

EFFECTS OF MONO- AND INTERCