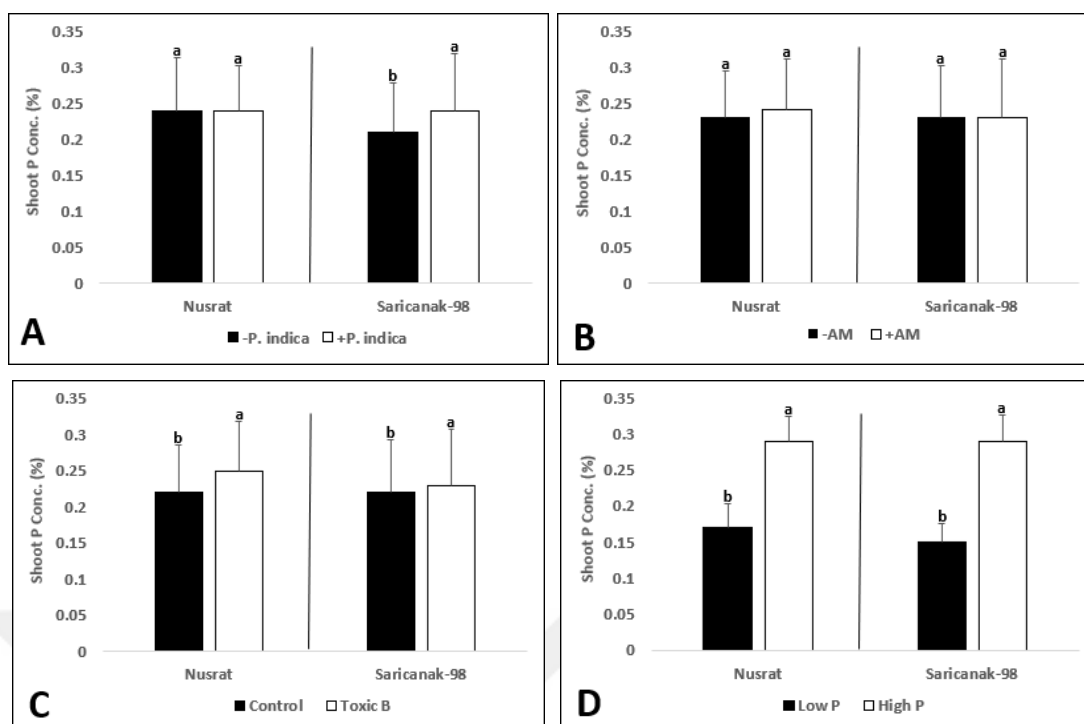


Figure 3.20: Shoot P concentrations (mg kg^{-1}) of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$ and toxic B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) and P supply (low P: $20 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$ and high P: $100 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$) under greenhouse conditions. Different letters indicate significant differences between means according to four-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

The shoot MDA concentration was significantly affected by all single effects, moreover, double (mycorrhizal inoculation X P supply and B supply X P supply), triple (*P. indica* inoculation X mycorrhizal inoculation X B supply), and quadruple (*P. indica* inoculation X mycorrhizal inoculation X B supply X P supply) interactions in Nusrat. In Saricanak-98, it was significantly affected by all single effects and triple (mycorrhizal inoculation X B supply X P supply) interaction (**Table 3.49**). *P. indica* inoculated plants showed reduced shoot MDA concentrations in both cultivars (**Figure 3.21A**). Although the presence of arbuscular mycorrhiza led to elevated shoot MDA concentration in Nusrat, it resulted in a reduction in Saricanak-98 (**Figure 3.21B**). Increasing B supply resulted in elevations in both cultivars (**Figure 3.21C**). Increasing P supply from the control to the high P level resulted in dose-

dependent increases of the shoot MDA concentrations for both cultivars and Saricanak-98 accumulated more MDA compared to Nusrat overall (**Figure 3.21D**).

The shoot proline concentration was considerably affected by only single effects of *P. indica* inoculation and P supply, double (*P. indica* inoculation X mycorrhizal inoculation, mycorrhizal inoculation X B supply, *P. indica* inoculation X P supply, mycorrhizal inoculation X P supply, and B supply X P supply), and triple (*P. indica* inoculation X mycorrhizal inoculation X P supply, *P. indica* inoculation X B supply X P supply) interactions in Nusrat. In Saricanak-98, it was significantly affected by only single effects of *P. indica* inoculation and P supply, double (*P. indica* inoculation X P supply and mycorrhizal inoculation X B supply) and triple (*P. indica* inoculation X mycorrhizal inoculation X B supply) interactions (**Table 3.49**). *P. indica* inoculated plants showed elevated shoot proline concentrations in both cultivars (**Figure 3.22A**). The presence of arbuscular mycorrhiza did not cause any significant change in terms of the shoot proline concentrations in both cultivars (**Figure 3.22B**). Besides that, increasing B supply did not significantly affect both cultivars (**Figure 3.22C**). Increasing the P supply from the control to the high P level resulted in dose-dependent increases of the shoot proline concentrations in both cultivars and Saricanak-98 accumulated more proline compared to Nusrat in general (**Figure 3.22D**).

Table 3.49: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on shoot MDA (nmol g⁻¹), proline concentration (%), total chlorophyll (nmol g⁻¹), and total carotenoids (nmol g⁻¹) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	MDA (nmol g ⁻¹)		Proline Conc. (%)		Total Chlorophyll (nmol g ⁻¹)		Total Carotenoids (nmol g ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.020	3.2	0.002	0.8	0.005	88	<0.001	22
	Arbuscular Mycorrhiza (AM)	1	<0.001	3.2	0.844	n.s.	0.250	n.s.	0.0735	n.s.
	Boron Supply (B)	1	<0.001	3.2	0.894	n.s.	0.050	88	0.346	n.s.
	Phosphorus Supply (P)	1	0.046	3.2	<0.001	0.8	0.092	n.s.	0.4465	n.s.
	I X AM	1	0.139	n.s.	<0.001	1.6	0.411	n.s.	0.0142	32
	I X B	1	0.884	n.s.	0.615	n.s.	0.029	122	0.0491	30
	AM X B	1	0.921	n.s.	0.004	1.2	0.614	n.s.	0.278	n.s.
	I X AM X B	1	0.040	10.4	0.082	n.s.	0.713	n.s.	0.9068	n.s.
	I X P	1	0.070	n.s.	0.004	1.6	0.048	126	0.2854	n.s.
	AM X P	1	0.014	6.2	0.002	1.6	0.215	n.s.	0.1256	n.s.
	I X AM x P	1	0.401	n.s.	<0.001	2.7	0.170	n.s.	0.9404	n.s.
	B X P	1	0.009	6.2	0.006	1.6	0.188	n.s.	0.2345	n.s.
	I X B X P	1	0.956	n.s.	0.003	2.7	0.033	261	0.3032	n.s.
	AM X B X P	1	0.445	n.s.	0.635	n.s.	0.353	n.s.	0.465	n.s.
	I X AM X B X P	1	0.042	16.9	0.526	n.s.	0.522	n.s.	0.539	n.s.
	Source of Variation	DF	MDA (nmol g ⁻¹)		Proline Conc. (%)		Total Chlorophyll (nmol g ⁻¹)		Total Carotenoids (nmol g ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum durum</i> cv. Saricanak-98	Inoculation (I)	1	0.004	3.3	<0.001	2.6	0.011	94	0.001	18
	Arbuscular Mycorrhiza (AM)	1	<0.001	3.3	0.894	n.s.	0.108	n.s.	0.898	n.s.
	Boron Supply (B)	1	0.001	3.3	0.300	n.s.	0.121	n.s.	0.558	n.s.
	Phosphorus Supply (P)	1	<0.001	3.3	<0.001	2.6	<0.001	94	<0.001	18
	I X AM	1	0.219	n.s.	0.339	n.s.	0.881	n.s.	0.059	n.s.
	I X B	1	0.059	n.s.	0.054	n.s.	0.057	n.s.	0.0254	26
	AM X B	1	0.150	n.s.	0.005	5.0	0.2628	n.s.	0.407	n.s.
	I X AM X B	1	0.623	n.s.	0.037	8.0	0.908	n.s.	0.557	n.s.
	I X P	1	0.543	n.s.	<0.001	4.7	0.001	138	0.020	28
	AM X P	1	0.945	n.s.	0.967	n.s.	0.879	n.s.	0.619	n.s.
	I X AM x P	1	0.156	n.s.	0.752	n.s.	0.019	279	0.0203	60
	B X P	1	0.501	n.s.	0.307	n.s.	0.879	n.s.	0.808	n.s.
	I X B X P	1	0.599	n.s.	0.089	n.s.	0.134	n.s.	0.345	n.s.
	AM X B X P	1	0.002	11.0	0.185	n.s.	0.524	n.s.	0.423	n.s.
	I X AM X B X P	1	0.311	n.s.	0.217	n.s.	0.072	n.s.	0.529	n.s.

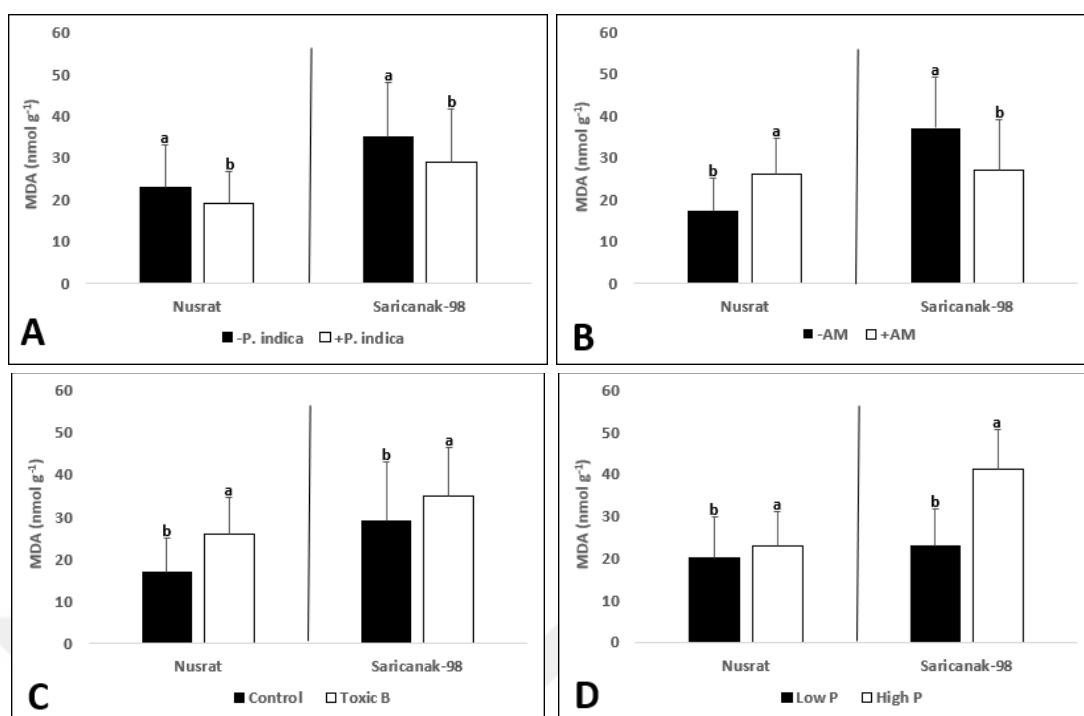


Figure 3.21: MDA concentration (nmol g⁻¹) of the third and fourth oldest leaves (pooled) of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃ and toxic B: 20 mg kg⁻¹ H₃BO₃) and P supply (low P: 20 mg kg⁻¹ KH₂PO₄ and high P: 100 mg kg⁻¹ KH₂PO₄) under greenhouse conditions. Different letters indicate significant differences between means according to four-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

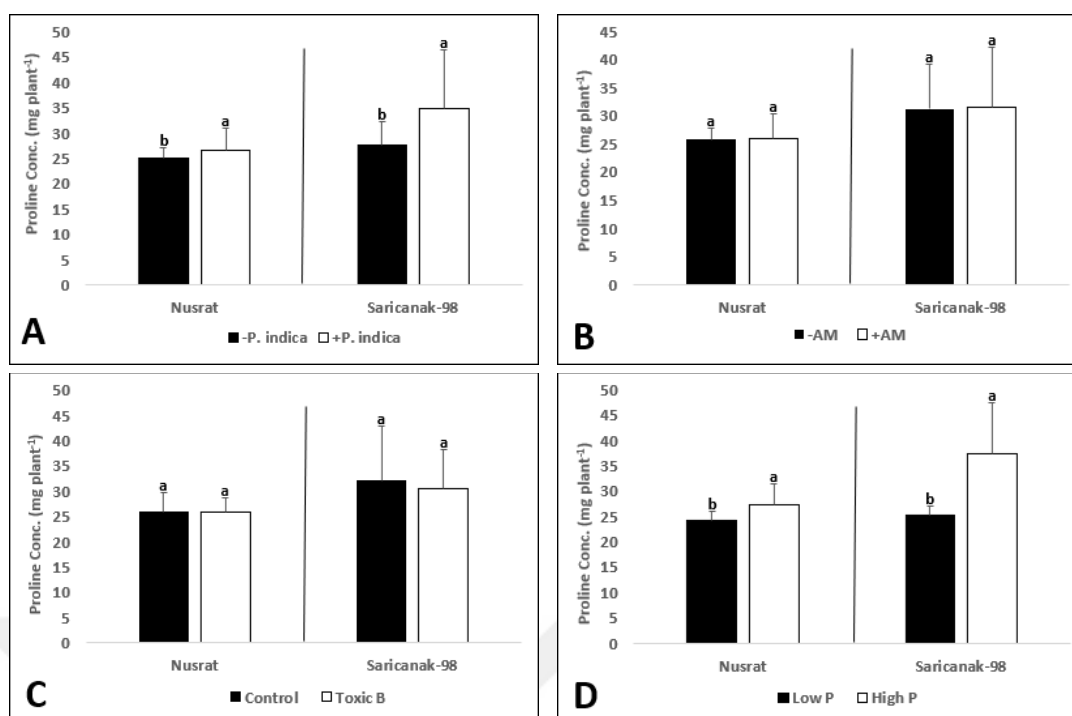


Figure 3.22: Proline concentration (mg plant⁻¹) of the third and fourth oldest leaves (pooled) of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃ and toxic B: 20 mg kg⁻¹ H₃BO₃) and P supply (low P: 20 mg kg⁻¹ KH₂PO₄ and high P: 100 mg kg⁻¹ KH₂PO₄) under greenhouse conditions. Different letters indicate significant differences between means according to four-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

The shoot chlorophyll concentration was considerably affected by only single effects of *P. indica* inoculation and B supply, double (*P. indica* inoculation X B supply, *P. indica* inoculation X P supply) and triple (*P. indica* inoculation X B supply X P supply) interactions in Nusrat. In Saricanak-98, it was significantly affected by only a single effect of *P. indica* inoculation and P supply, double (*P. indica* inoculation X P supply) and triple (*P. indica* inoculation X mycorrhizal inoculation X P supply) interactions (**Table 3.49**). *P. indica* inoculated plants showed elevated shoot chlorophyll concentrations in both cultivars (**Figure 3.23A**). Moreover, the presence of arbuscular mycorrhiza did not cause any significant change in terms of the shoot chlorophyll concentrations in both cultivars (**Figure 3.23B**). Even though increasing B supply caused reductions in Nusrat, it did not cause any change in Saricanak-98

(Figure 3.23C). Increasing the P supply from the low to the high P level did not significantly change the shoot chlorophyll concentrations in Nusrat; however, for Saricanak-98, it resulted in a dose-dependent increase and Saricanak-98 produced more chlorophyll compared to Nusrat overall (Figure 3.23D).

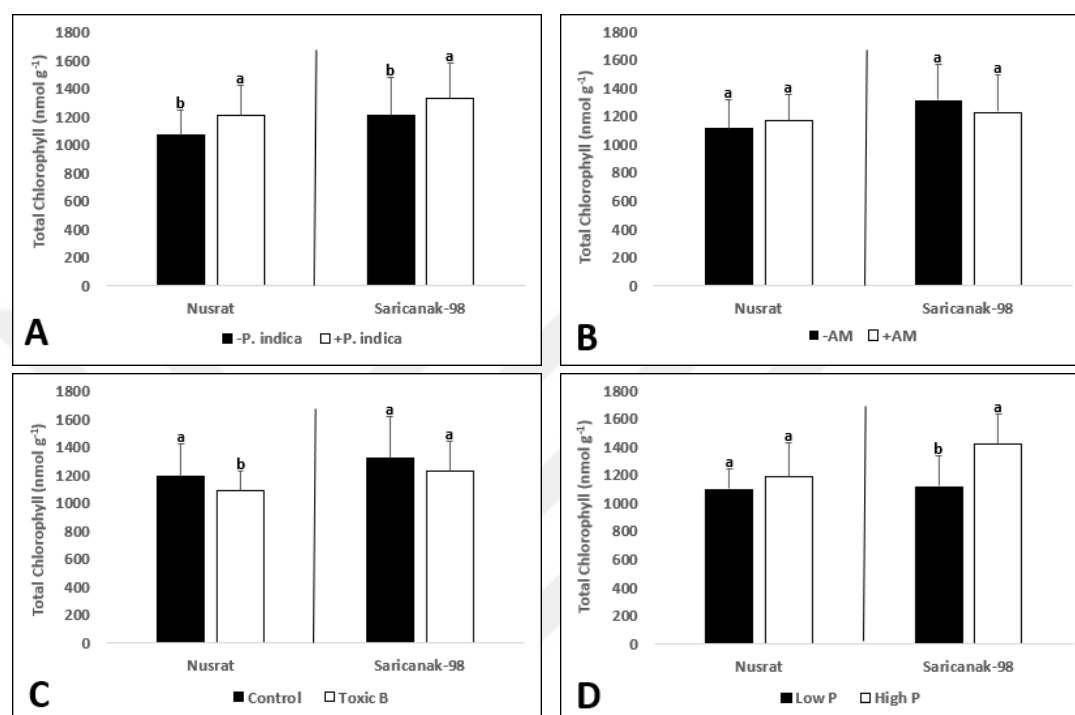


Figure 3.23: Total chlorophyll (nmol g⁻¹) of the second fully expanded leaves of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃ and toxic B: 20 mg kg⁻¹ H₃BO₃) and P supply (low P: 20 mg kg⁻¹ KH₂PO₄ and high P: 100 mg kg⁻¹ KH₂PO₄) under greenhouse conditions. Different letters indicate significant differences between means according to four-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

In Nusrat, the shoot carotenoid concentration was considerably affected by only single effects of *P. indica* inoculation, and double (*P. indica* inoculation X mycorrhizal inoculation and *P. indica* inoculation X B supply) interactions. In Saricanak-98, it was significantly affected by only single effects of *P. indica* inoculation and P supply, double (*P. indica* inoculation X B supply and *P. indica* inoculation X P supply), and triple (*P. indica* inoculation X mycorrhizal inoculation

X P supply) interactions (**Table 3.49**). *P. indica* inoculated plants showed elevated shoot carotenoid concentrations in both cultivars (**Figure 3.24A**). Moreover, the presence of arbuscular mycorrhiza did not cause any significant change in both cultivars (**Figure 3.24B**). Increasing B supply did not cause any change in shoot carotenoid concentrations in both cultivars (**Figure 3.24C**). Increasing P supply from the low to the high P level did not significantly change the shoot carotenoid concentrations in Nusrat; however, for Saricanak-98, it resulted in a dose-dependent increase and durum wheat produced more carotenoid compared to bread wheat overall (**Figure 3.24D**).

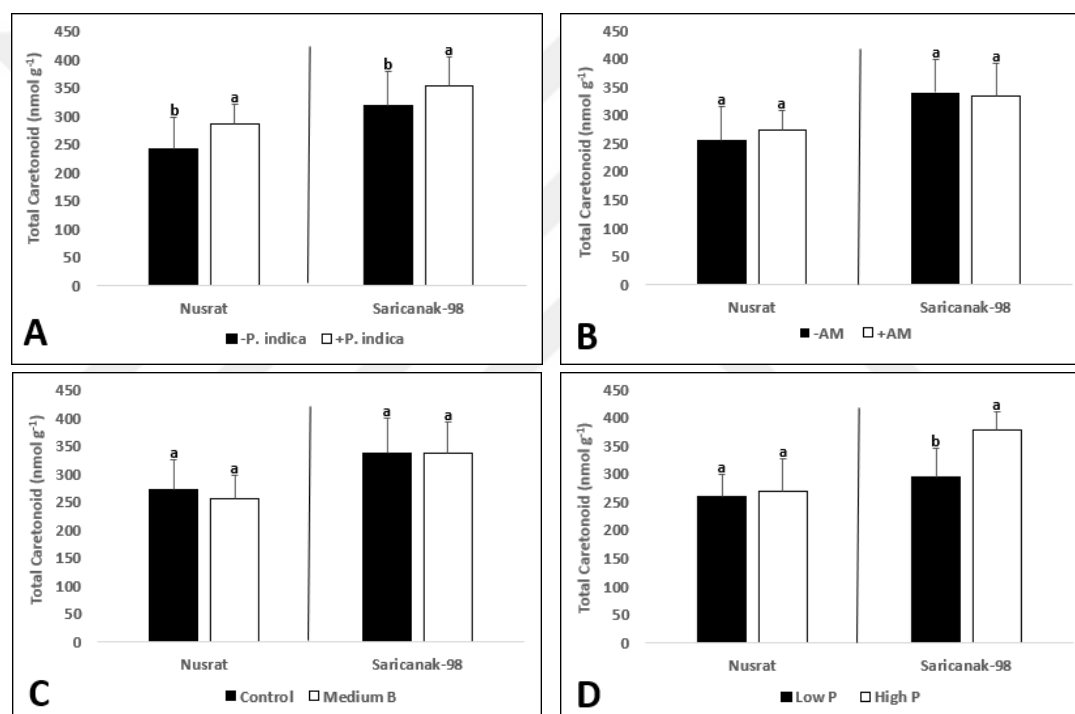


Figure 3.24: Total carotenoid (nmol g^{-1}) of the second fully expanded leaves of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$ and toxic B: $20 \text{ mg kg}^{-1} \text{ H}_3\text{BO}_3$) and P supply (low P: $20 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$ and high P: $100 \text{ mg kg}^{-1} \text{ KH}_2\text{PO}_4$) under greenhouse conditions. Different letters indicate significant differences between means according to four-way ANOVA and Tukey's HSD test ($p \leq 0.05$). Statistical analysis was carried out separately for Nusrat and Saricanak-98.

The shoot B content was significantly affected by the single effect of *P. indica* inoculation, B supply, and P supply, and double (*P. indica* inoculation X B supply, and B supply X P supply) interaction in Nusrat. In Saricanak-98, the shoot B content was significantly affected by the single effect of *P. indica* inoculation and B supply, double (*P. indica* inoculation X B supply, and *P. indica* inoculation X P supply), and triple (*P. indica* inoculation X B supply X P supply) interactions (**Table 3.50**). The shoot B content was significantly increased by the presence of *P. indica* in both cultivars. Increasing B levels from control to toxic resulted in dose-dependent elevations in the shoot B contents in either cultivar. Increasing P supply caused elevated shoot B content in Nusrat; however, in Saricanak-98, it did not cause any significant change (**Tables 3.50 and 3.51**).

The shoot Mn content was significantly affected by only the single effect of P supply, double (*P. indica* inoculation X mycorrhizal inoculation, *P. indica* inoculation X B supply, mycorrhizal inoculation X B supply), and triple (*P. indica* inoculation X mycorrhizal inoculation X P supply) interactions in Nusrat. In Saricanak-98, single effects of *P. indica* inoculation, mycorrhizal inoculation, and B treatment, double (*P. indica* inoculation X mycorrhizal inoculation, and *P. indica* inoculation X P supply) and triple (*P. indica* inoculation X B supply X P supply, and mycorrhizal inoculation X B supply X P supply) interactions significantly affected the shoot Mn contents (**Table 3.50**). The shoot Mn contents of Nusrat were not affected significantly by either *P. indica* or mycorrhizal inoculation. However, in Saricanak-98, although *P. indica* increased the shoot Mn contents, arbuscular mycorrhiza reduced it. In Nusrat, B toxicity did not cause any significant change; nevertheless, in Saricanak-98, it caused reductions in the shoot Mn contents. Whereas increasing the P supply resulted in elevated shoot Mn concentrations in Nusrat, it did not change in Saricanak-98 (**Tables 3.50 and 3.51**).

The shoot P content was significantly affected by only the single effect of P supply in Nusrat. On the other hand, in Saricanak-98, it was affected by single effects of *P. indica* inoculation, mycorrhizal inoculation, B supply and P supply, and double interaction of *P. indica* inoculation and mycorrhizal inoculation (**Table 3.50**). In Nusrat, increasing the P supply caused elevated P content. The shoot P content of Saricanak-98 was increased by *P. indica* but reduced by mycorrhiza. The shoot P

content of Saricanak-98 was reduced by B toxicity but improved by P supply (Tables 3.50 and 3.51).

Table 3.50: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on the shoot B ($\mu\text{g kg}^{-1}$), Mn ($\mu\text{g kg}^{-1}$), and P (mg plant^{-1}) contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	B ($\mu\text{g kg}^{-1}$)		Mn ($\mu\text{g kg}^{-1}$)		P (mg plant^{-1})	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	<0.001	30	0.369	n.s.	0.187	n.s.
	Arbuscular Mycorrhiza (AM)	1	0.424	n.s.	0.611	n.s.	0.640	n.s.
	Boron Supply (B)	1	<0.001	30	0.464	n.s.	0.944	n.s.
	Phosphorus Supply (P)	1	<0.001	30	0.010	17	<0.001	0.03
	I X AM	1	0.333	n.s.	<0.001	32	0.065	n.s.
	I X B	1	<0.001	57	0.001	32	0.073	n.s.
	AM X B	1	0.278	n.s.	0.002	32	0.170	n.s.
	I X AM X B	1	0.393	n.s.	0.469	n.s.	0.689	n.s.
	I X P	1	0.358	n.s.	0.229	n.s.	0.340	n.s.
	AM X P	1	0.819	n.s.	0.334	n.s.	0.441	n.s.
	I X AM x P	1	0.254	n.s.	0.044	52	0.586	n.s.
	B X P	1	<0.001	57	0.900	n.s.	0.464	n.s.
	I X B X P	1	0.364	n.s.	0.189	n.s.	0.984	n.s.
	AM X B X P	1	0.854	n.s.	0.112	n.s.	0.532	n.s.
	I X AM X B X P	1	0.256	n.s.	0.301	n.s.	0.374	n.s.
	Source of Variation	DF	B ($\mu\text{g kg}^{-1}$)		Mn ($\mu\text{g kg}^{-1}$)		P (mg plant^{-1})	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum durum</i> cv. Saricanak-98	Inoculation (I)	1	<0.001	29	<0.001	14	<0.001	0.01
	Arbuscular Mycorrhiza (AM)	1	0.385	n.s.	0.038	14	0.005	0.01
	Boron Supply (B)	1	<0.001	29	<0.001	14	0.001	0.01
	Phosphorus Supply (P)	1	0.067	n.s.	0.923	n.s.	<0.001	0.01
	I X AM	1	0.223	n.s.	<0.001	27	0.009	0.03
	I X B	1	<0.001	54	0.614	n.s.	0.147	n.s.
	AM X B	1	0.521	n.s.	0.881	n.s.	0.981	n.s.
	I X AM X B	1	0.266	n.s.	0.148	n.s.	0.458	n.s.
	I X P	1	<0.001	54	0.004	27	0.157	n.s.
	AM X P	1	0.070	n.s.	0.093	n.s.	0.358	n.s.
	I X AM x P	1	0.053	n.s.	0.258	n.s.	0.713	n.s.
	B X P	1	0.083	n.s.	0.153	n.s.	0.094	n.s.
	I X B X P	1	<0.001	93	0.002	44	0.587	n.s.
	AM X B X P	1	0.078	n.s.	0.002	44	0.158	n.s.
	I X AM X B X P	1	0.071	n.s.	0.907	n.s.	0.533	n.s.

Table 3.51: The shoot B ($\mu\text{g kg}^{-1}$), Mn ($\mu\text{g kg}^{-1}$), and P (mg plant^{-1}) contents of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants, inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{H}_3\text{BO}_3$ and toxic B: $20 \text{ mg kg}^{-1} \text{H}_3\text{BO}_3$) and P supply (low P: $20 \text{ mg kg}^{-1} \text{KH}_2\text{PO}_4$ and high P: $100 \text{ mg kg}^{-1} \text{KH}_2\text{PO}_4$) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Shoot Mineral Contents											
P Supply (mg kg^{-1})		B Supply (mg kg^{-1})		AM	<i>P. indica</i>	B ($\mu\text{g kg}^{-1}$)		Mn ($\mu\text{g kg}^{-1}$)		P (mg plant^{-1})	
Triticum aestivum cv. Nusrat	Low P	Control	(-)	(-)	(-)	17	$\pm$ 3	214	$\pm$ 40	0.17	$\pm$ 0.06
						15	$\pm$ 3	81	$\pm$ 18	0.12	$\pm$ 0.03
			(+))	(-)	(-)	12	$\pm$ 1	90	$\pm$ 26	0.11	$\pm$ 0.02
						13	$\pm$ 2	147	$\pm$ 14	0.12	$\pm$ 0.01
		Toxic	(-)	(-)	(-)	244	$\pm$ 73	142	$\pm$ 62	0.13	$\pm$ 0.05
						282	$\pm$ 65	85	$\pm$ 18	0.12	$\pm$ 0.03
			(+))	(-)	(-)	216	$\pm$ 20	96	$\pm$ 28	0.11	$\pm$ 0.01
						378	$\pm$ 20	239	$\pm$ 22	0.19	$\pm$ 0.03
	High P	Control	(-)	(-)	(-)	23	$\pm$ 4	223	$\pm$ 47	0.38	$\pm$ 0.06
						20	$\pm$ 5	109	$\pm$ 31	0.35	$\pm$ 0.12
			(+))	(-)	(-)	16	$\pm$ 3	127	$\pm$ 17	0.35	$\pm$ 0.07
						17	$\pm$ 4	169	$\pm$ 34	0.39	$\pm$ 0.04
		Toxic	(-)	(-)	(-)	374	$\pm$ 27	181	$\pm$ 32	0.31	$\pm$ 0.08
						536	$\pm$ 130	133	$\pm$ 20	0.37	$\pm$ 0.03
			(+))	(-)	(-)	403	$\pm$ 87	144	$\pm$ 41	0.34	$\pm$ 0.08
						551	$\pm$ 123	191	$\pm$ 42	0.40	$\pm$ 0.08
Triticum durum cv. Saricanak-98	Low P	Control	(-)	(-)	(-)	25	$\pm$ 5	200	$\pm$ 8	0.16	$\pm$ 0.02
						21	$\pm$ 4	178	$\pm$ 35	0.21	$\pm$ 0.03
			(+))	(-)	(-)	18	$\pm$ 3	92	$\pm$ 33	0.09	$\pm$ 0.03
						21	$\pm$ 3	187	$\pm$ 21	0.19	$\pm$ 0.04
		Toxic	(-)	(-)	(-)	356	$\pm$ 49	94	$\pm$ 37	0.12	$\pm$ 0.01
						510	$\pm$ 55	145	$\pm$ 5	0.20	$\pm$ 0.02
			(+))	(-)	(-)	192	$\pm$ 44	50	$\pm$ 7	0.08	$\pm$ 0.01
						524	$\pm$ 78	179	$\pm$ 36	0.20	$\pm$ 0.01
	High P	Control	(-)	(-)	(-)	26	$\pm$ 8	102	$\pm$ 14	0.37	$\pm$ 0.03
						23	$\pm$ 2	183	$\pm$ 25	0.44	$\pm$ 0.04
			(+))	(-)	(-)	24	$\pm$ 4	80	$\pm$ 4	0.33	$\pm$ 0.04
						20	$\pm$ 1	248	$\pm$ 23	0.46	$\pm$ 0.03
		Toxic	(-)	(-)	(-)	434	$\pm$ 71	108	$\pm$ 18	0.32	$\pm$ 0.03
						431	$\pm$ 147	173	$\pm$ 35	0.43	$\pm$ 0.05
			(+))	(-)	(-)	486	$\pm$ 12	59	$\pm$ 9	0.29	$\pm$ 0.02
						441	$\pm$ 102	166	$\pm$ 62	0.41	$\pm$ 0.03

P. indica inoculated plants did not result in any significant change in the shoot Ca concentrations in both cultivars. Similarly, in both cultivars, the presence of arbuscular mycorrhiza did not cause any significant change in the shoot Ca concentrations. Increasing B levels did not cause any change in the shoot Ca concentrations in both cultivars. However, increasing P supply from the low to the high P level resulted in dose-dependent increases in both cultivars (**Tables 3.52 and 3.53**).

P. indica inoculated plants did not result in any significant change in the shoot K concentrations in Nusrat; nevertheless, for Saricanak-98, the presence of *P. indica* caused reductions. The presence of arbuscular mycorrhiza did not cause any significant change in Nusrat; however, it resulted in elevated shoot K concentrations in Saricanak-98. Increasing B levels resulted in dose-dependent increases in both cultivars. On the other hand, increasing P supply from the low to the high P level resulted in dose-dependent decreases in both cultivars (**Tables 3.52 and 3.53**).

Whereas the presence of *P. indica* did not result in any considerable change in Nusrat, it reduced the shoot Mg concentrations in Saricanak-98. On the other hand, arbuscular mycorrhiza-inoculated plants belonging to both cultivars showed significantly elevated shoot Mg concentrations. Considering B toxicity, increasing B levels reduced the shoot Mg concentrations in Nusrat; however, it did not lead to any significant change in Saricanak-98. Moreover, increasing P supply from the low to the high P level caused dose-dependent increases in both cultivars (**Tables 3.52 and 3.53**).

While *P. indica* inoculation improved the shoot S concentrations in Nusrat, the plants belonging to Saricanak-98 did not show any considerable change with the endophytic fungus in terms of the essential nutrient. Moreover, the presence of arbuscular mycorrhiza caused elevated shoot S concentrations in both cultivars. However, increasing B levels did not lead to any significant change in the shoot S concentrations. Increasing P supply from the low to the high P level caused dose-dependent increases in both cultivars (**Tables 3.52 and 3.53**).

Table 3.52: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on selected shoot macronutrient concentrations of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Ca (%)		K (%)		Mg (%)		S (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.293	n.s.	0.271	n.s.	0.897	n.s.	0.003	0.02
	Arbuscular Mycorrhiza (AM)	1	0.557	n.s.	0.117	n.s.	0.026	0.010	<0.001	0.02
	Boron Supply (B)	1	0.787	n.s.	0.002	0.18	0.001	0.010	0.086	n.s.
	Phosphorus Supply (P)	1	0.018	0.02	<0.001	0.18	<0.001	0.010	<0.001	0.02
	I X AM	1	0.120	n.s.	0.058	n.s.	0.370	n.s.	<0.001	0.04
	I X B	1	0.382	n.s.	0.578	n.s.	0.169	n.s.	0.295	n.s.
	AM X B	1	0.513	n.s.	0.820	n.s.	0.410	n.s.	0.348	n.s.
	I X AM X B	1	0.271	n.s.	0.156	n.s.	0.998	0.030	0.883	n.s.
	I X P	1	0.078	n.s.	0.624	n.s.	0.144	n.s.	0.596	n.s.
	AM X P	1	0.437	n.s.	0.998	n.s.	0.158	n.s.	<0.001	0.04
	I X AM x P	1	0.007	0.08	0.494	n.s.	<0.001	n.s.	0.100	n.s.
	B X P	1	0.383	n.s.	0.078	n.s.	0.098	n.s.	0.746	n.s.
	I X B X P	1	0.811	n.s.	0.565	n.s.	0.912	n.s.	0.897	n.s.
	AM X B X P	1	0.010	0.05	0.346	n.s.	0.387	n.s.	0.558	n.s.
	I X AM X B X P	1	0.894	n.s.	0.363	n.s.	0.355	n.s.	0.230	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Ca (%)		K (%)		Mg (%)		S (%)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.241	n.s.	0.038	0.12	0.007	0.003	0.812	n.s.
	Arbuscular Mycorrhiza (AM)	1	0.456	n.s.	<0.001	0.12	0.018	0.003	<0.001	0.02
	Boron Supply (B)	1	0.201	n.s.	<0.001	0.12	0.244	n.s.	0.200	n.s.
	Phosphorus Supply (P)	1	<0.001	0.01	<0.001	0.12	<0.001	0.003	<0.001	0.02
	I X AM	1	<0.001	0.03	<0.001	0.23	0.058	n.s.	0.7395	n.s.
	I X B	1	0.009	0.03	0.053	n.s.	0.005	0.010	0.031	0.02
	AM X B	1	0.434	n.s.	0.055	n.s.	0.599	n.s.	0.075	n.s.
	I X AM X B	1	0.049	0.06	0.113	n.s.	0.6846	n.s.	0.2352	n.s.
	I X P	1	0.360	n.s.	<0.001	0.23	0.8524	n.s.	0.3315	n.s.
	AM X P	1	0.118	n.s.	0.571	n.s.	0.0133	0.010	<0.001	0.02
	I X AM x P	1	0.380	n.s.	0.016	0.40	0.1171	n.s.	0.7723	n.s.
	B X P	1	0.466	n.s.	0.392	n.s.	0.2918	n.s.	0.0144	0.02
	I X B X P	1	0.535	n.s.	0.989	n.s.	0.0518	n.s.	0.3963	n.s.
	AM X B X P	1	0.547	n.s.	0.386	n.s.	0.995	n.s.	0.0047	0.06
	I X AM X B X P	1	0.841	n.s.	0.565	n.s.	0.8164	n.s.	0.1541	n.s.

Table 3.53: Selected shoot macronutrient concentrations of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃ and toxic B: 20 mg kg⁻¹ H₃BO₃) and P supply (low P: 20 mg kg⁻¹ KH₂PO₄ and high P: 100 mg kg⁻¹ KH₂PO₄) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

		P Supply (mg kg ⁻¹)	B Supply (mg kg ⁻¹)	AM	<i>P. indica</i>	Ca (%)		K (%)		Mg (%)		S (%)	
Triticum aestivum cv. Nusrat	Low P	Control	(-)	(-)	(-)	0.61	± 0.04	3.55	± 0.62	0.224	± 0.024	0.35	± 0.04
						0.60	± 0.02	3.79	± 0.28	0.190	± 0.010	0.34	± 0.03
			(+)	(-)	(-)	0.59	± 0.08	3.96	± 0.25	0.194	± 0.019	0.31	± 0.01
						0.64	± 0.02	3.81	± 0.52	0.229	± 0.021	0.42	± 0.03
		Toxic	(-)	(-)	(-)	0.62	± 0.10	4.16	± 0.68	0.202	± 0.048	0.39	± 0.09
						0.67	± 0.02	4.29	± 0.24	0.194	± 0.006	0.34	± 0.02
			(+)	(-)	(-)	0.56	± 0.02	4.54	± 0.21	0.182	± 0.009	0.34	± 0.01
						0.63	± 0.02	4.08	± 0.22	0.222	± 0.011	0.44	± 0.03
	High P	Control	(-)	(-)	(-)	0.67	± 0.04	3.45	± 0.27	0.235	± 0.012	0.42	± 0.03
						0.69	± 0.06	3.19	± 0.14	0.247	± 0.018	0.42	± 0.03
			(+)	(-)	(-)	0.67	± 0.04	3.38	± 0.20	0.271	± 0.014	0.48	± 0.05
						0.60	± 0.04	3.34	± 0.19	0.232	± 0.009	0.56	± 0.04
		Toxic	(-)	(-)	(-)	0.58	± 0.13	3.26	± 0.66	0.199	± 0.030	0.42	± 0.04
						0.66	± 0.04	3.45	± 0.27	0.214	± 0.007	0.43	± 0.02
			(+)	(-)	(-)	0.71	± 0.05	3.87	± 0.35	0.242	± 0.031	0.54	± 0.10
						0.63	± 0.05	3.37	± 0.29	0.226	± 0.010	0.57	± 0.05
Triticum durum cv. Saricanak-98	Low P	Control	(-)	(-)	(-)	0.64	± 0.03	3.38	± 0.07	0.171	± 0.019	0.37	± 0.03
						0.70	± 0.04	3.47	± 0.45	0.147	± 0.016	0.38	± 0.02
			(+)	(-)	(-)	0.70	± 0.04	4.10	± 0.16	0.173	± 0.015	0.38	± 0.03
						0.62	± 0.03	3.01	± 0.20	0.148	± 0.011	0.37	± 0.02
		Toxic	(-)	(-)	(-)	0.63	± 0.05	3.63	± 0.49	0.150	± 0.013	0.35	± 0.03
						0.72	± 0.03	3.70	± 0.23	0.158	± 0.013	0.40	± 0.01
			(+)	(-)	(-)	0.66	± 0.01	4.21	± 0.13	0.149	± 0.005	0.39	± 0.02
						0.68	± 0.03	3.66	± 0.37	0.154	± 0.009	0.41	± 0.02
	High P	Control	(-)	(-)	(-)	0.77	± 0.07	2.89	± 0.15	0.168	± 0.015	0.48	± 0.03
						0.81	± 0.05	3.06	± 0.14	0.166	± 0.007	0.46	± 0.05
			(+)	(-)	(-)	0.84	± 0.03	3.18	± 0.05	0.195	± 0.008	0.67	± 0.12
						0.76	± 0.01	2.96	± 0.10	0.174	± 0.014	0.59	± 0.03
		Toxic	(-)	(-)	(-)	0.78	± 0.03	2.86	± 0.12	0.164	± 0.010	0.48	± 0.06
						0.81	± 0.05	3.15	± 0.32	0.172	± 0.013	0.48	± 0.06
			(+)	(-)	(-)	0.83	± 0.02	3.37	± 0.12	0.192	± 0.008	0.50	± 0.02
						0.84	± 0.06	3.53	± 0.36	0.173	± 0.010	0.56	± 0.02

P. indica inoculated plants did not result in any significant change in the shoot Cu concentrations in both cultivars. Similar to *P. indica* inoculation, the presence of arbuscular mycorrhiza did not lead to either positive or negative effects in the shoot Cu concentrations (**Tables 3.54 and 3.55**).

The shoot Fe concentration was significantly affected by single effects of B supply and P supply and double interaction of *P. indica* inoculation and P supply in Nusrat. In Saricanak-98, single effects of *P. indica* inoculation and P supply, and double interaction of *P. indica* inoculation and P supply affected the shoot Mn contents in Saricanak-98 (**Table 3.54**). In Nusrat, *P. indica* inoculation did not result in any considerable change; nevertheless, in Saricanak-98, the plant inoculated with *P. indica* showed reduced shoot Fe concentrations. In both cultivars, mycorrhizal inoculation did not cause any significant change. In Nusrat, increasing B level reduced the shoot Fe concentrations whereas in Saricanak-98, any significant change did not happen. Increasing the P supply from the low to the high P level caused dose-dependent reductions in both cultivars (**Tables 3.54 and 3.55**).

The shoot Zn concentration was significantly affected by only the single effect of P supply treatment, double (*P. indica* inoculation X mycorrhizal inoculation, and B supply X P supply), and triple (*P. indica* inoculation X mycorrhizal inoculation X P supply) interactions in Nusrat. In Saricanak-98, single effects of *P. indica* inoculation, mycorrhizal inoculation, and P supply treatments, and double (*P. indica* inoculation X B supply, mycorrhizal inoculation X B supply, mycorrhizal inoculation X P supply) interactions significantly affected the shoot Mn concentrations (**Table 3.54**). Even though the presence of *P. indica* did not result in any significant change in the shoot Zn concentrations in Nusrat, *P. indica* caused elevations in Saricanak-98. The presence of mycorrhizal inoculation did not result in any significant change in Nusrat while *P. indica* caused reductions in Saricanak-98. B toxicity did not cause any marked changes in both cultivars. Increasing P supply from the low to the high P level caused dose-dependent decreases in both cultivars (**Tables 3.54 and 3.55**).

Table 3.54: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on selected shoot micronutrient concentrations of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.942	n.s.	0.283	n.s.	0.094	n.s.
	Arbuscular Mycorrhiza (AM)	1	0.290	n.s.	0.311	n.s.	0.386	n.s.
	Boron Supply (B)	1	<0.001	0.64	0.027	14	0.406	n.s.
	Phosphorus Supply (P)	1	0.028	0.64	0.007	14	<0.001	1.9
	I X AM	1	0.123	n.s.	0.792	n.s.	<0.001	3.5
	I X B	1	0.975	n.s.	0.406	n.s.	0.690	n.s.
	AM X B	1	0.643	n.s.	0.731	n.s.	0.275	n.s.
	I X AM X B	1	0.996	n.s.	0.145	n.s.	0.567	n.s.
	I X P	1	0.505	n.s.	0.005	26	0.990	n.s.
	AM X P	1	0.106	n.s.	0.655	n.s.	0.312	n.s.
	I X AM x P	1	<0.001	1.96	0.121	n.s.	0.007	6.1
	B X P	1	0.188	n.s.	0.244	n.s.	0.027	3.5
	I X B X P	1	0.370	n.s.	0.061	n.s.	0.732	n.s.
	AM X B X P	1	0.416	n.s.	0.150	n.s.	0.417	n.s.
	I X AM X B X P	1	0.533	n.s.	0.234	n.s.	0.395	n.s.
<i>Triticum durum</i> cv. Saricanak-98								

Table 3.55: Selected shoot macronutrient concentrations of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃ and toxic B: 20 mg kg⁻¹ H₃BO₃) and P supply (low P: 20 mg kg⁻¹ KH₂PO₄ and high P: 100 mg kg⁻¹ KH₂PO₄) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

Shoot Mineral Concentrations											
P Supply (mg kg ⁻¹)	B Supply (mg kg ⁻¹)	AM	<i>P. indica</i>	Cu (mg kg ⁻¹)			Fe (mg kg ⁻¹)			Zn (mg kg ⁻¹)	
<i>Triticum aestivum</i> cv. Nusrat	Low P	Control	(-)	(-)	10.11	± 1.80	146	± 34	27.6	± 7.1	
			(+)	(+)	9.12	± 0.68	151	± 25	19.7	± 2.3	
			(-)	(-)	8.53	± 1.06	130	± 31	21.4	± 1.7	
			(+)	(+)	10.55	± 1.50	190	± 42	25.9	± 8.9	
		Toxic	(-)	(-)	12.76	± 2.53	147	± 37	30.1	± 6.9	
			(+)	(+)	10.81	± 0.48	150	± 45	19.5	± 1.6	
	High P	Control	(-)	(-)	10.55	± 0.70	152	± 26	24.8	± 2.7	
			(+)	(+)	12.42	± 1.07	137	± 27	32.1	± 3.1	
			(-)	(-)	10.49	± 1.02	156	± 12	23.6	± 2.7	
			(+)	(+)	10.51	± 0.64	131	± 16	18.8	± 1.3	
		Toxic	(-)	(-)	11.46	± 0.91	165	± 27	20.7	± 1.7	
			(+)	(+)	10.46	± 0.21	118	± 25	20.8	± 1.5	
<i>Triticum durum</i> cv. Saricanak-98	Low P	Control	(-)	(-)	10.79	± 2.18	110	± 21	20.9	± 3.3	
			(+)	(+)	11.81	± 0.62	104	± 21	18.1	± 1.1	
			(-)	(-)	12.97	± 1.74	148	± 12	19.2	± 4.3	
			(+)	(+)	12.17	± 0.54	112	± 13	20.1	± 1.4	
		Toxic	(-)	(-)	11.09	± 0.85	140	± 40	32.7	± 3.9	
			(+)	(+)	12.39	± 0.26	105	± 13	34.5	± 2.8	
	High P	Control	(-)	(-)	10.96	± 1.03	191	± 52	22.4	± 2.7	
			(+)	(+)	10.68	± 0.87	108	± 27	26.2	± 3.8	
			(-)	(-)	11.16	± 1.35	124	± 3	24.0	± 3.8	
			(+)	(+)	13.18	± 0.47	111	± 8	33.7	± 3.8	
		Toxic	(-)	(-)	12.46	± 0.88	154	± 19	23.3	± 5.3	
			(+)	(+)	12.36	± 0.69	126	± 31	31.9	± 4.1	
Low P	Control	(-)	(-)	11.82	± 0.71	123	± 25	21.7	± 1.4		
		(+)	(+)	12.53	± 1.47	125	± 19	24.8	± 1.3		
		(-)	(-)	13.72	± 0.48	125	± 15	21.6	± 0.6		
		(+)	(+)	11.73	± 0.36	97	± 24	25.6	± 1.3		
	Toxic	(-)	(-)	13.53	± 0.55	114	± 19	20.6	± 2.7		
		(+)	(+)	14.30	± 0.99	109	± 16	24.4	± 1.7		
High P	Control	(-)	(-)	16.05	± 0.75	120	± 11	20.6	± 0.9		
		(+)	(+)	15.51	± 0.98	127	± 33	28.9	± 3.3		
		(-)	(-)								
		(+)	(+)								
	Toxic	(-)	(-)								
		(+)	(+)								

P. indica inoculation and mycorrhizal inoculation did not cause any significant changes in the shoot Ca contents in Nusrat. Whereas *P. indica* inoculation resulted in elevated shoot Ca contents, mycorrhizal inoculation led to reductions in Saricanak-98. Moreover, B toxicity decreased the shoot Ca contents in both cultivars. Increasing P supply from the low to the high P level caused dose-dependent increases in both cultivars (**Tables 3.56 and 3.57**).

P. indica inoculation, mycorrhizal inoculation, and B toxicity did not cause any significant changes in the shoot K contents in Nusrat. Whereas *P. indica* inoculation resulted in elevated shoot K contents, mycorrhizal inoculation led to reductions in Saricanak-98. B toxicity reduced the contents in Saricanak-98. Increasing P supply from the low to the high P level caused dose-dependent increases in both cultivars (**Tables 3.56 and 3.57**).

P. indica and mycorrhizal inoculation did not result in any significant effects in terms of shoot Mg contents in Nusrat. For Saricanak-98, although *P. indica* inoculation enhanced the shoot Mg contents, mycorrhizal inoculation did not result in any significant changes. The shoot Mg contents were reduced by B toxicity and increased by P supply in both cultivars (**Tables 3.56 and 3.57**).

The shoot S contents of both cultivars were improved by *P. indica* inoculation. The presence of mycorrhizal inoculum resulted in increased shoot S contents in Nusrat; however, it did not cause any marked effect in Saricanak-98. While B toxicity did not affect the shoot S contents in Nusrat, it caused significant reductions in Saricanak-98. In both cultivars, the contents were improved by P supply (**Tables 3.56 and 3.57**).

Table 3.56: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on selected shoot macronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.051	n.s.	0.232	n.s.	0.224	n.s.	0.009	0.05
	Arbuscular Mycorrhiza (AM)	1	0.360	n.s.	0.731	n.s.	0.574	n.s.	0.011	0.05
	Boron Supply (B)	1	0.005	0.05	0.314	n.s.	<0.001	0.022	0.265	n.s.
	Phosphorus Supply (P)	1	<0.001	0.05	<0.001	0.314	<0.001	0.022	<0.001	0.05
	I X AM	1	0.113	n.s.	0.103	n.s.	0.027	0.030	0.001	0.10
	I X B	1	0.011	0.10	0.043	0.439	0.016	0.040	0.321	n.s.
	AM X B	1	0.081	n.s.	0.152	n.s.	0.162	n.s.	0.338	n.s.
	I X AM X B	1	0.441	n.s.	0.357	n.s.	0.900	n.s.	0.860	n.s.
	I X P	1	0.378	n.s.	0.349	n.s.	0.378	n.s.	0.168	n.s.
	AM X P	1	0.370	n.s.	0.424	n.s.	0.152	n.s.	0.011	0.10
	I X AM x P	1	0.053	n.s.	0.718	n.s.	0.029	0.069	0.920	n.s.
	B X P	1	0.133	n.s.	0.157	n.s.	0.054	n.s.	0.348	n.s.
	I X B X P	1	0.523	n.s.	0.508	n.s.	0.485	n.s.	0.501	n.s.
	AM X B X P	1	0.355	n.s.	0.084	n.s.	0.118	n.s.	0.198	n.s.
	I X AM X B X P	1	0.614	n.s.	0.224	n.s.	0.823	n.s.	0.475	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	0.05	<0.001	0.18	<0.001	0.012	<0.001	0.04
	Arbuscular Mycorrhiza (AM)	1	<0.001	0.05	0.015	0.18	0.093	n.s.	0.258	n.s.
	Boron Supply (B)	1	<0.001	0.05	<0.001	0.18	<0.001	0.012	<0.001	0.04
	Phosphorus Supply (P)	1	<0.001	0.05	<0.001	0.18	<0.001	0.012	<0.001	0.04
	I X AM	1	0.003	0.09	<0.001	0.33	0.003	0.023	<0.001	0.07
	I X B	1	0.246	n.s.	0.020	0.33	0.143	n.s.	0.250	n.s.
	AM X B	1	0.729	n.s.	0.578	n.s.	0.405	n.s.	0.029	0.07
	I X AM X B	1	0.617	n.s.	0.762	n.s.	0.115	n.s.	0.694	n.s.
	I X P	1	0.020	0.09	0.081	n.s.	0.857	n.s.	0.124	n.s.
	AM X P	1	0.246	n.s.	0.014	0.34	0.062	n.s.	0.003	0.074
	I X AM x P	1	0.264	n.s.	0.126	n.s.	0.040	0.041	0.783	n.s.
	B X P	1	0.844	n.s.	0.820	n.s.	0.874	n.s.	0.030	0.074
	I X B X P	1	0.066	n.s.	0.015	0.55	0.036	0.041	0.423	n.s.
	AM X B X P	1	0.405	n.s.	0.860	n.s.	0.263	n.s.	0.007	0.126
	I X AM X B X P	1	0.928	n.s.	0.778	n.s.	0.938	n.s.	0.617	n.s.

Table 3.57: Selected shoot macronutrient contents of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃ and toxic B: 20 mg kg⁻¹ H₃BO₃) and P supply (low P: 20 mg kg⁻¹ KH₂PO₄ and high P: 100 mg kg⁻¹ KH₂PO₄) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	P Supply (mg kg ⁻¹)	B Supply (mg kg ⁻¹)	AM	<i>P. indica</i>	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)		
<i>Triticum aestivum</i> cv. Nusrat	Low P	Control	(-)	(-)	0.63	± 0.15	3.57	± 0.43	0.23	± 0.06	0.36	± 0.09	
				(+)	0.46	± 0.09	2.91	± 0.53	0.15	± 0.03	0.26	± 0.05	
			(+))	(-)	0.40	± 0.06	2.72	± 0.56	0.13	± 0.03	0.21	± 0.04	
		(+)		0.44	± 0.06	2.60	± 0.09	0.16	± 0.00	0.29	± 0.02		
		Toxic	(-)	0.42	± 0.15	2.75	± 0.68	0.14	± 0.05	0.26	± 0.09		
			(+)	0.45	± 0.08	2.86	± 0.56	0.13	± 0.03	0.23	± 0.06		
	High P		Control	(-)	0.35	± 0.03	2.82	± 0.16	0.11	± 0.01	0.21	± 0.03	
		(+)		0.56	± 0.10	3.65	± 0.47	0.20	± 0.03	0.40	± 0.08		
		Toxic		(-)	0.86	± 0.13	4.40	± 0.63	0.30	± 0.05	0.55	± 0.11	
			(+)	0.86	± 0.18	3.98	± 1.07	0.31	± 0.08	0.53	± 0.16		
			Control	(-)	0.81	± 0.07	4.09	± 0.43	0.33	± 0.03	0.58	± 0.11	
		(+)		0.86	± 0.09	4.75	± 0.59	0.33	± 0.02	0.80	± 0.06		
High P	Toxic	(-)		0.62	± 0.12	3.51	± 0.65	0.22	± 0.06	0.47	± 0.12		
		(+)	0.80	± 0.07	4.20	± 0.47	0.26	± 0.02	0.52	± 0.05			
		Control	(-)	0.69	± 0.09	3.78	± 0.78	0.24	± 0.06	0.53	± 0.16		
	(+)		0.78	± 0.12	4.20	± 0.79	0.28	± 0.05	0.72	± 0.20			
		P Supply (mg kg ⁻¹)	B Supply (mg kg ⁻¹)	AM	<i>P. indica</i>	Ca (mg plant ⁻¹)		K (mg plant ⁻¹)		Mg (mg plant ⁻¹)		S (mg plant ⁻¹)	
<i>Triticum durum</i> cv. Saricanak-98	Low P	Control	(-)	(-)	0.73	± 0.03	3.83	± 0.26	0.19	± 0.01	0.42	± 0.02	
				(+)	0.80	± 0.15	3.92	± 0.39	0.17	± 0.04	0.43	± 0.09	
			(+))	(-)	0.49	± 0.12	2.86	± 0.59	0.12	± 0.04	0.27	± 0.09	
		(+)		0.79	± 0.09	3.85	± 0.64	0.19	± 0.03	0.48	± 0.10		
		Toxic	(-)	0.50	± 0.02	2.89	± 0.06	0.12	± 0.00	0.28	± 0.02		
			(+)	0.75	± 0.03	3.85	± 0.27	0.16	± 0.01	0.41	± 0.01		
	High P		Control	(-)	0.32	± 0.02	2.06	± 0.10	0.07	± 0.00	0.19	± 0.02	
		(+)		0.73	± 0.09	3.92	± 0.22	0.17	± 0.03	0.44	± 0.06		
		Toxic		(-)	1.05	± 0.11	3.95	± 0.39	0.23	± 0.03	0.65	± 0.06	
			(+)	1.16	± 0.08	4.40	± 0.34	0.24	± 0.02	0.66	± 0.04		
			Control	(-)	0.97	± 0.05	3.69	± 0.27	0.23	± 0.02	0.78	± 0.14	
		(+)		1.19	± 0.06	4.59	± 0.26	0.27	± 0.02	0.92	± 0.07		
High P	Toxic	(-)		0.95	± 0.11	3.48	± 0.32	0.20	± 0.02	0.59	± 0.12		
		(+)	1.04	± 0.14	4.01	± 0.25	0.22	± 0.04	0.61	± 0.09			
		Control	(-)	0.83	± 0.02	3.40	± 0.05	0.19	± 0.01	0.50	± 0.02		
	(+)		1.00	± 0.12	4.17	± 0.40	0.21	± 0.03	0.67	± 0.12			

For Nusrat, neither *P. indica* nor mycorrhizal inoculation led to any significant effects in terms of shoot Cu content. On the other hand, even though *P. indica* increased the shoot Cu contents in Saricanak-98, arbuscular mycorrhiza reduced it. However, B toxicity did not cause any significant change in both cultivars. The shoot Cu contents of both cultivars were improved by increasing P supply from the low to the high P level (**Tables 3.58 and 3.59**).

The shoot Fe content was significantly affected by single effects of B and P treatments in both cultivars. Neither *P. indica* nor mycorrhizal inoculation caused any considerable changes in the shoot Fe contents of both cultivars. While B toxicity reduced the shoot Fe content in both cultivars, P supply enhanced it. On the other hand, no significant interaction occurred between the main effects (**Tables 3.58 and 3.59**).

The shoot Mn content was significantly affected by only the single effect of P supply, double (*P. indica* inoculation X mycorrhizal inoculation, *P. indica* inoculation X B supply, mycorrhizal inoculation X B supply), and triple (*P. indica* inoculation X mycorrhizal inoculation X P supply) interactions in Nusrat. In Saricanak-98, single effects of *P. indica* inoculation, mycorrhizal inoculation, and B treatment, double (*P. indica* inoculation X mycorrhizal inoculation, and *P. indica* inoculation X P supply) and triple (*P. indica* inoculation X B supply X P supply, and mycorrhizal inoculation X B supply X P supply) interactions significantly affected the shoot Mn contents (**Table 3.58**). The shoot Mn contents of Nusrat were not affected markedly by either *P. indica* or mycorrhizal inoculation. However, in Saricanak-98, although *P. indica* increased the shoot Mn contents, arbuscular mycorrhiza reduced it. In Nusrat, B toxicity did not cause any significant change; nevertheless, in Saricanak-98, it caused reductions. Whereas increasing P supply resulted in elevated shoot Mn concentrations in Nusrat, it did not change in Saricanak-98 (**Tables 3.58 and 3.59**).

The shoot Zn content was significantly affected by only the single effect of P supply, double (*P. indica* inoculation X mycorrhizal inoculation, and *P. indica* inoculation X B supply), and triple (mycorrhizal inoculation X B supply X P supply) interactions in Nusrat. In Saricanak-98, single effects of *P. indica* inoculation, mycorrhizal inoculation, and B treatment, double (*P. indica* inoculation X mycorrhizal inoculation, *P. indica* inoculation X B supply, mycorrhizal inoculation X B supply,

P. indica inoculation X P supply, and mycorrhizal inoculation X P supply), and triple (*P. indica* inoculation X B supply X P supply, and mycorrhizal inoculation X B supply X P supply) interactions remarkably affected the shoot Mn contents (**Table 3.58**). The shoot Zn contents of Nusrat were not affected considerably by either *P. indica* or mycorrhizal inoculation. However, in Saricanak-98, although *P. indica* increased the shoot Zn contents, arbuscular mycorrhiza reduced it. In Nusrat, B toxicity did not cause any significant change; nevertheless, it caused reductions in the shoot Zn contents in Saricanak-98. Whilst increasing P supply resulted in elevated shoot Zn concentrations in Nusrat, it did not change in Saricanak-98 (**Tables 3.58 and 3.59**).



Table 3.58: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on selected shoot micronutrient contents of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Cu ($\mu\text{g kg}^{-1}$)		Fe ($\mu\text{g kg}^{-1}$)		Mn ($\mu\text{g kg}^{-1}$)		Zn ($\mu\text{g kg}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.097	n.s.	0.692	n.s.	0.369	n.s.	0.933	n.s.
	Arbuscular Mycorrhiza (AM)	1	0.670	n.s.	0.936	n.s.	0.611	n.s.	0.974	n.s.
	Boron Supply (B)	1	0.710	n.s.	<0.001	18	0.464	n.s.	0.1107	n.s.
	Phosphorus Supply (P)	1	<0.001	1.09	<0.001	18	0.010	17	<0.001	2.59
	I X AM	1	0.011	2.06	0.188	n.s.	<0.001	32	<0.001	4.91
	I X B	1	0.030	2.06	0.251	n.s.	0.001	32	0.031	4.91
	AM X B	1	0.162	n.s.	0.163	n.s.	0.002	32	0.062	n.s.
	I X AM X B	1	0.933	n.s.	0.524	n.s.	0.469	n.s.	0.862	n.s.
	I X P	1	0.291	n.s.	0.165	n.s.	0.229	n.s.	0.513	n.s.
	AM X P	1	0.152	n.s.	0.350	n.s.	0.334	n.s.	0.994	n.s.
	I X AM x P	1	0.038	3.33	0.129	n.s.	0.044	54	0.116	n.s.
	B X P	1	0.356	n.s.	0.051	n.s.	0.900	n.s.	0.054	n.s.
	I X B X P	1	0.872	n.s.	0.399	n.s.	0.189	n.s.	0.389	n.s.
	AM X B X P	1	0.135	n.s.	0.769	n.s.	0.112	n.s.	0.023	8.27
	I X AM X B X P	1	0.532	n.s.	0.684	n.s.	0.301	n.s.	0.420	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Cu ($\mu\text{g kg}^{-1}$)		Fe ($\mu\text{g kg}^{-1}$)		Mn ($\mu\text{g kg}^{-1}$)		Zn ($\mu\text{g kg}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	0.82	0.141	n.s.	<0.001	14	<0.001	2.04
	Arbuscular Mycorrhiza (AM)	1	0.010	0.82	0.301	n.s.	0.038	14	<0.001	2.04
	Boron Supply (B)	1	0.164	n.s.	0.002	16	<0.001	14	<0.001	2.04
	Phosphorus Supply (P)	1	<0.001	0.82	0.001	16	0.923	n.s.	0.062	n.s.
	I X AM	1	0.024	1.54	0.086	n.s.	<0.001	27	<0.001	3.82
	I X B	1	0.170	n.s.	0.060	n.s.	0.614	n.s.	0.035	3.82
	AM X B	1	0.559	n.s.	0.575	n.s.	0.881	n.s.	0.041	3.74
	I X AM X B	1	0.553	n.s.	0.569	n.s.	0.148	n.s.	0.226	n.s.
	I X P	1	0.001	1.54	0.998	n.s.	0.004	27	0.030	3.82
	AM X P	1	0.014	1.57	0.380	n.s.	0.093	n.s.	<0.001	3.90
	I X AM x P	1	0.101	n.s.	0.276	n.s.	0.258	n.s.	0.483	n.s.
	B X P	1	0.031	1.57	0.570	n.s.	0.153	n.s.	0.362	n.s.
	I X B X P	1	0.186	n.s.	0.146	n.s.	0.002	44	0.017	6.57
	AM X B X P	1	0.426	n.s.	0.560	n.s.	0.002	44	0.021	6.31
	I X AM X B X P	1	0.606	n.s.	0.622	n.s.	0.907	n.s.	0.336	n.s.

Table 3.59: Selected shoot micronutrient contents of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃ and toxic B: 20 mg kg⁻¹ H₃BO₃) and P supply (low P: 20 mg kg⁻¹ KH₂PO₄ and high P: 100 mg kg⁻¹ KH₂PO₄) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

		P Supply (mg kg ⁻¹)	B Supply (mg kg ⁻¹)	AM	<i>P. indica</i>	Cu (mg kg ⁻¹)		Fe (mg kg ⁻¹)		Mn (mg kg ⁻¹)		Zn (mg kg ⁻¹)	
Triticum aestivum cv. Nusrat	Low P	Control	(-)	(-)	(-)	10.3	± 2.4	146	± 23	214	± 40	27.8	± 6.9
						7.1	± 1.7	118	± 40	81	± 18	15.3	± 4.3
			(+))	(-)	(-)	5.9	± 1.3	90	± 30	90	± 26	14.8	± 3.9
						7.2	± 0.2	130	± 22	147	± 14	17.4	± 4.0
		Toxic	(-)	(-)	(-)	8.4	± 2.2	103	± 56	142	± 62	20.0	± 6.5
						7.2	± 1.2	98	± 26	85	± 18	13.0	± 2.5
			(+))	(-)	(-)	6.6	± 0.8	94	± 13	96	± 28	15.5	± 3.1
						11.1	± 1.2	121	± 16	239	± 22	28.6	± 2.6
	High P	Control	(-)	(-)	(-)	13.4	± 2.4	200	± 35	223	± 47	30.0	± 5.0
						13.1	± 3.7	167	± 63	109	± 31	23.5	± 6.9
			(+))	(-)	(-)	13.8	± 1.4	199	± 38	127	± 17	25.1	± 3.8
						14.9	± 1.4	167	± 29	169	± 34	29.6	± 4.1
		Toxic	(-)	(-)	(-)	11.7	± 2.4	120	± 31	181	± 32	23.4	± 7.3
						14.4	± 1.4	126	± 21	133	± 20	22.0	± 1.1
			(+))	(-)	(-)	12.7	± 3.1	142	± 13	144	± 41	19.0	± 6.3
						15.2	± 3.1	140	± 33	191	± 42	25.4	± 6.0
Triticum durum cv. Saricanak-98	Low P	Control	(-)	(-)	(-)	12.5	± 0.5	156	± 37	200	± 8	37.1	± 5.7
						14.2	± 2.5	119	± 16	178	± 35	39.3	± 5.4
			(+))	(-)	(-)	7.7	± 1.9	133	± 39	92	± 33	16.0	± 5.6
						13.6	± 1.5	140	± 46	187	± 21	33.6	± 7.3
		Toxic	(-)	(-)	(-)	8.9	± 0.1	100	± 15	94	± 37	19.0	± 1.2
						13.7	± 0.4	115	± 8	145	± 5	35.1	± 4.3
			(+))	(-)	(-)	6.1	± 0.9	76	± 12	50	± 7	11.6	± 3.4
						13.3	± 1.6	137	± 45	179	± 36	34.1	± 1.0
	High P	Control	(-)	(-)	(-)	16.2	± 1.8	166	± 17	102	± 14	29.6	± 1.7
						17.9	± 0.9	178	± 20	183	± 25	35.6	± 2.7
			(+))	(-)	(-)	15.9	± 0.7	146	± 22	80	± 4	25.1	± 1.6
						18.2	± 1.1	150	± 36	248	± 23	39.7	± 3.1
		Toxic	(-)	(-)	(-)	16.5	± 2.0	141	± 36	108	± 18	25.2	± 5.1
						18.4	± 2.4	143	± 42	173	± 35	31.2	± 2.8
			(+))	(-)	(-)	16.2	± 0.7	121	± 12	59	± 9	20.7	± 0.6
						18.5	± 2.8	150	± 33	166	± 62	34.2	± 3.6

P. indica inoculated plants had significantly higher REL compared to non-inoculated plants in both cultivars. Although mycorrhizal inoculation did not cause any significant change in Nusrat, it decreased the shoot REL in Saricanak-98. In both cultivars, B toxicity caused increases in the shoot REL. Whereas increasing P supply resulted in elevated shoot REL in Nusrat, it did not cause any change in Saricanak-98 (**Tables 3.60 and 3.61**).

According to collective data displayed in **Tables 3.60 and 3.61**, *P. indica* inoculated plants belonging to both cultivars had significantly lower GB in comparison with non-inoculated plants. In Nusrat, arbuscular mycorrhiza had no significant effect on the shoot GB accumulation; nevertheless, it caused more GB accumulation when compared to control plants in Saricanak-98. In Nusrat, B toxicity resulted in elevation in the shoot GB accumulation, but it did not cause any change in Saricanak-98. Increasing P supply resulted in significant GB accumulations in the shoots compared to control groups in both cultivars.

In both cultivars, *P. indica* inoculated plants did not show any remarkable effect on TEAC in the plant shoots when compared to non-inoculated plants. Opposite to *P. indica*, the plants inoculated with mycorrhizal inoculum showed increases in comparison with the plants without mycorrhizal inoculum in both cultivars. As for B toxicity, it did not cause any considerable change in the shoot TEAC levels in both cultivars. Even though, in Nusrat, P supply did not change TEAC in the shoot, it resulted in elevated TEAC in Saricanak-98 (**Tables 3.60 and 3.61**).

The presence of *P. indica* did not cause any significant effect on PAL levels of the shoot in both cultivars, as shown in **Tables 3.60 and 3.61**. In Nusrat, mycorrhizal inoculation of the plants caused elevated PAL levels in the shoot; on the contrary, in Saricanak-98, it caused a reduction. In comparison with control, B toxicity did not show any marked effect on the PAL level of the shoots in both cultivars. On the other hand, increasing P supply resulted in elevations of PAL levels in the shoots of both cultivars.

Table 3.60. Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications supply as well as their interactions on the shoot REL (%), GB (%), TAC ($\mu\text{mol TE g}^{-1}\text{ FW}$), and Transsinnamic acid ($\mu\text{g g}^{-1}\text{ FW hour}^{-1}$) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	REL (%)		GB (%)		TAC ($\mu\text{mol TE g}^{-1}\text{ FW}$)		Transsinnamic acid ($\mu\text{g g}^{-1}\text{ FW hour}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	<0.001	2.1	<0.001	0.03	0.227	n.s.	0.354	n.s.
	Arbuscular Mycorrhiza (AM)	1	0.289	n.s.	0.337	n.s.	<0.001	3.4	<0.001	1.8
	Boron Supply (B)	1	<0.001	2.1	0.039	0.03	0.102	n.s.	0.557	n.s.
	Phosphorus Supply (P)	1	0.002	2.1	<0.001	0.03	0.698	n.s.	<0.001	1.8
	I X AM	1	0.766	n.s.	0.176	n.s.	0.537	n.s.	0.414	n.s.
	I X B	1	<0.001	4.0	0.006	0.05	0.051	n.s.	0.613	n.s.
	AM X B	1	0.003	4.0	0.500	n.s.	0.002	6.2	0.0035	3.4
	I X AM X B	1	0.108	n.s.	0.029	0.09	0.071	n.s.	0.981	n.s.
	I X P	1	0.181	n.s.	0.069	n.s.	0.879	n.s.	0.780	n.s.
	AM X P	1	0.158	n.s.	<0.001	0.05	0.145	n.s.	0.007	3.4
	I X AM x P	1	0.817	n.s.	0.362	n.s.	0.598	n.s.	0.222	n.s.
	B X P	1	0.037	4.0	0.946	n.s.	0.626	n.s.	0.006	3.4
	I X B X P	1	0.093	n.s.	0.051	n.s.	0.420	n.s.	0.641	n.s.
	AM X B X P	1	0.496	n.s.	0.005	0.09	0.284	n.s.	0.024	5.5
	I X AM X B X P	1	0.813	n.s.	0.650	n.s.	0.997	n.s.	0.571	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	REL (%)		GB (%)		TAC ($\mu\text{mol TE g}^{-1}\text{ FW}$)		Transsinnamic acid ($\mu\text{g g}^{-1}\text{ FW hour}^{-1}$)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	<0.001	1.3	<0.001	0.03	0.948	n.s.	0.848	n.s.
	Arbuscular Mycorrhiza (AM)	1	0.016	1.3	<0.001	0.03	<0.001	2.7	0.032	2.8
	Boron Supply (B)	1	<0.001	1.3	0.573	n.s.	0.874	n.s.	0.091	n.s.
	Phosphorus Supply (P)	1	0.086	n.s.	<0.001	0.03	<0.001	2.7	<0.001	2.8
	I X AM	1	0.091	n.s.	0.388	n.s.	0.295	n.s.	0.417	n.s.
	I X B	1	0.110	n.s.	0.043	0.06	0.703	n.s.	0.911	n.s.
	AM X B	1	0.002	2.6	0.142	n.s.	0.071	n.s.	0.971	n.s.
	I X AM X B	1	0.101	n.s.	0.514	n.s.	0.723	n.s.	0.820	n.s.
	I X P	1	0.795	n.s.	0.780	n.s.	0.098	n.s.	0.602	n.s.
	AM X P	1	0.013	2.6	0.619	n.s.	<0.001	5.1	0.198	n.s.
	I X AM x P	1	0.046	4.4	0.166	n.s.	0.934	n.s.	0.2772	n.s.
	B X P	1	0.177	n.s.	0.632	n.s.	0.482	n.s.	0.774	n.s.
	I X B X P	1	0.519	n.s.	0.224	n.s.	0.714	n.s.	0.402	n.s.
	AM X B X P	1	0.326	n.s.	0.267	n.s.	0.048	8.9	0.588	n.s.
	I X AM X B X P	1	0.031	6.8	0.294	n.s.	0.603	n.s.	0.792	n.s.

Table 3.61: The shoot REL (%), GB (%), TAC ($\mu\text{mol TE g}^{-1}\text{ FW}$), and Transsinnamic acid ($\mu\text{g g}^{-1}\text{ FW hour}^{-1}$) activities of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: $1\text{ mg kg}^{-1}\text{ H}_3\text{BO}_3$ and toxic B: $20\text{ mg kg}^{-1}\text{ H}_3\text{BO}_3$) and P supply (low P: $20\text{ mg kg}^{-1}\text{ KH}_2\text{PO}_4$ and high P: $100\text{ mg kg}^{-1}\text{ KH}_2\text{PO}_4$) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	P Supply (mg kg^{-1})	B Supply (mg kg^{-1})	AM	<i>P. indica</i>	REL (%)		GB (%)		TAC ($\mu\text{mol TE g}^{-1}\text{ FW}$)		Transsinnamic acid ($\mu\text{g g}^{-1}\text{ FW hour}^{-1}$)	
<i>Triticum aestivum</i> cv. Nusrat	Low P	Control	(-)	(-)	5.8	± 0.8	0.80	± 0.04	27.4	± 1.3	26.4	± 4.1
				(+)	3.6	± 0.9	0.72	± 0.04	26.6	± 1.5	24.3	± 3.6
			(+))	(-)	5.8	± 0.4	0.67	± 0.07	35.4	± 0.2	28.5	± 4.6
				(+)	6.4	± 0.9	0.71	± 0.03	28.4	± 0.2	26.6	± 4.7
		Toxic	(-)	(-)	15.5	± 3.4	0.79	± 0.04	28.6	± 0.0	28.9	± 3.8
				(+)	23.1	± 8.8	0.74	± 0.02	25.4	± 0.0	29.6	± 1.9
			(+))	(-)	10.3	± 0.9	0.78	± 0.07	37.6	± 0.2	30.6	± 1.0
				(+)	14.7	± 3.9	0.74	± 0.07	41.2	± 0.2	29.5	± 2.1
	High P	Control	(-)	(-)	6.6	± 1.4	0.84	± 0.05	29.5	± 0.1	28.6	± 5.3
				(+)	3.6	± 0.6	0.78	± 0.08	23.4	± 0.1	26.6	± 1.3
			(+))	(-)	8.2	± 1.0	0.91	± 0.04	37.0	± 0.2	38.9	± 4.2
				(+)	8.1	± 0.7	0.95	± 0.09	28.5	± 0.2	39.6	± 4.3
		Toxic	(-)	(-)	15.2	± 7.5	0.96	± 0.11	22.3	± 0.1	31.9	± 2.1
				(+)	30.5	± 5.5	0.83	± 0.04	19.6	± 0.1	28.9	± 4.6
			(+))	(-)	15.6	± 4.2	1.01	± 0.03	38.5	± 0.2	31.2	± 4.3
				(+)	25.6	± 9.3	0.81	± 0.05	46.2	± 0.2	33.1	± 1.2
<i>Triticum durum</i> cv. Saricanak-98	Low P	Control	(-)	(-)	5.3	± 1.4	0.77	± 0.04	21.4	± 0.1	21.4	± 4.3
				(+)	7.3	± 2.1	0.65	± 0.05	16.6	± 0.1	23.7	± 3.6
			(+))	(-)	4.9	± 0.6	0.82	± 0.05	27.0	± 0.3	21.4	± 4.6
				(+)	6.6	± 0.6	0.78	± 0.04	27.3	± 0.3	22.8	± 2.8
		Toxic	(-)	(-)	15.5	± 2.0	0.78	± 0.04	16.1	± 0.1	25.7	± 7.4
				(+)	18.2	± 4.2	0.69	± 0.09	13.7	± 0.1	25.1	± 6.2
			(+))	(-)	8.4	± 2.1	0.88	± 0.08	34.7	± 0.3	23.9	± 2.5
				(+)	12.3	± 1.5	0.74	± 0.04	32.6	± 0.3	22.7	± 3.7
	High P	Control	(-)	(-)	6.7	± 1.5	0.81	± 0.07	15.5	± 0.1	37.7	± 5.2
				(+)	8.0	± 1.5	0.81	± 0.05	17.6	± 0.1	32.1	± 5.3
			(+))	(-)	8.3	± 2.0	0.96	± 0.14	39.2	± 0.3	28.5	± 3.3
				(+)	9.8	± 2.1	0.87	± 0.07	44.0	± 0.3	29.8	± 1.9
		Toxic	(-)	(-)	9.7	± 3.3	0.92	± 0.06	16.2	± 0.2	38.1	± 9.4
				(+)	19.6	± 8.4	0.79	± 0.04	15.8	± 0.2	36.0	± 10.2
			(+))	(-)	13.5	± 2.2	0.97	± 0.07	39.0	± 0.3	31.7	± 5.0
				(+)	12.7	± 0.5	0.77	± 0.13	42.2	± 0.3	33.8	± 2.3

There was no remarkable change in the shoot SOD activity in Nusrat due to the presence of *P. indica* or arbuscular mycorrhiza. In Saricanak-98, *P. indica* caused an elevation; however, mycorrhiza caused a reduction. Whereas B toxicity caused a decrease in the shoot SOD activity in Nusrat, it did not result in any change in Saricanak-98. Whereas P supply did not cause any significant change in Nusrat, it caused elevated shoot SOD activity in Saricanak-98 (**Tables 3.62 and 3.63**).

Regarding CAT activities of both cultivars, *P. indica* and mycorrhizal inoculation did not result in any significant changes. B toxicity decreased the shoot CAT activity in Nusrat; however, it did not cause any change in Saricanak-98. Increasing P supply caused elevated CAT activity in the shoot of both cultivars (**Tables 3.62 and 3.63**).

The presence of *P. indica* or mycorrhizal inoculum, in Nusrat, caused elevated APX activity in the shoot, but in Saricanak-98 it had no significant effect on that of activity. Whereas B toxicity caused an elevated APX activity in the shoot of Nusrat cultivar, it decreased the activity in Saricanak-98. Increasing P supply caused elevated shoot APX activities in both cultivars (**Tables 3.62 and 3.63**).

Compared to corresponding control plants in Nusrat and Saricanak-98, respectively, *P. indica* inoculated plants belonging to Nusrat showed reduced GRX activity in the shoot; on the contrary, the plants belonging to Saricanak-98 showed elevated shoot GRX activity in the presence of *P. indica*. In Nusrat, mycorrhizal inoculum did not affect the shoot GRX activity; however, the activity of which was increased by mycorrhiza in Saricanak-98. B toxicity caused an increase in the shoot GRX activity in Nusrat but had no significant effect on the GRX activity of shoot in Saricanak-98. Increasing P supply caused elevated shoot GRX activities in both cultivars (**Tables 3.62 and 3.63**).

Table 3.62: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on shoot activities of SOD ($\text{U g}^{-1} \text{FW}$), CAT ($-\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$), APX ($\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$), and GRX ($\mu\text{mol}_{\text{NADPH}} \text{ g}^{-1} \text{FW min}^{-1}$) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected $\text{HSD}_{0.05}$ test scores.

	Source of Variation	DF	SOD Activity ($\text{U g}^{-1} \text{FW}$)		CAT Activity ($-\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$)		APX Activity ($\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$)		GRX Activity ($\mu\text{mol}_{\text{NADPH}} \text{ g}^{-1} \text{FW min}^{-1}$)	
			F Pr.	$\text{HSD}_{0.05}$	F Pr.	$\text{HSD}_{0.05}$	F Pr.	$\text{HSD}_{0.05}$	F Pr.	$\text{HSD}_{0.05}$
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.196	n.s.	0.177	n.s.	0.017	6	0.004	0.06
	Arbuscular Mycorrhiza (AM)	1	0.624	n.s.	0.851	n.s.	<0.001	6	0.147	n.s.
	Boron Supply (B)	1	0.048	19	0.028	0.84	<0.001	6	0.007	0.06
	Phosphorus Supply (P)	1	0.916	n.s.	<0.001	0.84	<0.001	6	0.006	0.06
	I X AM	1	0.757	n.s.	<0.001	1.62	0.003	12	0.251	n.s.
	I X B	1	0.097	n.s.	0.121	n.s.	0.010	12	0.019	0.12
	AM X B	1	0.220	n.s.	0.428	n.s.	<0.001	12	0.289	n.s.
	I X AM X B	1	0.418	n.s.	0.746	n.s.	<0.001	20	0.509	n.s.
	I X P	1	0.022	26	0.050	1.62	0.766	n.s.	0.435	n.s.
	AM X P	1	0.102	n.s.	0.076	n.s.	0.004	12	0.131	n.s.
	I X AM x P	1	0.951	n.s.	0.008	2.78	0.015	20	0.003	0.21
	B X P	1	0.514	n.s.	0.801	n.s.	0.067	n.s.	0.016	0.21
	I X B X P	1	0.336	n.s.	0.369	n.s.	0.053	n.s.	0.533	n.s.
	AM X B X P	1	0.355	n.s.	0.460	n.s.	0.004	20	0.037	0.21
	I X AM X B X P	1	0.420	n.s.	0.985	n.s.	0.177	n.s.	0.239	n.s.
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	SOD Activity ($\text{U g}^{-1} \text{FW}$)		CAT Activity ($-\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$)		APX Activity ($\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$)		GRX Activity ($\mu\text{mol}_{\text{NADPH}} \text{ g}^{-1} \text{FW min}^{-1}$)	
			F Pr.	$\text{HSD}_{0.05}$	F Pr.	$\text{HSD}_{0.05}$	F Pr.	$\text{HSD}_{0.05}$	F Pr.	$\text{HSD}_{0.05}$
	Inoculation (I)	1	0.027	17	0.544	n.s.	0.125	n.s.	0.035	0.05
	Arbuscular Mycorrhiza (AM)	1	0.001	17	0.276	n.s.	0.057	n.s.	0.019	0.05
	Boron Supply (B)	1	0.109	n.s.	0.762	n.s.	0.009	13	0.506	n.s.
	Phosphorus Supply (P)	1	<0.001	17	<0.001	0.74	0.006	13	<0.001	0.05
	I X AM	1	0.777	n.s.	0.614	n.s.	0.051	n.s.	0.519	n.s.
	I X B	1	0.847	n.s.	0.050	n.s.	0.180	n.s.	0.686	n.s.
	AM X B	1	0.399	n.s.	0.560	n.s.	0.049	24	0.126	n.s.
	I X AM X B	1	0.938	n.s.	0.040	1.54	0.116	n.s.	0.275	n.s.
	I X P	1	0.094	n.s.	0.360	n.s.	0.578	n.s.	<0.001	0.10
	AM X P	1	0.143	n.s.	0.001	1.49	<0.001	n.s.	0.048	0.10
	I X AM x P	1	0.703	n.s.	0.280	n.s.	0.922	n.s.	0.9863	n.s.
	B X P	1	0.107	n.s.	0.736	n.s.	0.269	n.s.	0.781	n.s.
	I X B X P	1	0.546	n.s.	0.518	n.s.	0.051	n.s.	0.208	n.s.
	AM X B X P	1	0.998	n.s.	0.450	n.s.	0.647	n.s.	0.211	n.s.
	I X AM X B X P	1	0.317	n.s.	0.538	n.s.	0.185	n.s.	0.709	n.s.

Table 3.63: The shoot SOD ($\text{U g}^{-1} \text{FW}$), CAT ($-\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$), APX ($\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$), and GRX ($\mu\text{mol}_{\text{NADPH}} \text{ g}^{-1} \text{FW min}^{-1}$) activities of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: $1 \text{ mg kg}^{-1} \text{H}_3\text{BO}_3$ and toxic B: $20 \text{ mg kg}^{-1} \text{H}_3\text{BO}_3$) and P supply (low P: $20 \text{ mg kg}^{-1} \text{KH}_2\text{PO}_4$ and high P: $100 \text{ mg kg}^{-1} \text{KH}_2\text{PO}_4$) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	P Supply (mg kg^{-1})	B Supply (mg kg^{-1})	AM	<i>P. indica</i>	SOD Activity ($\text{U g}^{-1} \text{FW}$)		CAT Activity ($-\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$)		APX Activity ($\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$)		GRX Activity ($\mu\text{mol}_{\text{NADPH}} \text{ g}^{-1} \text{FW min}^{-1}$)	
<i>Triticum aestivum</i> cv. Nusrat	Low P	Control	(-)	(-)	211	± 18	6.94	± 0.77	41.8	± 7.1	1.05	± 0.12
				(+)	163	± 40	6.41	± 1.02	20.6	± 6.9	0.87	± 0.13
			(+))	(-)	139	± 23	3.10	± 0.62	42.9	± 4.0	0.73	± 0.10
				(+)	134	± 19	8.60	± 1.95	77.1	± 19.1	0.96	± 0.25
		Toxic	(-)	(-)	124	± 67	7.04	± 0.56	50.8	± 13.1	1.16	± 0.27
				(+)	158	± 13	4.66	± 0.43	48.9	± 12.6	0.90	± 0.08
			(+))	(-)	143	± 20	3.41	± 1.19	58.5	± 8.9	1.18	± 0.05
				(+)	139	± 16	6.55	± 0.71	79.7	± 6.0	1.08	± 0.06
	High P	Control	(-)	(-)	140	± 77	8.94	± 1.81	69.9	± 23.4	1.01	± 0.17
				(+)	170	± 19	8.28	± 2.31	45.2	± 11.9	1.02	± 0.09
			(+))	(-)	153	± 34	9.61	± 3.09	81.8	± 6.9	1.23	± 0.12
				(+)	176	± 39	10.29	± 1.89	93.9	± 8.0	1.08	± 0.07
		Toxic	(-)	(-)	117	± 58	8.66	± 2.26	89.0	± 21.7	1.13	± 0.06
				(+)	161	± 22	7.73	± 1.16	128.4	± 15.2	0.99	± 0.08
			(+))	(-)	134	± 20	8.32	± 2.10	82.5	± 14.2	1.25	± 0.14
				(+)	173	± 11	8.14	± 0.95	92.9	± 5.7	1.01	± 0.06
<i>Triticum durum</i> cv. Saricanak-98	P Supply (mg kg^{-1})	B Supply (mg kg^{-1})	AM	<i>P. indica</i>	SOD Activity ($\text{U g}^{-1} \text{FW}$)		CAT Activity ($-\text{mmol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$)		APX Activity ($\mu\text{mol H}_2\text{O}_2 \text{ g}^{-1} \text{FW min}^{-1}$)		GRX ($\mu\text{mol}_{\text{NADPH}} \text{ g}^{-1} \text{FW min}^{-1}$)	
	Low P	Control	(-)	(-)	161	± 96	6.68	± 0.95	165.9	± 78.4	0.78	± 0.22
				(+)	176	± 11	5.75	± 1.45	142.2	± 17.7	1.00	± 0.08
			(+))	(-)	166	± 39	5.44	± 1.04	82.1	± 23.9	0.71	± 0.05
				(+)	153	± 9	4.59	± 0.73	89.5	± 11.7	0.93	± 0.06
		Toxic	(-)	(-)	173	± 22	6.74	± 1.34	100.4	± 33.5	0.71	± 0.15
				(+)	179	± 27	6.69	± 0.96	124.4	± 19.7	0.88	± 0.11
			(+))	(-)	147	± 16	3.34	± 1.11	52.8	± 11.4	0.84	± 0.01
				(+)	158	± 13	5.65	± 1.42	101.1	± 12.4	0.93	± 0.04
	High P	Control	(-)	(-)	219	± 13	6.53	± 2.07	134.4	± 33.1	1.19	± 0.08
				(+)	250	± 35	6.69	± 1.00	113.3	± 16.7	1.09	± 0.08
			(+))	(-)	174	± 10	8.60	± 1.92	118.3	± 8.1	1.29	± 0.09
				(+)	225	± 22	6.24	± 0.84	161.0	± 17.2	1.23	± 0.07
		Toxic	(-)	(-)	196	± 23	7.23	± 1.09	98.4	± 19.7	1.09	± 0.15
				(+)	227	± 42	6.35	± 1.64	102.2	± 31.8	1.12	± 0.16
			(+))	(-)	154	± 26	7.35	± 2.07	141.7	± 13.3	1.29	± 0.11
				(+)	174	± 13	8.11	± 1.94	137.2	± 16.5	1.20	± 0.10

Protein concentrations of the shoots were not affected by *P. indica* in both cultivars. Whereas arbuscular mycorrhiza reduced the protein concentration in Nusrat, it caused elevations in Saricanak-98. Protein concentrations were decreased and not affected by B toxicity in Nusrat and Saricanak-98, respectively. Increasing P supply did not cause any significant changes in Nusrat; however, it resulted in elevated shoot protein concentration in Saricanak-98 (**Tables 3.64 and 3.65**).

POX activity was affected by neither *P. indica* nor mycorrhizal inoculation in Nusrat; however, in Saricanak-98, the activity was reduced by both *P. indica* and mycorrhizal inoculation. B toxicity caused elevated shoot POX activities in both cultivars. Increasing P supply did not cause any significant effect in Nusrat and caused a reduction in Saricanak-98 (**Tables 3.64 and 3.65**).

Table 3.64: Four-way analysis of variance (ANOVA) of the effects of *P. indica* inoculation, arbuscular mycorrhiza inoculation, boron, and phosphorus applications as well as their interactions on the shoot protein concentration (%) and POX activity (mmol Tetraguaiacol g⁻¹ FW min⁻¹) of bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants grown under greenhouse conditions (at 49 DAS): DF (degrees of freedom), F Pr. (F value probabilities) and Tukey's protected HSD_{0.05} test scores.

	Source of Variation	DF	Protein Conc. (%)		POX. Act. (mmol Tetraguaiacol g ⁻¹ FW min ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
<i>Triticum aestivum</i> cv. Nusrat	Inoculation (I)	1	0.535	n.s.	0.076	n.s.
	Arbuscular Mycorrhiza (AM)	1	<0.001	0.15	0.094	n.s.
	Boron Supply (B)	1	<0.001	0.15	0.014	2.79
	Phosphorus Supply (P)	1	0.642	n.s.	0.077	n.s.
	I X AM	1	0.146	n.s.	0.079	n.s.
	I X B	1	0.852	n.s.	0.040	5.24
	AM X B	1	0.166	n.s.	<0.001	5.24
	I X AM X B	1	0.484	n.s.	0.057	n.s.
	I X P	1	0.624	n.s.	0.211	n.s.
	AM X P	1	<0.001	0.28	<0.001	5.34
	I X AM x P	1	0.091	n.s.	0.021	8.68
	B X P	1	<0.001	0.28	0.011	5.34
	I X B X P	1	0.499	n.s.	0.006	8.68
	AM X B X P	1	0.929	n.s.	0.003	8.68
	I X AM X B X P	1	0.206	n.s.	0.043	14.00
<i>Triticum durum</i> cv. Saricanak-98	Source of Variation	DF	Protein Conc. (%)		POX. Act. (mmol Tetraguaiacol g ⁻¹ FW min ⁻¹)	
			F Pr.	HSD _{0.05}	F Pr.	HSD _{0.05}
	Inoculation (I)	1	0.209	n.s.	<0.001	4.06
	Arbuscular Mycorrhiza (AM)	1	<0.001	0.18	<0.001	4.06
	Boron Supply (B)	1	0.767	n.s.	0.029	4.06
	Phosphorus Supply (P)	1	<0.001	0.18	<0.001	4.06
	I X AM	1	0.076	n.s.	<0.001	7.68
	I X B	1	0.242	n.s.	0.058	n.s.
	AM X B	1	0.977	n.s.	0.095	n.s.
	I X AM X B	1	0.283	n.s.	0.056	n.s.
	I X P	1	0.025	0.34	0.021	7.68
	AM X P	1	0.377	n.s.	<0.001	7.68
	I X AM x P	1	0.745	n.s.	0.028	13.44
	B X P	1	0.211	n.s.	0.069	n.s.
	I X B X P	1	0.977	n.s.	0.264	n.s.
	AM X B X P	1	0.221	n.s.	0.092	n.s.
	I X AM X B X P	1	0.229	n.s.	0.095	n.s.

Table 3.65: The shoot protein concentration (%) and POX activity (mmol Tetraguaiacol g⁻¹ FW min⁻¹) of 49-day-old bread wheat (*Triticum aestivum* cv. Nusrat) and durum wheat (*Triticum durum* cv. Saricanak-98) plants inoculated and non-inoculated with *P. indica* and arbuscular mycorrhiza grown in autoclaved soil at different levels of soil B stress (control: 1 mg kg⁻¹ H₃BO₃ and toxic B: 20 mg kg⁻¹ H₃BO₃) and P supply (low P: 20 mg kg⁻¹ KH₂PO₄ and high P: 100 mg kg⁻¹ KH₂PO₄) under greenhouse conditions. Data points represent means and standard deviations of four replicates.

	P Supply (mg kg ⁻¹)	B Supply (mg kg ⁻¹)	AM	<i>P. indica</i>	Protein Conc. (%)		POX. Act. (mmol Tetraguaiacol g ⁻¹ FW min ⁻¹)		
<i>Triticum aestivum</i> cv. Nusrat	Low P	Control	(-)	(-)	4.99	± 0.26	13.01	±	0.92
				(+)	4.78	± 0.15	14.22	±	1.92
			(+))	(-)	3.87	± 0.35	18.63	±	3.86
				(+)	3.92	± 0.10	21.14	±	3.24
		Toxic	(-)	(-)	4.70	± 0.23	16.28	±	3.31
				(+)	4.92	± 0.15	14.95	±	1.84
			(+))	(-)	4.10	± 0.11	17.28	±	2.47
				(+)	3.99	± 0.36	17.95	±	4.09
	High P	Control	(-)	(-)	4.85	± 0.21	15.87	±	3.58
				(+)	4.60	± 0.43	13.37	±	0.89
			(+))	(-)	4.53	± 0.37	17.96	±	4.42
				(+)	4.53	± 0.37	15.03	±	3.69
		Toxic	(-)	(-)	4.25	± 0.45	21.30	±	7.96
				(+)	3.84	± 0.52	44.14	±	17.08
			(+))	(-)	4.04	± 0.19	13.11	±	3.50
				(+)	4.68	± 0.40	12.94	±	1.90
<i>Triticum durum</i> cv. Saricanak-98	Low P	Control	(-)	(-)	3.98	± 0.30	44.37	±	21.02
				(+)	3.64	± 0.16	27.69	±	9.59
			(+))	(-)	4.07	± 0.07	10.96	±	2.11
				(+)	4.44	± 0.13	10.25	±	0.46
		Toxic	(-)	(-)	3.85	± 0.05	73.32	±	19.92
				(+)	3.72	± 0.25	29.31	±	10.96
			(+))	(-)	4.60	± 0.40	11.17	±	1.74
				(+)	4.32	± 0.03	12.85	±	3.69
	High P	Control	(-)	(-)	4.22	± 0.65	27.69	±	10.45
				(+)	4.48	± 0.29	18.42	±	1.69
			(+))	(-)	4.54	± 0.49	11.93	±	1.06
				(+)	5.17	± 0.46	14.04	±	1.05
		Toxic	(-)	(-)	4.31	± 0.65	30.63	±	3.25
				(+)	4.32	± 0.20	17.00	±	1.93
			(+))	(-)	4.39	± 0.39	13.89	±	2.68
				(+)	4.82	± 0.75	13.73	±	2.14

4. DISCUSSION

4.1. The First Soil Experiment: An Endeavour from *P. indica* to Reduce Serious Injury Caused by B Toxicity at Vegetative Stage

Even though *Piriformospora indica* is a remarkably promising plant growth-promoting microorganism, practical implications of this endophyte in agricultural production need the elucidation of its crop-specific physiological effects. While *P. indica* root colonisation may be particularly beneficial under stress conditions, its potential in terms of B toxicity has not yet been investigated.

In crop plants, the most practically relevant consequences of stress are decreases in growth, yield and/or quality. According to experimental studies, plants grown under toxic B levels exhibit impaired growth of shoots and roots [Nable, 1988; Metwally et al., 2012; Al-Huqail et al., 2020]. It has been reported that *P. indica* inoculation increases the biomass production of a wide range of crops [Varma et al., 1999; Peškan-Berghöfer et al., 2004; Oelmüller et al., 2009; Kundu and Vadassery, 2022]. In this work, to investigate the potential contribution of *P. indica* to the growth wheat in different B toxicity levels, bread and durum wheat plants were inoculated with the endophyte in both autoclaved and non-autoclaved soils. The present investigation demonstrated that, irrespective of B toxicity, the shoot dry matter of *P. indica* inoculated plants are significantly higher than that of non-inoculated plants (Tables 3.1 and 3.2, Figure 3.1).

Hitherto, whereas the possible advantages of *P. indica* have never been documented in the context of B toxicity in wheat, a plethora of research has been executed on crop plants, encompassing wheat, under various stress conditions. To illustrate, under Cd toxic conditions, *P. indica* colonized wheat plants displayed enhanced growth and chlorophyll content in comparison to those non-colonized. In addition to that, although the Cd concentration was elevated in roots, it was decreased in the shoot in the presence of the fungal endophyte [Shahabivand et al., 2012]. Another research carried out on wheat plants revealed that *P. indica* has the capacity to protect the plant from damage caused by *Fusarium disease* (crown rot disease) at the seedling stage [Rabiey et al., 2015]. Experimental input of Zarea et al. (2012) showed that

under salinity stress, *P. indica* inoculation resulted in enhanced wheat (*Triticum aestivum* L.) growth irrespective of the salinity, and the symbiotic association-mediated high salinity tolerance was documented. Furthermore, a study carried out by **Jogawat et al. (2013)** demonstrated that, *P. indica* inoculated rice seedlings displayed improved root and shoot lengths as well as fresh and dry weights under salt stress conditions. The aforementioned findings in terms of dry weight are congruent with the results obtained from the first experiment (**Tables 3.1 and 3.2**). In other words, the dry matter differences between *P. indica* inoculated and non-inoculated plants were owing to beneficial effects derived from *P. indica*-host association; thus, the plant growth promotion, which is one of the typical effects of the fungal endophyte, is observed in this experiment (**Table 3.2 and Fig 3.1**).

Plant growth can be improved by soil sterilization via autoclaving or other techniques due to the elimination of soil-borne pathogens and competing microorganisms [**Meredith and Anderson, 1992; Endlweber and Scheu, 2006; Mahmood et al., 2014**]. An experimental study by **Darbar and Lakzian (2007)** to reveal the effect of different soil sterilization methods such as autoclaving, fumigation and ultraviolet and microwave irradiation on soil chemical and biological properties (soil acidity, electrical conductivity, cation exchange capacity, optical density, extractable carbon and nitrogen, and colony forming unit) was executed under laboratory conditions. The 2007 study reported that all the sterilization methods markedly decreased pH and increased electrical conductivity, optical density, extractable carbon, and nitrogen in comparison with native soil samples. Moreover, the autoclaving method was the most effective in eliminating soil microorganisms; on the other hand, ultraviolet irradiation was the least effective method [**Darbar and Lakzian, 2007**].

However, soil autoclaving technique cannot be applied to the field. The present investigation found that soil sterilization considerably caused increased shoot dry matters irrespective of *P. indica* which is consistent with the literature (**Table 3.2 and Figure 3.1**). To the best of my knowledge, to date, there has not been any published research that compares native and sterile soils in the scope of the growth-promoting and stress-alleviating effects of *P. indica* against B toxicity. Nonetheless, an experimental study carried out to reveal the effect of arbuscular mycorrhiza on wheat plants grown in sterilized and non-sterilized soils demonstrated that the shoot dry

weights of plants were significantly enhanced by soil sterilization [Ova et al., 2015]. Besides that, according to experimental input by Gholami et al. (2009) the inoculation effect of PGPR *Azospirillum brasilense* (DSM 1690) on the growth of maize was significantly higher in sterile soil in comparison to those grown in non-sterile soil.

Chlorosis and necrosis on older leaves are common toxicity symptoms of plants under B toxic conditions. Furthermore, plant root and shoot elongation are decreased under such conditions [Nable et al., 1990a; Marschner, 2011; Roessner et al., 2006; Rehman et al., 2018] and the results obtained from this experiment are congruent with these findings. After being loaded into xylem, B is transported by this vascular system to shoot in a mechanism helped by the transpiration stream, which is indispensable for the long-distance translocation of solutes occurring in the xylem [Raven, 1980; Shelp et al., 1995]. Because the accumulation of B takes place at the site of a high transpiration rate (i.e., margins of large mature leaves), this long-distance transport has also been regarded as passive. Along the transpiration stream, B is transported and accumulates at its end; as a consequence, this element has a considerably higher concentration rate in leaf tips and margins in comparison with the rest of the leaf [Brown and Hu, 1996; Brown and Shelp, 1997]. B can additionally be transported via phloem to both vegetative and reproductive tissues [Shelp et al., 1995; Matoh and Ochiai, 2005]; nevertheless, this capacity diverges between species [Brown and Shelp, 1997]. The species (namely, wheat, barley, canola, and walnut) translocating sucrose in their phloem exhibit low B mobility, thus; older leaves of these species have relatively higher B concentrations. This experiment showed that the leaves belonging to both bread and durum wheat cultivars turned chlorotic and necrotic under B toxic conditions. Furthermore, these symptoms are more noticeable in older leaves, and increasing B toxicity gradually exacerbated these symptoms, as expected (Figure. 3.1).

In this work, *P. indica* inoculation significantly decreased the leaf MDA concentrations and REL while toxic B levels caused increased levels of MDA and REL in either cultivar (Figs 3.4 and 3.5). Hence, it can be interpreted that B toxicity disrupted the membrane integrity of the plant leaves and the ameliorative effects of *P. indica* emerged in both cultivars. An investigation reported that B-toxicity treatments caused reduced growth and elevated amounts of H₂O₂ and MDA in root and shoot

tissues of wheat cultivars [Metwally et al., 2012]. Furthermore, the experimental inputs were documented by Karabal et al. (2003), indicating increased MDA concentrations and REL in a dose-dependent manner in leaves in comparison with control groups in response to B toxicity in both sensitive and tolerant barley cultivars (*Hordeum vulgare* L.) which were consistent with our findings. Hitherto, under stress conditions, the link between *P. indica* and MDA concentration has been the subject of various works. For instance, the recent investigations found that *P. indica* inoculation of plants leads to an elevation in proline content and decrease in MDA accumulation in corn (*Zea mays* L.) and rice (*Oryza sativa* L.) plants, respectively [Tsai et al., 2020; Xu et al., 2017]. Moreover, studies documented that under drought stress, the presence of *P. indica* leads to decreases in the level of electrolyte leakage, thereby re-confirming the importance of this endophyte in preventing oxidative damage caused by ROS [Tyagi et al., 2017; Ghabooli et al., 2020].

Green plants depend on photosynthesis for growth and development, and environmental stress can adversely affect this process. In the chloroplast, a reduction in chlorophyll content leads to structural alterations, which hampers photosynthetic performance [Shu et al., 2014]. Excess of B causes various effects on plants, such as a decrease in leaf chlorophyll content and photosynthetic rate [Nable et al., 1997; Reid, 2007]; consequently, the reductions in chlorophyll concentration under B toxicity can be regarded as a quantitative parameter to determine plant tolerance to B stress. The current study paid particular attention to chlorophyll concentrations because they have pivotal roles in photosynthesis and photoprotection. According to Tables 3.1 and 3.3, the chlorophyll level was improved in *P. indica* inoculated plants of durum wheat while it did not have any significant change in that of Nusrat. This effect presumably originated from differences in tolerance/resistance B toxicity mechanisms in either cultivar. According to experimental inputs of both Yau et al. (1995) and Kalayci et al. (1998), durum wheat is highly sensitive to B toxicity, and durum cultivars were documented to be more sensitive than most bread wheat cultivars. Hence, it may suggest that B toxicity affects bread wheat to such an extent that it allows chlorophyll to sustain its essential metabolic activity and survive without the aid of *P. indica*; nonetheless, durum wheat requires the endophytic fungus under such negative conditions. An experimental study conducted by Ogunwole et al. (2015) demonstrated that toxic B levels caused significantly

decreased chlorophyll a, b, and total chlorophyll concentrations, and according to their interpretations, this diminution could be due to oxidative injuries and chlorosis, which resulted in enhanced chlorophyll degradation or inhibited chlorophyll synthesis. Additionally, an elevation in the MDA and H₂O₂ contents of plants exposed to B toxicity was earlier documented in chickpea (*Cicer arietinum* L.) and tomato (*Solanum lycopersicum* L.) plants [Ardic et al., 2009; Cervilla et al., 2007 and 2009]. These results were attributed to oxidative stress and membrane peroxidation which may increase chlorophyll degradation and reduce chlorophyll synthesis. The findings above associated with REL and MDA are congruent with our results. Furthermore, Jogawat et al. (2013) conducted a study on rice (*Oryza sativa* L.) plants to ascertain resistibility against salinity stress with or without *P. indica*. In this sense, the content of Chl a, Chl b, and carotenoids was analysed in 200- and 300-mM salt-treated plants with *P. indica* inoculation. Based on their results, the presence of the fungal inoculum resulted in a marked elevation in the amount of these pigments in comparison with those grown in the absence of *P. indica* under the same salt conditions thus helping rice plants by protecting them against salinity stress. Additionally, this study showed that Chl and carotenoids can be degraded because of high sodium ion toxicity during salt stress. In the current study, the shoot carotenoid contents of both Nusrat and Saricanak-98 were improved by *P. indica* compared to those grown without the fungal inoculum (Tables 3.7 and 3.10) and these findings are congruent with aforementioned study. On the other hand, soil sterilization did not cause any significant effects on carotenoid contents of both cultivars indicating that removing biotic stress factors from the soil did not cause any further beneficial effects in tested cultivars. In the final accounting, B toxicity generally reduced the carotenoid contents of both bread and durum cultivars indicating carotenoid contents degraded by abiotic stress as expected (Table 3.10). Various investigations have also reported that photosynthetic improvements, encompassing but not limited to improvement in chlorophyll levels and the photochemical efficiency of photosystem II, can be responsible for the monitored beneficial effects of *P. indica* against salinity [Ghorbani et al., 2018; Zarea et al., 2012; Sharma et al., 2017; Abdelaziz et al., 2019].

Although B is an essential micronutrient for all seed plants, its adequacy range is notably narrow, and for this reason, excess B can easily lead to toxic effects on plants

[Saleem et al., 2011; Miwa et al., 2014]. Here, wheat boron uptake was not affected by *P. indica* whereas significantly reduced by soil sterilization (Table 3.4 and Figure 3.6B). There is a plethora of studies on the subject of both *P. indica* and toxicity. For instance, according to experimental inputs by Ghorbani et al. (2018), *P. indica* inoculated tomato (*Solanum lycopersicum* L.) plants showed reduced absorption and transport of Na⁺ to the shoot in comparison with those grown without the fungal endophyte against NaCl stress. *P. indica* has been also known to lessen the toxicity caused by heavy metals [Bhardwaj, 2011]. To illustrate, another study conducted on sunflower plants (*Helianthus annuus* L.) revealed that *P. indica* displayed the ability to immobilize Cd in the root and to lessen Cd concentrations in the stem and leaf of plants under Cd toxic conditions [Shahabivand et al., 2017]. The experimental findings of these studies reveal that the fungal endophyte has the potential to ease toxicity induced by ions and heavy metals in the host plant. As a counterexample, this experiment showed that the shoot B concentrations of both cultivars were not significantly affected by *P. indica*, and the beneficial effects of *P. indica* associated with B absorption and transport can be varied amongst cultivars (Figure 3.6A). In spite of the fact that the shoot B concentrations were not changed by *P. indica*, the experimental plants subjected to B toxicity benefited from the ameliorative effects of *P. indica* in various ways (such as lowering MDA concentration and REL), thereby maintaining growth and development. Moreover, B-tolerant varieties of some species display a diminution in the B concentration of their leaves compared to those non-tolerant [Nable et al., 1990a], and one of the possible scenarios for tolerant varieties is likely to absorb less B into the roots and shoots. In the current experiment, because less B uptake was carried out into the roots and shoots, B was accumulated less in bread wheat leaves than in durum wheat (Figure 3.6). The ability to maintain low concentrations of B in plant tissues seems to be a main mechanism which is partially attributed to the active efflux of B from the roots and this mechanism is similar for all tested species [Hayes and Reid, 2004; Schnurbusch et al., 2010]. Consequently, overall, B toxicity-tolerant genotypes accumulate less B than those non-tolerant, suggesting that exclusion rather than internal tolerance mechanisms are in charge [Schnurbusch et al., 2010].

P is a crucial mineral nutrient for plant growth, which is acquired from the environment either directly by their roots or indirectly via symbiotic associations, for

example, those with mycorrhizal fungi [Koide, 1991; Marschner, 2011]. The present investigation found that the shoot P concentrations were considerably lessened by the endophytic fungus whereas enhanced by soil sterilization (Table 3.4 and Figs. 3.8A-B). Furthermore, in terms of B toxicity, increasing B levels resulted in elevated shoot P concentrations in bread and durum wheat cultivars. The experimental studies indicated that *P. indica*, particularly in the semiarid and arid regions, is essential in terms of P-acquisition by the root, also, a phosphate transporter (PiPT) and a considerable amount of acid phosphatase (i.e., phosphate solubilizing enzyme in the soil) from *P. indica* has been documented [Yadav et al., 2010]. Thus far, plant growth promotion has been associated with P uptake [Shahollari et al., 2005]. Contrary to the above findings from the literature, the shoot P concentrations were decreased by *P. indica* whereas plant growths were bettered by *P. indica* inoculation in this experiment (Table 3.2 and Figure 3.8). For optimal growth, the P requirement varies between 0.3 to 0.5 % dry weight throughout the vegetative stage of growth [Marschner, 2011], and in this study, the P concentrations are within this range. *P. indica* inoculation may “dilute” P in the above-ground parts of plants via encouraging shoot growth, which is one of the possible explanations. Upon calculating the P contents of the cultivars, it was found that *P. indica* caused a significant elevation in P contents (Tables 3.11 and 3.13). Moreover, based on the literature, the B and P absorptions have an antagonistic relationship. To name another example, under some extreme conditions of excess B toxicity or B deficiency, the inhibited P absorption and transport to the above-ground part were documented in *Vicia faba* and Spanish groundnut plants [Robertson and Loughman, 1974; Blamey and Chapman, 1979], which were in the same line with our findings.

For plant growth, Mn is a vital nutrient and has diverse roles encompassing activating a large number of enzymes, on the other hand, there are only a few Mn-containing enzymes in plants i.e., the Mn-protein in PS II, the Mn-containing superoxide dismutase (MnSOD) and oxalate oxidase [Marschner, 2011]. *P. indica* has been reported to make micronutrients (i.e., particularly Zn, Fe, Mn, and Cu) available to the plant [Gosal et al., 2010; Achatz et al., 2010; Gosal et al., 2008; Gosal et al., 2007]. Nevertheless, here, *P. indica* significantly lessens the shoot Mn concentrations in Nusrat but not that of Sariacank-98 (Table 3.4 and Figure 3.7).

This experiment revealed that the effect of *P. indica* varied between wheat cultivars, and it can decrease Mn uptake under Mn toxic conditions in the case of bread wheat. Because of eliminating the Mn-oxidizing bacteria that convert plant-available Mn^{2+} into unavailable higher oxides, soil sterilization may result in Mn toxicity [Boyd, 1971; Williams-Linera and Ewel, 1984]. Here, soil sterilization, implying the removal of soil microorganisms, caused dramatic elevations (3X) of Mn uptake which is in agreement with the results obtained from the literature. For instance, Miransari et al. (2009) executed an experimental work to reveal the effects of arbuscular mycorrhiza, soil sterilization, and soil compaction on wheat (*Triticum aestivum* L.) nutrient uptake and found that soil sterilization considerably boosted the uptake of Mn, Zn, and Cu. Considering Nusrat is more tolerant to B toxicity in comparison with Saricanak-98, the fact that toxic B levels significantly increased Mn accumulation in this cultivar in the present study is notable. In plants, B promotes Mn accumulation in general [Lopez-Lefebvre et al., 2002; Mozafar, 1989; Alvarez-Tinaut, 1980]; therefore, higher shoot Mn accumulation can be attributed to toxic B levels in Nusrat. In the case of Saricanak-98, however, toxic B levels had no such effect (Table 3.4 and Figure 3.7).

In the course of colonization, extracellular and intracellular hyphae is formed by *P. indica*. Whereas the external hyphae branch out of the colonized root, the internal hyphae penetrate the root cortex, encompassing the root epidermis [Verma et al., 2022]. A high-affinity Fe transporter (PiFTR) from *P. indica* was identified by a study aiming to investigate the role of a *P. indica* Fe transporter on rice Fe uptake under low and rich Fe conditions with or without *P. indica*. PiFTR expression profile in the internal and external hyphae was characterized and analysed by qRT PCR. Compared to those in the external hyphae, PiFTR expression of the internal hyphae showed a 1.78-fold increase. Furthermore, PiFTR expression was induced when *P. indica* was grown under low Fe conditions suggesting Fe transporters involved Fe uptake and radiolabelled Fe confirming that the amount of Fe present in rice plants was exclusively transferred by *P. indica* [Verma et al., 2022]. In this study, in terms of Fe supply, the shoot Fe concentrations of plants were in the optimum range and Fe-limited conditions were not present (Tables 3.7 and 3.9). Moreover, the shoot Fe content of Nusrat was increased by *P. indica*; however, in the case of Saricanak-98, this root endosymbiotic fungus did not cause any significant

change. Surprisingly, the shoot Fe concentrations of both tested cultivars are not in line with the abovementioned findings. On the other hand, for Nusrat upon checking the shoot DW (**Table 3.2**), and considering the B tolerance, these results are notable. In plants, as the exogenously applied B concentration increases, Fe accumulation shows varying trends. When the B level is lower than the toxic level, Fe accumulation increases with increasing B level; however, a significant decrease happens in the case of extreme B level, implying that a proper amount of B might indirectly affect the Fe uptake via enhancing plant growth to increase the demand for Fe [Lopez-Lefebvre et al., 2002; Mozafar, 1989; Alvarez-Tinaut et al., 1980]. According to an experimental study conducted on *Arabidopsis* plants (*Arabidopsis thaliana* (L.) Heynh.) by Peng et al. (2021) revealed that via promoting the reuse of Fe found in apoplast in the roots of experimental plants, B also aids in the long-distance transport of Fe from the roots to the aboveground part which is probably one of the possible interaction mechanisms between Fe and B. According to these findings, the interaction between B and Fe likely depends on both B levels and plant species. The present study revealed that B treatments did not cause any significant change in durum; on the other hand, in bread, only the medium level caused a reduction in the shoot Fe concentration (**Tables 3.7** and **3.9**). As a result, B supply may alleviate Fe toxicity only in the medium level of Nusrat, which is more tolerant to B, but not in that of Saricanak-98.

P. indica has been deemed to extract, mobilize, and translocate micronutrients especially Zn, Fe, Mn, and Cu from soil and turns them into plant available forms [Gosal et al., 2010; Achatz et al., 2010; Gosal et al., 2008; Gosal et al., 2007]. For example, as reported by Padash et al. (2016), the more pronounced Mn and Zn concentrations in lettuce (*Lactuca sativa* L. cv. Siyahoo) plant's leaves grown in soil inoculated with *P. indica* found compared to those grown in soil non-inoculated with the fungal endophyte. In the current study, *P. indica* did not affect the shoot Zn concentration of Nusrat but reduced that of Saricanak-98 (**Table 3.4** and **Figure 3.9**). Upon calculating the shoot Zn content of both tested cultivars, the presence of fungal endophyte resulted in elevated shoot Zn content in Nusrat but did not cause any significant change in Saricanak-98 (**Tables 3.16** and **3.17**). These findings are notable considering the B tolerance of Nusrat. Autoclaving process is known to elevate available/exchangeable/extractable nutrients such as N (NH_4^+ and NO_3^-), P

[Darbar and Lakzian, 2007], Mg, Mn, and Fe [Lopez and Wollum, 1976; Wolf et al., 1989]. According to a study conducted on wheat (*Triticum aestivum* L.) reported that soil sterilization significantly enhances the uptake of Mn, Zn and Cu [Miransari et al., 2009]. The current investigation found that soil sterilization increased the shoot Zn concentrations of both tested cultivars in comparison with those grown in native soil and these findings are congruent with the literature. Thus far, there have been studies conducted on plants to reveal the interaction between B and Zn showing B accumulation has an antagonistic relationship to Zn accumulation, in general [Hosseini et al., 2007]. However, the interaction mechanism between B and Zn has not yet been deciphered [Long and Peng, 2023]. Under Zn deficit conditions, B accumulation increased in the experimental plants [Graham et al., 1987; Sing et al., 1990], whereas it was reduced by the application of Zn fertilizers [Gunes and Alpaslan, 2000; Rajaei et al., 2009]. A study conducted on corn plants (*Zea mays* L. single cross CS201) showed that Zn accumulation reduced with an increase in B concentration [Mozafar, 1989]. Thus, in cultivated lands, the addition of Zn fertilizers can effectively relieve crop plants in terms of B toxicity under excess B conditions [Hosseini et al., 2007]. However, as a counterexample, according to experimental input by Lopez-Lefebvre et al. (2002), B treatments were not able to reduce the accumulation of Zn in *Nicotiana tabacum* plants. Another experimental study conducted on black mustard (*Brassica nigra* W. D. J. Koch) demonstrated that under extreme B deficient and toxic conditions, there is a synergistic interaction between Zn and B and Zn toxicity symptoms are exacerbated by excess B [Sinha et al., 2000]. In the present study, increasing the B level from the control to the high resulted in inconsistent shoot Zn concentrations in either cultivar, suggesting that the interaction pattern between B and Zn might display different trends depending on plant species and the B level.

From a plant's nutritional point of view in the current investigation, *P. indica* colonization resulted in significantly increased macronutrient contents in the leaves of both Nusrat and Saricanak-98, such as P, K, Ca, Mg, and S (Tables 3.5 and 3.6). Because N, P, K, Ca, Mg, and S are crucial for improved plant growth and biomass accumulation [Marschner, 2011; Macuphe et al., 2021] and were markedly affected by *P. indica* inoculation in this study, it is, therefore, not surprisingly the results in the current study showed that the fungus had significant effect on plant dry

weights and vigour. Correspondingly, the endophytic fungus positively affected the chlorophyll contents of both cultivars in the current study, and amongst macronutrients, N, Ca, K, Mg, and S are crucial nutrients having roles in the synthesis of chlorophyll [Marschner, 2011; Narayan et al., 2021]. Besides that, the trend of the shoot micronutrient contents of both tested cultivars was quite apparent and intriguing, as shown in **Tables 3.4, 3.7, 3.8, 3.9** and **Figures 3.6, 3.7, 3.9**. There were statistical differences ($p < 0.05$) between *P. indica* inoculated and non-inoculated plants in five micronutrients (B, Cu, Fe, Mn, and Zn) in Nusrat and in three micronutrients (B, Cu, and Mn) in Saricanak-98 with non-inoculated plants having consistently lower levels of corresponding nutrients of both cultivars than *P. indica*-inoculated plants. *P. indica* can enhance the uptake of these nutrients [Gosal et al., 2010; Achatz et al., 2010]. Whereas these elements are required to be in small amounts, they play physiologically crucial roles in plants. For instance, biosynthesis of chlorophyll requires Fe [Vergara et al., 2019]; B is a crucial microelement in the metabolism of nucleic acid, carbohydrates and protein [Rout and Sahoo, 2015]; Cu has essential roles in the physiological processes of plants, for instance, photosynthesis, respiration, carbohydrate distribution, nitrogen fixation, metabolism of protein, and antioxidant activity [Uluisik et al., 2018], and Mn is crucial for plant development and metabolism [Ruscitti et al., 2017]. The deficiency or excess of the above-mentioned micronutrients can cause deleterious effects on plants [Schmidt and Husted, 2019; Luciano et al., 2017]. To illustrate, a deficiency of Fe micronutrient can result in chlorosis on new leaves and decrease sugar metabolic enzymes [Hasanuzzaman et al., 2017]. Further, an experimental study was carried out to reveal the effect of *P. indica* in the uptake of Zn by wheat plants in a Zn-deficient media and showed that *P. indica* inoculation positively affected Zn uptake [Abadi and Sepehri, 2016]. According to their interpretation, the elevation of Zn concentration in *P. indica* colonized plants could be associated with the improvement of carotenoid and chlorophyll contents thus enhancing photosynthetic rate by stomatal conductance, subsequently. In terms of soil sterilization, the trend of the shoot micro and macronutrient contents of both tested cultivars was quite interesting. There were statistical differences ($p < 0.05$) between the experimental plants grown in autoclaved soil and those grown in non-autoclaved soil in all macro (Ca, K, Mg, P, and S) and micronutrient (B, Cu, Fe, Mn, and Zn) contents in both tested cultivars with the plants grown in native soil having consistently lower levels of

corresponding nutrients of both cultivars in comparison with those grown in sterile soil. As a result, removing the native soil microorganisms (i.e., biotic stress factors) from the soil resulted in increased nutrient contents.

The most prevalent strategy of plants so as to tolerate stressful conditions is the accumulation of compatible osmolytes such as proline, glycine betaine, and free sugars [Augé, 2001; Delauney and Verma, 1993]. According to a study conducted on tomato plants (*Solanum lycopersicum* L.) by Ghorbani et al. (2018) to show the effect of *P. indica* under salinity stress revealed that *P. indica* inoculated tomato plants displayed significantly reduced glycine betaine accumulation at 100 and 150 mM NaCl concentrations; however, at 0 and 50 mM NaCl levels the endosymbiotic fungus increased glycine betaine accumulation in leaves. Co-ordinately, with increasing salt levels, proline content in leaves increased dose-dependent manner, however, reduced with the presence of *P. indica* in comparison with those grown with the absence of *P. indica* in all treatments except control. Moreover, proline and GB contents in leaves and roots were markedly increased by salt stress. Additionally, *P. indica* inoculated roots showed markedly elevated proline and GB contents all salt treatment group except the control. According to another experimental study conducted on two wheat (*Triticum aestivum* L.) cultivars (B-tolerant cv. Bolal-2973 and B-sensitive cv. Atay-85) under B toxic conditions revealed that proline and glycine betaine contents were not significantly changed in leaf and root tissues of both cultivars after B toxicity stress. Furthermore, neither cultivar's leaf nor root tissues showed any differentially regulated genes associated with the metabolisms of proline or glycine betaine, according to the findings [Kayihan et al., 2017]. In the current investigation, *P. indica* inoculation reduced the glycine betaine accumulation in both tested cultivars and only high B level resulted in elevated GB levels in both cultivars (Tables 3.20 and 3.21), indicating that under current experimental conditions, leaves GB parameters of both tested cultivars were found to be not applicable to reveal the stress-alleviating effects of *P. indica* and B toxicity effects of plants. Moreover, considering the aforementioned findings, it appears that the root contents of proline and GB might be useful parameters to elucidate the effects of *P. indica*. On the other hand, it requires more comprehensive studies to completely ascertain the mechanism of how *P. indica* is aiding the plant in maintaining osmotic

balance. According to the literature, there has not been any study comparing the difference between sterilized and non-sterilized soil in terms of proline and GB.

ROS are formed when B reaches toxic levels, as in most ionic stresses [Cervilla et al., 2007]. Therefore, a high external B level might enable the transport of boric acid into the cell which might be subsequently converted to borate to a limited extent due to the high internal pH of the cytosol [Wimmer et al., 2002]. Thus, this could lead to the release of free ROS, such as $O_2^{\cdot-}$, $OH^{\cdot-}$, and H_2O_2 [Dat et al., 2000]. Membrane lipids and proteins are oxidatively damaged by these ROS which are strong oxidizing agents [Molassiotis et al., 2006]. Plants have hence, osmo-protective solutes (i.e., proline and glycine-betaine), nonenzymatic antioxidants (i.e., carotenoids, glutathione, ascorbate), and enzymatic antioxidants, such as CAT, SOD, POX, and GRX to surmount abiotic stresses such as B toxicity [Seth and Aery, 2017].

As is well known, GRX also has a critical role in oxidative stress via converting the oxidized glutathione (GSSG) to reduced glutathione (GSH) and retaining a high GSH/ GSSG ratio [Fadzilla et al., 1997; Irihimovitch and Shapira, 2000]. In the current study, B toxicity caused no marked effect on the shoot GRX concentrations in Saricanak-98; however, only the medium B level markedly reduced the shoot GRX activity in Nusrat (Tables 3.18 and 3.19). Experimental inputs documented by Masood et al. (2012), showed that compared to the control group, high B did not cause any considerable change in the shoot leaf GRX concentration on wheat (*Triticum aestivum* L. cv. Thasos) plants which is in accord with the outputs of this study in general. In the case of endophytic fungus, the present investigation showed that the shoot GRX activity was positively affected by *P. indica* in durum wheat but was not associated with the endophytic fungus in bread wheat. A study conducted on wheat plants to show the beneficial effects of *P. indica* against Cd toxicity revealed that the presence of this endosymbiotic fungus positively affects the activity of GRX activity, [Shahabivand et al. 2016] which is congruent with the outputs of current study.

The POX is a crucial enzyme system that is particularly involved in the H_2O_2 detoxification. Here, an increment in POX activity was observed at only a high applied concentration of B in Nusrat; however, B toxicity caused no effect on POX activity in Saricanak-98 (Tables 3.22 and 3.23). Seth and Aery (2017) explored that the shoot POX activity of wheat (*Triticum aestivum* L. var. Raj 4037) plants

increased at high B concentrations whereas reduced at low B doses. Accordingly, another study carried out by **Kayihan et al. (2017)** found that B toxicity has an impact on gene expression of enzymes that are associated with antioxidant response in wheat, most of which were peroxidases. Given the existing body of evidence reported in the studies of **Seth and Aery (2017)** and **Kayihan et al. (2017)**, they support and are in accord with the current investigation in B-tolerant Nusrat.

The APX is another crucial enzyme in the ascorbate-glutathione cycle and reduces H_2O_2 by employing ascorbate as an electron donor [**Mittler, 2002**]. In this investigation, in the B tolerant-Nusrat cultivar, the shoot APX as well as POX activities were developed by *P. indica* whereas either activity did not show any significant change caused by the fungal infection in Saricanak-98 (**Tables 3.18 and 3.19**). On the other hand, B toxicity caused inconsistent activities of APX in both cultivars. **Yaghoubian et al. (2014)** reported that wheat (*Triticum aestivum* L. cv. Azar2) plants exposed to drought showed enhanced antioxidant enzyme activity (CAT, APX, and POX) with increasing water stress which is not in accord with the outputs of this study. On the other hand, in the same study, the presence of *P. indica* caused markedly increased antioxidant enzyme activities such as CAT, APX and POX, and leaf chlorophyll content in comparison with those grown in the absence of fungal inoculum against drought. Taking into account the APX and POX activities of Nusrat in terms of *P. indica*, these findings are the same lane as the results belonging to the current investigation. In the final accounting, the higher POX and APX activities in *P. indica* inoculated plants of B-tolerant Nusrat would be partly interpreted that those antioxidant enzymes were employed to keep H_2O_2 concentrations lower in protecting the plants against oxidative damage, in turn enhancing B tolerance. By contrast, *P. indica* inoculated plants did not show any significant change in the shoot of POX and APX concentrations in Saricanak-98.

Dismutation of O_2^- to H_2O_2 was carried out by the SOD and $H_2O_2^-$ to O_2 and H_2O was carried out by CAT, subsequently [**De Gara et al., 2004; Shahabivand et al., 2016**]. A significant reduction with *P. indica* inoculation in the B-tolerant Nusrat cultivar suggests that detoxification of H_2O_2 by SOD enzymatic antioxidant is not sufficient under B stress (**Tables 3.18 and 3.19**). However, for Saricanak-98, no significant effects in the presence of fungal endophyte were observed which needs more study to enlighten in a broad manner. Moreover, the shoot SOD activity of

durum wheat resulted in inconsistent concentrations with increasing B levels whereas only the medium B level caused a reduction in the activity of SOD in bread wheat. Previously, an experimental work from **Karabal et al. (2003)** found that barley plants subjected to excess B caused no marked effect on the shoot SOD activity. To name another example, experimental inputs reported by **Supanjani (2006)**, showed that the shoot SOD and CAT activities of hot pepper (*Capsicum annuum* L. cv. Nokkwang) plants were reduced under increasing B toxicity and salinity. As a result, experimental inputs from **Karabal et al. (2003)** and **Supanjani (2006)** support and are in agreement with the outputs belonging to the shoot SOD activity obtained from the current study.

On the other hand, these outputs related to antioxidant responses are not in accord with the findings of **Molassiotis et al. (2006)** who reported elevated SOD, CAT, and APX activities in apple rootstock under B toxicity. Besides that, increased SOD and APX activities in barley under B-toxic conditions [**Karabal et al., 2003**] and in wheat under saline conditions [**Sairam et al., 2005**] have been documented. Similarly, **Gunes et al. (2007)** found that the wheat plants grown in B toxic soil showed elevated SOD, CAT, and APX activities. In terms of endophytic fungus used in this study, the experimental plants grown with *P. indica* showed elevated shoot SOD activities in Nusrat and had no impact that of Saricanak-98, though (**Tables 3.18 and 3.19**). **Shahabivand et al. (2016)** carried out a pot trial to investigate the effects of *P. indica* and arbuscular mycorrhizal fungus (*Funneliformis mosseae*) on some enzymatic activities in leaves and roots of wheat (*Triticum aestivum* L. cv. Sardari39) under Cd stress. According to their outputs, the presence of *P. indica* and *F. mosseae* + *P. indica* resulted in reduced H₂O₂ and MDA contents, and elevated proline accumulation in wheat leaves and roots. Moreover, the SOD activity in leaves and roots increased at lower Cd concentrations whereas at higher Cd concentrations the rate decreased. In terms of endosymbiotic fungus, the presence of *P. indica* caused elevated SOD activities in leaves as well as roots against Cd toxicity. In the current investigation, the activity of SOD increased in *P. indica* inoculated Nusrat cultivar which is in agreement with the study of **Shahabivand et al. (2016)**. Taking into account endophytic development, early plant growth stage could be contingent on both host genotype and nutrient supply [**Chepllick, 2008; Taghinasab et al., 2018**]. It can be interpreted as both the ameliorative effects of *P.*

indica and the toxic effects of B on the shoot SOD activity were not manifested, and this enzyme was not employed under these conditions for Saricanak-98.

Here, the shoot TEAC levels of both tested cultivars were not affected B toxicity in comparison with the control groups; on the other hand, were reduced by *P. indica* (Tables 3.20 and 3.21). Further, the presence of endosymbiotic fungus did not result in marked changes in the shoot CAT activities of both Nusrat and Saricanak-98 and the shoot CAT activities of both cultivars responded to B toxic levels in the same way as that of TEAC. The increased TEAC levels likely takes place due to the production of phenolic compounds or other by-products of B [Revilla et al., 1985; Masood et al., 2012], particularly when the oxidative damage to the membrane is elevated under B toxicity. Therefore, B has the ability to interact with membrane compounds and increase low molecular weight TEAC overall. Numerous metabolic pathways, including, *inter alia*, cell wall production, lignification, glucose metabolism, phenol metabolism, and plasma membrane integrity, are known to be affected by B [Marschner, 2011; Mühling et al., 1998; Dordas and Brown, 2005]. However, the study conducted by Masood et al. (2012) showed that the shoot TEAC and CAT levels were not significantly influenced by B toxicity although high B level resulted in necrotic leaf spots and these results are congruent with the results reported in the present study Tables 3.18 and 3.19, 3.20, and 3.21; Figure 3.1. A study was carried out to investigate the effects of the endophytic entomopathogenic fungus *Beauveria bassiana* (strain: SM3) on the physiology and nutritive value of Lettuce (*Lactuca sativa* L. cv. Green Oak) against the pathogenic aphid *Myzus persicae* [Macuphe et al., 2021]. Although *L. sativa* is a highly demanded vegetable crop the plant is susceptible to aphid infestation. One of the objectives of this study is to assess the effect of *B. bassiana* on growth, macronutrient and micronutrient contents, and antioxidant contents of *L. sativa*. Besides the control group, plants were exposed to four fungal conidial concentrations: $0,1 \times 10^6$, 1×10^7 and 1×10^8 conidia mL⁻¹ by drenching in a greenhouse experiment. Plants inoculated with fungal inoculum showed significantly higher TEAC levels at the control and highest conidial concentration (1×10^8 conidia mL⁻¹) than the lower conidial concentrations ($0,1 \times 10^6$, and 1×10^7). Moreover, in this study, tissue Cu level was markedly associated with TEAC. In the current study, concentration of 9.65×10^5 mL⁻¹ was used to inoculate the plant roots, and TEAC levels of both cultivars and Cu level of bread

wheat were considerably reduced by *P. indica*. On the other hand, Saricanak-98 cultivar was not influenced by the endophytic fungus and the reason for this study is unclear. It is necessary to carry out further studies to ascertain the relationship between *P. indica* and TEAC in plants. Conidial concentrations used to infect plant roots may cause significant differences between in plants.

4.2. The Second Soil Experiment: *P. indica*-Ameliorated Yield Parameters Against Excess B Independently of P Uptake

In the present study, considerable *P. indica*-mediated improvement in grain number, and straw dry weight were observed for both bread and durum wheat cultivars (**Table 3.25, Figs. 3.12, and 3.13**) which are congruent with the studies reporting *P. indica*-mediated yield increases in wheat [**Taghinasab et al., 2018**] and barley [**Waller et al., 2005**]. Nonetheless, the number of investigations in which cereals (i.e., wheat and barley) were grown to full maturity in the presence or absence of symbiosis with *P. indica* and marked yield elevations were documented are limited. Because most studies published have only focused on the effects monitored at the vegetative stage [**Hosseini et al., 2017; Baltruschat et al., 2008**] and none of these studies documenting yield effects were dependent on B toxicity. As a matter of fact, the elevated grain numbers and straw dry matter could be the first indication of colonization by an endophyte in studies where plants were grown to full maturity. Here, soil sterilization resulted in increased yield parameters irrespective of the endophytic fungus probably owing to the elimination of soil-borne pathogens as explained before. Overall, soil sterilization caused marked effects on the shoot growth as well as yield parameters (**Figs. 3.12, 3.13, and 3.14**), suggesting the native soil was a considerable source of biotic stress.

An excessive amount of B hampers starch biosynthesis or forms B-carbohydrate complexes thus causing delayed grain formation [**Das, 2014**] and on account of the negative effects of toxic B upon pollen development and pollen tube growth, seed and fruit set can be hindered [**Fang et al., 2016**]. The straw dry matter was not influenced by B toxicity in both tested cultivars (**Table 3.25, and Figure 3.12**). Previously, **Serfling et al. (2007)**, demonstrated that although the straw biomass of wheat was boosted by *P. indica*, considerable increment in grain yield was not found.

Here, on the one hand, the straw biomass was enhanced significantly by *P. indica*; on the other hand, grain yield did not (**Figs. 3.12**, and **3.14**). In conclusion, straw dry weight can stay relatively high even when grain yield is restricted, as happened in the present investigation. Even though the straw B concentration was decreased by *P. indica*, the grain B concentration was not influenced in Nusrat. In the case of Saricanak-98, the straw B concentration was not affected by the fungal endophyte, but the grain B concentration reduced (**Tables 3.25**, and **3.26**; **Figs. 3.11**, and **3.15**). This study revealed the effect of *P. indica* on the translocation of B differentiated in either cultivar. Further, grain levels were negligibly small in comparison to straw B concentrations, which makes sense considering the limited phloem mobility of B in wheat [Stangoulis et al., 2010].

In retrospect, Taghinasab et al. (2018), conducted an experimental work in wheat (*Triticum aestivum* L., cv. Bobwhite) to comparatively investigate the effect of the fungal endophytes *Trametes versicolor* and *P. indica* in P-deprived and P-rich conditions on P uptake and yield. According to their experimental inputs, *P. indica* enhanced grain P content in both P-deprived and P-rich conditions, by 30% and 16% respectively. Moreover, in P-deprived conditions, the *P. indica*-colonized plants showed increased straw P content of 33%. As for grain yield, a marked *P. indica*-mediated elevation was observed under the high-P conditions while *P. indica* did not cause any effect in the P-deprived condition. In the current study, the presence of *P. indica* did not show any significant changes in both grain and straw P concentrations in both tested cultivars (**Tables 3.27**, and **3.28**). Further, *P. indica* did not have a marked impact on straw P content of Nusrat; nonetheless, increased that of Saricanak-98. According to the study of Taghinasab et al. (2018), *P. indica*-mediated wheat yield improvements were associated with increased P uptake. Further, according to experimental inputs by Baghaie and Aghilizefreesi (2020), under soil Pb pollution, *P. indica* inoculated wheat cultivars genotypes (*Triticum aestivum* L. cvs. Falat, and Durum) showed significantly higher N and P concentrations in grains than *P. indica* non-inoculated plants. Moreover, the grain N and P uptakes were reduced by Pb-polluted soil. Evidently, under conditions of the current study, *P. indica* did not improve P uptake which also correlated with unaffected grain yields even though it increased the straw P content of Saricanak-98. As shown **Figure 3.10**, the developmental stage of Saricanak-98 was ahead of the

Nusrat at 50 DAS. Besides that, the grain yield of Saricanak-98 was much higher than that of B tolerant-Nusrat as displayed in **Figure 3.14**. It is, therefore, conceivable that *P. indica* must play an important role in developmental stage stem elongation and spike formation in durum wheat. Overall, even though a major advantage of the fungal endophyte in symbiosis is obtaining P for host plants, their usefulness in terms of grain yield in these conditions is ambiguous. In this sense, beneficial effects of the endophyte could be dependent on genotype and nutrient supply.

Considering *P. indica*, previous studies focusing on yield or long-term effects have not so far documented the micro and macronutrients of grain and straw except grain N, P, and Fe contents [Taghinasab et al., 2018; Baghaie and Aghilizefreesi, 2020]. To name an example, according to experimental inputs of Baghaie and Aghilizefreesi (2020), the grain Fe content of wheat plants was increased by *P. indica*, in the current study however, *P. indica* colonization did not result in any significant change in both wheat cultivars (Tables 3.35, and 3.36). Here, along with P, the grain macronutrient such as Ca, K, Mg, and S contents of both bread and durum wheat cultivars were influenced neither negatively nor positively by *P. indica* irrespective of B toxicity (Tables 3.37, and 3.38). Besides that, the grain Cu, and Mn contents were not changed by the presence of the endophytic fungus in both cultivars (Tables 3.35, and 3.36). On the other hand, soil sterilization resulted in markedly higher (3x) in the grain as well as straw Mn contents of both cultivars in comparison with those grown in native soil as happened in the vegetative stage of both cultivars which is intriguing. As explained previous pages, because of eliminating the Mn-oxidizing bacteria that convert plant-available Mn^{2+} into unavailable higher oxides, soil sterilization may result in Mn toxicity [Boyd 1971; Williams-Linera and Ewel 1984]. Straw Cu, Fe, and Mg contents of both cultivars were not affected by *P. indica* (Tables 3.45, 3.46, 3.47, and 3.48). Taken together, these results indicate that the endophyte had negligible effects on the grain as well as straw macro or micronutrients of both tested cultivars and B toxicity had inconsistent effects on these parameters.

4.3. The Third Soil Experiment: Beneficial Effects of Fungal-Host Interplays Depending on the Environmental Conditions

The symbiosis of arbuscular mycorrhiza (AM) fungi with plant roots has been demonstrated to be advantageous in tolerating and dealing with unfavourable conditions in different plant species [Beltrano and Ronco, 2008; Liu et al., 2016] including wheat plants [Abdi et al., 2021; Sonmez et al., 2009]. It was previously documented that AM-plant symbiosis causes to better plant growth and morphology characteristics, osmotic regulation, water uptake, antioxidant activity and root hydraulic characteristics, *inter alia* [Harrison, 2005; Chitarra et al., 2016; Hashem et al., 2016; Goh et al., 2013; Berta et al., 1995; Lu and Koide, 1994]. To name an example, AM-mitigated B toxicity tolerance was reported in *Puccinellia tenuiflora* under the combined stresses of salt and drought [Liu et al., 2018]. The current experiment aimed to unveil comparatively the effects of AM and *P. indica* on both bread and durum wheat cultivars supplemented with P against B toxicity in sterile soil conditions.

Here, uninoculated plants did not show mycorrhizal and *P. indica* colonisations in the roots of both tested cultivars. Structural characteristics of both AM and *P. indica* were monitored in the roots belonging to inoculated plants of bread and durum wheat thus both fungal endophytes are infective to wheat (Figure 3.16).

Not surprisingly, although the shoot dry matters were reduced by B toxicity, improved by P supply in dose-dependent manner in both Nusrat and Saricanak-98 (Table 3.48 and Figure 3.17C-D). Reductions in the shoot dry matters are one of the prevalent effects of B toxicity [Camacho-Cristobal et al., 2008] and it also appeared in the first experiment. Moreover, adequate P application from the early stage of growth is vital for optimal growth production [Taghinasab et al., 2018] which is congruent with the output of this experiment (Figure 3.17D). Mycorrhizal relationships can also be negative depending on environmental conditions and costs in terms of symbiosis [Smith and Smith, 2011]. As for fungal relationships, although AM colonization caused neither positive nor negative effects on the shoot dry matters of both tested cultivars, *P. indica* colonization improved it in Saricanak-98 but not that of B-tolerant Nusrat (Table 3.48 and Figure 3.17A-B). In retrospect, Sonmez et al., (2009) reported that under B toxic condition AM-colonized wheat

plants reduce the shoot B concentration and according to experimental inputs of **Liu et al. (2018)**, AM inoculation alleviated boron toxicity in *Puccinellia tenuiflora* plants via increasing biomass and reducing tissue B concentrations. On the other hand, AM fungi confer plant tolerance to heavy metals stress by enhancing plant growth [**Gaur and Adholeya, 2004**] and in the current study, AM-induced shoot dry weight improvements were not found in both cultivars even though tissue B concentrations of the abovementioned studies belonging to **Sonmez et al.** and **Liu et al.** are not in conformity with the output of this experiment (**Table 3.48, Figs. 3.17A and 3.18A**). Although as tackled in the first soil experiment, *P. indica* alleviated B toxic concentrations in shoots, in the current experiment it can be inferred that soil sterilization and nutrient regimes might likely affect certain aspects of the symbiotic relationships of *P. indica* and AM.

In the present experiment, the highest shoot B concentrations were with the high B levels; however, the lowest concentrations were in the controls in both cultivars, and durum wheat accumulated more B compared to bread overall (**Table 3.48 and Figure 3.18**). Interestingly, AM did not significantly affect the B concentration of both tested cultivars as opposed to the study of **Sonmez et al. (2009)** in which AM inoculation significantly reduced shoot B concentrations. As tackled in preceding pages, B is a member of the metalloid subgroup III and it shows intermediate properties between metals and non-metals [**Marschner, 2011**]. In retrospect, even though B is not a heavy metal, AM fungi inoculation was studied as an inexpensive remediation method in B-contaminated soils [**Havener, 2013**]. AM demonstrates the capacity to tolerate particular metals by means of a number of mechanisms, ranging from selective binding to total toxin immobilisation [**Leyval et al., 1997**]. There have been a bunch of works to reveal the role of AM on metal hyperaccumulating plants such as those documented by **Regvar et al. (2003)** and **Wang et al. (2007)**. From these studies, controversial outputs were obtained. Some findings divulged elevated heavy metal concentrations in AM-inoculated plants [**Leyval et al., 1997; Tonin et al., 2001**], but, on the other hand, others revealed decreased heavy metal uptake [**Zhu et al., 2001**]. Besides that, based on growing reports, AM can aid the phytoremediation of metal contamination through making metals more available for plant uptake or through decreasing metal toxicity in host plants [**Coninx et al., 2017**]. Surprisingly, *P. indica* elevated the shoot B concentration in B tolerant-

Nusrat, but not that of Saricanak-98 (**Figure 3.18A**), and these outputs are controversial, especially compared to the study tackled in the discussion of the first soil experiment. Even though this could be a bold argument, *P. indica*-mediated remediation of B contamination by increasing B uptake might be possible in sterile soil and under conditions of excess B. However, the mechanism by which *P. indica* confers the host plants has not yet been fully unveiled. High P level caused reduced B accumulation in Saricanak-98 (**Figure 3.18D**). According to studies, the relationship between P and B uptake is antagonistic, which is likely because of sharing the same absorption and transport system, thereby causing a competitive relationship [Kaya et al., 2009; Gunes et al., 2000; Long and Peng, 2023]. However, Long and Peng, interpreted that complex physiological processes underlie the interactions between B and other elements.

Regarding the shoot chlorophyll contents of both tested cultivars, while *P. indica* had positive effects on it, arbuscular mycorrhiza caused neither positive nor negative changes (**Table 3.49** and **Figure 3.23**). Moreover, the shoot chlorophyll concentration is also considerably affected by triple interactions between *P. indica*, B toxicity and P supply in Nusrat and at high P level and control B level, the presence of *P. indica* confers higher chlorophyll biosynthesis in leaves. In Saricanak-98, the shoot chlorophyll concentration is also considerably affected by triple interactions of *P. indica*, AM fungi, and P supply. At high P level, the presence or absence of mycorrhizal and endophytic fungal symbiosis did not cause any significant changes; however, resulted in the highest shoot chlorophyll biosynthesis, probably thanks to high P (**Table 3.49** and **Figure 3.23D**). Previous studies showed positive effects of *P. indica* on chlorophyll content of plants as tackled in previous section belonging to the first soil experiment and the current results are in the line with those studies [Jogawat et al., 2013; Shahabivand et al., 2012]. Yet, former studies also showed that the photosynthetic capacity of leaves also can be improved by AM [Yang et al., 2007; Davis et al., 2014; Huang et al., 2023]. To put this more concretely, according to Abdi et al. (2021), under drought conditions, AM-inoculated wheat plants significantly increased the amount of Chl a and b in leaves compared to those not inoculated, and the ameliorative effect of AM appeared in that way. Further, in the same study, total chlorophyll content was negatively influenced against drought stress.

Under drought conditions, a similar experimental study conducted on wheat plants inoculated with *P. indica* and *G. mosseae* either alone or in combination reported that the amounts of chlorophyll *a*, *b* and *a+b* were improved by both symbiotic fungi [Yaghoubian et al., 2014]. In the current study, however, the amount of chlorophyll in both tested cultivars were not affected by double interaction of *P. indica* and AM fungi (Table 3.49 and Figure 3.23A-B). Under B toxic conditions, diminished leaf chlorophyll contents and photosynthetic rates have been documented by a number of studies and loss of chlorophyll is the one of the early symptoms of the B toxicity [Ghanati et al., 2005; Metwally et al., 2012; Hajiboland et al., 2015; Ogunwole et al., 2015]. Here, in Nusrat, B toxicity reduced the chlorophyll content; however, had no effect on that of Saricanak-98 (Figure 3.23C). The shoot chlorophyll content was not affected by P supply in Nusrat but increased in Saricanak-98, and Saricanak-98 produced more chlorophyll as compared to Nusrat overall (Figure 3.23D). Considering the crucial role of P in chlorophyll synthesis in plant cells [Marschner, 2011], in durum wheat, more P uptake likely ended up with more chlorophyll content; however, in Nusrat chlorophyll degraded because of B toxicity. However, chlorophyll degradation in Nusrat happened to such an extent that allows the plants to maintain growth and development.

In the current investigation, the carotenoid biosynthesis in leaves of both cultivars was significantly increased by *P. indica* (Table 3.49 and Figure 3.24), and these inputs are corroborated by the studies conducted under various stress conditions previously [Sharma et al., 2017; Abdelaziz et al., 2019; Albqmi et al., 2023; Walter et al., 2010; Strack and Fester, 2006]. Additionally, in Saricanak-98, at the high level of P supply, the leaf carotenoid levels in inoculated plants with *P. indica* and AM fungi together was found to be the highest suggesting that at high P condition, co-inoculation of AM and *P. indica* symbiosis causes a synergetic effect on carotenoid biosynthesis (Table 3.49). Surprisingly, B toxicity did not cause decreased carotenoid levels in both tested cultivars (Table 3.49 and Figure 3.24C) and these results are in line with a previous study conducted on wheat cultivars [Metwally et al., 2012]. Therefore, it can be interpreted that the effects of B toxicity did not appear as a degradation of carotenoid level, and both *P. indica* and AM fungi improved carotenoid production irrespective of B toxicity. Considering the crucial role of carotenoids in chloroplasts, these outputs are intriguing. Formerly, Kumar et

al. (2019) conducted a study on sorghum plant, which belongs to Poaceae family and is a staple crop as wheat, to unveil the effects of polyamines and AM under Cd toxic conditions. In this study, the presence of AM imparted considerable mitigation of Cd-induced toxicity in sorghum plants via the defensive role of AM in plants by improving chlorophyll and carotenoid contents in the leaves.

The higher membrane stability is often associated with lower MDA concentrations [Abid et al., 2016] due to lipid peroxidation. Apart from first soil experiment, in this experiment the shoot MDA concentrations were also reduced by *P. indica* in both Nusrat and Saricanak-98 (Table 3.49 and Figure 3.21A). Further, the presence of AM resulted in a reduced shoot MDA concentration as well as REL in Saricanak-98 (Tables 3.60, 3.61 and Figure 3.21B). The shoot MDA levels of Nusrat were significantly affected by quadrable interactions of *P. indica*, AM fungi, B toxicity, and P supply treatments, and both high B and P levels, *P. indica* non-inoculated but AM inoculated plants showed the highest MDA concentrations in their leaves (Table 3.49). As for Saricanak-98, MDA levels were significantly affected by triple interactions of AM fungi, B toxicity, and P supply, and at high levels of B and P, non-mycorrhizal plants displayed the highest MDA concentrations in their leaves under B toxic conditions. According to Simón-Grao et al., (2019), *Rhizophagus irregularis* inoculated Carrizo citrange rootstock plants had lower MDA concentrations than that of non-inoculated plants under B toxic conditions and these outputs are congruent with result of this experiment. In the same study, excess B reduced vegetative growth both AM-inoculated and non-inoculated rootstock plants and the decrease was greater in non-inoculated plants in comparison with AM-inoculated plants. Further, at high level B, plants cells of experimental plants produced highest levels of MDA in both inoculation groups. Under salinity stress, mycorrhization resulted in reduced MDA accumulation in wheat leaves according to experimental inputs of Talaat and Shawky (2014). In the current study, increasing B levels caused elevations in the MDA levels of both tested cultivars (Figure 3.21C) which is also in accord with the results in the abovementioned study along with vegetative growth. Similarly, AM-inoculated wheat plants showed reduced MDA accumulation, according to an experimental study conducted under drought conditions previously by Abdi et al. (2021) which is also in agreement with the outputs of the present investigation regarding AM and *P. indica* symbiosis. Besides

that, in this study, POX and CAT enzyme activities, which are associated with reducing the concentration of H₂O₂, were not generally affected by *P. indica* and AM fungi regardless of B toxicity (**Tables 3.62, 3.63, 3.64 and 3.65**). These results agree with the findings of **Simón-Grao et al. (2019)**, where although MDA levels were reduced by mycorrhization, H₂O₂ concentrations were not affected. In retrospect, oxidative stress and membrane peroxidation were attributed to elevated chlorophyll degradation and reduced chlorophyll synthesis [**Ardic et al., 2009; Cervilla et al., 2007 and 2009**]. Taking together, results of MDA in both tested cultivars in the current study showed that both *P. indica* and AM fungi reduced the shoot MDA levels which can be the reason for increased chlorophyll and carotenoid levels.

Proline biosynthesis of plants is considered one of the widespread responses to stress [**Parvaiz and Satyawati, 2008; Ashraf and Foolad, 2007**]. Previously, investigations have been focused on the function of proline regarding the process of osmotic adjustment, the detoxification of ROS, and the protection of membranes against lipid peroxidation [**Martinez-Cuenca et al., 2015**]. A study conducted on citrus plants under excess B conditions showed reduced proline levels in the leaves. It can be inferred that proline can likely be considered a negative biomarker of the degree of tolerance to B stress [**Simón-Grao et al., 2019**]. Hence, higher proline concentration in the leaves indicates lower B toxicity. Here, as displayed by four-way ANOVA, the shoot proline levels of Nusrat were significantly affected by triple interactions of *P. indica*, B toxicity, and P supply treatments showing at high P level, the shoot proline accumulation in *P. indica* inoculated plants caused the highest proline levels (**Table 3.49 and Figure 3.22**). The shoot proline levels of Nusrat were also significantly affected by triple interactions of *P. indica*, AM fungi, and P supply treatments showing at high P supply, co-inoculation of AM fungi and *P. indica* caused the highest proline levels in leaves. The shoot proline levels of Saricanak-98 were significantly affected by triple interactions of *P. indica*, AM fungi and B toxicity treatments showing at control B level, the shoot proline accumulation in *P. indica* inoculated plants with AM fungi caused the highest level. However, mycorrhization did not significantly affect the proline concentration in the leaves of both tested cultivars (**Figure 3.22A**). These results agree with former study where proline accumulation was not affected by AM fungi [**Simón-Grao et al., 2019**]. B toxicity did not change the shoot proline concentration of both tested cultivars

(**Figure 3.22B**). However, the outputs obtained in the current study on the proline levels support the idea that Nusrat cultivar is more tolerant to excess B as compared to Saricanak-98, as the concentration of proline in Saricanak-98 is greater than that of Nusrat. Under B toxicity, an elevation in proline level is noteworthy because a reduction in the proline level can cause higher lipid peroxidation and, thus, membrane damage [Molassiotis et al., 2006]. On the other hand, in the current study, B toxicity caused increased shoot MDA levels in both cultivars thereby reduced chlorophyll and carotenoid biosynthesis. Hence, there is some discrepancy between proline and MDA levels. Proline is constantly regarded as a ROS antagonist which causes a reduction in oxidative stress [Kohneharhi and Demir, 2023]. In both Nusrat and Saricanak-98, given that B toxicity did not cause significant changes in the shoot proline levels, the shoot enzyme activities in this experiment were conflicting or aberrant. It can, therefore, be inferred that these enzymes were not leveraged by the experimental plants. Taking into account this experiment conducted in sterile soil condition, removal of the native soil microflora may likely affect the beneficial aspects of both fungi. Formerly, improved the performance of *G. mosseae* and *G. fasciculatum* was found in the autoclaved substrate soil mixed with soil microbiota as compared to autoclaved substrate [Al-Khaliel, 2010]. Similar findings were also documented by Azcon et al. (1990). They revealed that whereas the infection of *G. mosseae* increased by soil microorganisms, the establishment of *G. fasciculatum* was reduced in the roots of *Medicago sativa*. As of this writing, the integrated function of *P. indica* and AM with the native soil microflora in terms of infection and symbiosis is still unclear. *P. indica* caused boosted proline as well as diminished MDA levels of both cultivars (**Figs 3.21 and 3.22**), and similar results were observed in corn (*Zea mays* L.) and rice (*Oryza sativa* L.) plants, against water stress and drought, respectively [Tsai et al., 2020; Xu et al., 2017]. Therefore, regardless of B toxicity, although *P. indica* positively affects the shoot proline level, mycorrhization did cause neither negative nor positive effects. As for P supply, high P increased the shoot proline levels of both tested cultivars (**Figure 3.22D**) which is in agreement with the outputs of Al-Karaki et al. (1996) who conducted an experiment on sorghum [*Sorghum bicolor* (L.) Moench] and bean (*Phaseolus vulgaris* L. and *P. acutifolius* A. Gray) cultivars under water stress conditions to reveal phosphorus effect on proline accumulation. According to their experimental inputs, when grown with high P supply, the leaves of sorghum plants accumulated

highest proline whereas bean leaves had highest proline when grown with low P supply. It may suggest that high proline accumulation in leaves of plants grown in at high P levels might be an adaptive response to the current experimental conditions for either cultivar.

In bread wheat, both fungal endophytes boosted the shoot APX activity whereas in durum wheat, they caused no remarkable changes (**Tables 3.62 and 3.63**). Enhanced APX activities in wheat inoculated with *P. indica* and *G. mosseae* against drought in sterilized soil were previously documented by **Yaghoubian et al. (2014)** and also tackled in the discussion of the first experiment. In the first experiment POX and APX activities were also increased by *P. indica* in Nusrat; conversely, not affected in Saricanak-98. Thus, a greater POX and APX activities in leaves of bread wheat signifies a tolerance to B toxicity which supports the notion of tolerance in Nusrat wheat against B toxicity. According to **Alscher (1989) and Sreenivasulu et al. (2000)**, elevation in APX activity has been to be thanks to transcriptional and translational modifications. Concomitant with the outputs of this experiments, **Rani et al. (2018)** also documented that AM colonization improved the APX and POX activities under control and drought conditions in both drought susceptible and tolerant wheat varieties. On the other hand, it was revealed that these activities were higher in tolerant variety as compared to susceptible variety under drought condition. In addition, similar results were also found that AM colonization boosted the APX activity under control and drought conditions in date palm, and soybean plants [**Benhiba et al., 2015; Porcel et al., 2003**].

The shoot APX activity was increased by B toxicity in B tolerant-Nusrat; however, it was reduced in Saricanak-98 (**Tables 3.62 and 3.63**). The coordination of increased APX as well as POX activities in Nusrat might indicate improvement in ROS detoxification and, thus better protective mechanisms in comparison with Saricanak-98. **Sharma et al. (2016)** conducted an experiment on Cd-resistant and Cd-sensitive wheat genotypes to investigate the effects of AM fungi (*Rhizophagus intraradices*) under different Cd concentrations (0, 100, 200 and 300 mg Cd kg⁻¹ soil) for 30 days. They found that Cd toxicity resulted in elevated lipid peroxidation and reduced plant growth; however, AM inoculated Cd-resistant wheat genotype displayed higher APX activity. In the present study, APX activity in inoculated plants with *P. indica* and AM fungi together was more than in non-inoculated plants grown under B toxic

conditions. This result is verified in a study conducted on wheat plants under drought conditions to investigate comparatively the effects of *P. indica* and *Glomus mosseae* in sterile soil [Yaghoubian et al., 2014]. At high P, increased shoot APX activities were found in both tested cultivars (Tables 3.62 and 3.63). Moreover, the shoot APX activities were markedly affected by triple interactions of *P. indica*, AM fungi, P supply and at high P level, the inoculation of *P. indica* and AM caused the highest proline concentrations in Nusrat. Double interactions of AM fungi and P supply and supplementation with high level P and the presence of AM fungi (AM+P) resulted in highest shoot APX activity in Saricanak-98. A study conducted on *Nicotiana tabacum* under drought conditions with or without AM fungi (*Glomus versiforme*) showed that the supplementation of P and inoculation of AM (AM+P) elevated the shoot APX concentrations under drought as well as control conditions [Begum et al., 2020] which is congruent with the current experimental results. However, in the present experiment, there are conflicting outputs such as the shoot SOD and CAT activities in both cultivars. Either SOD and CAT enzyme activities of both Nusrat and Saricanak-98 were not remarkably affected by neither *P. indica* nor AM fungi (Tables 3.62 and 3.63). The study conducted by Begum et al. (2020), showed that SOD and CAT activities of *Nicotiana tabacum* plants improved by AM fungi. Besides that, *P. indica* did not also change both enzyme activities contrary to experimental inputs of Shahabivand et al. (2016) showing under Cd toxicity, the presence of both AM and *P. indica* increased SOD activities of wheat leaves. In this sense, AM and *P. indica*-mediated SOD and CAT enzyme activities were not employed by host plants under B toxic conditions suggesting the current experimental conditions were not appropriate to reveal ameliorative effects of these enzymes thereby protective mechanisms via both fungal agents.

Here, the shoot P concentrations of both cultivars were not affected by AM fungi (Table 3.48 and Figure 3.20B), under B toxic conditions although the elevated host plant P nutrition by AM fungi has been partly attributed to the enhanced development of AM in plants [Kaya et al., 2009]. Additionally, AM colonization did not significantly affect the shoot dry matters of both tested cultivars as tackled in Table 3.48 and Figure 3.17B. P has been one of the crucial regulators of AM symbiosis formation and efficiency because root colonization, and P uptake via fungal pathway and growth responses hampered with increasing soil P availability

[Smith and Read, 2010; Richardson et al., 2011]. Plants can, however, also influence the symbiosis through a variety of means, including hyphal branching and fungal metabolic activity stimulation [Bücking and Shachar-Hill, 2005; Besserer et al., 2006]. As tackled precedential pages, depending on environmental conditions and costs of symbiosis, mycorrhizal interplays can also have negative trends [Smith and Smith, 2011]. Regarding *P. indica* symbiosis, although the fungal endophyte did not change the P uptake in Nusrat, it improved Saricanak-98 (**Figure 3.20A**). Different trends in P uptake of both cultivars might likely depend on the plant's tolerance to B, therefore, the P uptake of Nusrat was not affected under B toxic conditions.

As tackled in **Tables 3.52** and **3.53**, amongst the macronutrient concentrations studied in the present experiment, AM inoculation led to an increase in the shoot Mg and S concentrations in both cultivars and the K concentration in Saricanak-98. Besides that, *P. indica* symbiosis resulted in an increase in the shoot S concentration in Saricanak-98. Both *P. indica* and AM-inoculated plants of tested cultivars did not show any discernible differences in the Ca concentration. B toxicity caused an increase in the K concentration and no marked effects in the Ca and S concentrations in both cultivars. The shoot Mg concentration was reduced in Nusrat and not affected in Saricanak-98 under B toxic level. As for P supplementation, high P level led to an increase in Ca, Mg, and S concentrations; however, a decrease in the K concentration in either cultivar. Hitherto, the interplays of B with K, Mg, and S in plants have not been investigated much. As it also appeared in the shoot concentrations of the current study, increased B concentration promoted the uptake of K in leaves as well as roots in tobacco; however, reduced the tissue Mg uptake in tomato plants [Lopez-Lefebvre et al., 2002]. On the other hand, no remarkable effect of B level on Mg uptake was found in maize [Mozafar, 1989] which is congruent with the shoot Mg concentration of Saricanak-98. Additionally, it appeared that there is also an interaction between B and S. Although increasing B concentrations did not affect the shoot S concentrations in this experiment, S can markedly increase the B absorption in *Brassica juncea* and *Helianthus annuus*, and the S uptake is elevated by higher B levels [Karthikeyan and Shukla, 2008]; however, the specific mechanism has not been unveiled so far.

As tackled in **Tables 3.54**, **3.55** and **3.48**, and **Figure 3.19A**, the shoot Zn and Mn concentrations were improved by *P. indica* in durum wheat. However, the Mn

concentration in bread wheat and the Fe concentration in durum wheat were reduced by the *P. indica*. Both *P. indica* and AM-inoculated plants of tested cultivars did not show any discernible differences in the shoot Cu concentrations (**Tables 3.54**, and **3.55**). Although, toxic B level caused a reduction in the Fe concentration in Nusrat and the Mn concentration in Saricanak-98, increased Mn, and Cu concentrations in Nusrat. The shoot Zn concentrations, however, was not significantly changed by B toxicity. As regards to P supplementation, the shoot Fe, Mn, and Zn concentrations of both cultivars were reduced by increasing P supply. It is possible that P supplementation diluted Fe, Mn, and Zn in above-ground parts of plants by promoting shoot growth. Having calculated these micronutrient contents of the cultivars, it appears that the Fe, Mn, and Zn contents were boosted by P supply remarkably. On the other hand, P supply in either cultivar led to an increase in the shoot Cu concentration.

5. CONCLUSION

In the contemporary era, unfavourable climatic conditions severely affect agriculture and crop productivity, thus influencing global food security. Hereby, to alleviate these deleterious effects and protect the yield capacity of crop plants, sustainable management approaches are urgently needed. The novel methods that can boost plant growth and yield by enhancing the physiological and biochemical characteristics or strengthening the indigenous tolerance mechanisms could be advantageous to prevent yield losses substantially. In the current dissertation, the roles of *P. indica* as well as AM fungi and their effects on the tolerance mechanisms under B toxic conditions in two wheat cultivars, have been investigated to provide novel insight.

Piriformospora indica, as a growth-promoting microorganism, has remarkable potential whilst the mechanism by which *P. indica* confers host benefits has not been thoroughly understood. In terms of the first and second experiments, these pot trials provided insight into the extent and mechanisms of the contribution of *P. indica* to bread as well as durum wheat concerning growth and yield parameters in the presence or absence of B toxicity as an abiotic stress factor which had formerly not been studied in relation to *P. indica*. It was found for the first time that wheat growth and yield parameters can be considerably enhanced by *P. indica* under B toxicity even though the extent of the benefit conferred by *P. indica* is not proportional to stress severity. Moreover, generally speaking, it revealed that the antioxidant enzyme activities were not stimulated by *P. indica* against B toxicity. Soil sterilization was not required to reveal the beneficial effects of *P. indica* suggesting that *P. indica* was not suppressed by the native soil microflora. Inoculation with *P. indica* helped wheat plants by alleviating cell membrane damage but not necessarily by decreasing toxic B concentrations in the shoot or improving the concentrations of potentially limiting vital plant nutrients such as P.

Regarding the third pot trial, both *P. indica* and AM fungi were implemented to comparatively reveal their potential effects of wheat plants under B toxicity with P supplementation in sterile soil which has not been studied so far. It was reported that both AM fungi and *P. indica* did not affect either positively or negatively on shoot dry matter in both tested cultivars. Intriguingly, the shoot B and P concentrations in both cultivars were not affected by AM fungi. Inoculation with *P. indica* did not

cause changed P uptake in B-tolerant Nusrat; however, improved in Saricanak-98. Besides that, irrespective of B toxicity, although the chlorophyll biosynthesis was improved by *P. indica* inoculation, was not affected by AM fungi. The carotenoid biosynthesis in both tested cultivars was improved in both AM fungi and *P. indica* inoculated plants. Symbiotic relationships of AM fungi and *P. indica* helped host plants via alleviating cell membrane damage caused by B toxicity. APX enzyme activities of both tested experimental cultivars were positively affected in the presence of *P. indica* as well as AM fungi, irrespective of B conditions.

In contrast to AM fungi, since the production of *P. indica* can be relatively easily carried out on an industrial scale, it can be suggested that this fungal may be practically applicable endophyte as a biofertilizer for sustainable agriculture under adverse soil conditions, such as B toxicity. In the final accounting, more investigations are, however, required to validate the potential of both *P. indica* and AM fungi under field conditions.

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BIOGRAPHY

He commenced on a bachelor's (BSc) in 2008 in Firat University Biology department. While studying for his BSc, he developed a keen interest in plant physiology and nutrition (i.e., crop plants) and he found it very intriguing and exciting, specifically its influence on today's World. After graduating, he embarked on a master's degree (MSc) in 2014 in the scope of a project in plant science which was completed in December 2016 in the Biology Department of Düzce University. In this manner, he studied the effect of aluminium toxicity on *Bryophyllum Daigremontianum*, which is a medicinal plant as well, by using physiological methods and molecular markers (RAPD and ISSR). He also worked on a project which was financially supported by TÜBİTAK during his MSc period. Thanks to the progress of his BSc and MSc, it is glaring that studying and doing research are endeavours, thus he wanted to engage in even more. With the advent of his MSc graduation, he embarked on his Ph.D. in the areas of plant physiology and nutrition at the Biotechnology Institution of Gebze Technical University in 2017. To do so, his Ph.D. project and studies had financially been supported by YÖK as well as TÜBİTAK until his scientific works were finalized. Throughout his Ph.D., he used the endophytic fungus *P. indica* as a biological agent to improve the boron toxicity tolerance in two different wheat cultivars in greenhouse conditions. After finishing the project in which he had gotten involved, he was qualified with ERASMUS grant to broaden his perspective and gain different points of view, thus he went to Germany as a trainee for three months in 2022 so as to work with another research group who were also studying with *P. indica* at the Institute of Phytopathology of Justus Liebig University Giessen, Germany. As of this writing, he is also qualified with another ERASMUS grant and has received an invitation from the Department of Agricultural, Food and Environmental Sciences of Marche Polytechnic University, Italy. He has been working as an English teacher at a private course for over a year to feel financially secure, thereby fulfilling his scientific desire.

PUBLICATIONS AND PRESENTATIONS FROM THE THESIS

Kaval A., Yılmaz H., Tunca Gedik S., Yıldız Kutman B., Kutman Ü. B., (2023), “The Fungal Root Endophyte *Serendipita indica* (*Piriformospora indica*) Enhances Bread and Durum Wheat Performance under Boron Toxicity at Both Vegetative and Generative Stages of Development through Mechanisms Unrelated to Mineral Homeostasis”, *Biology*, 12, 1098.

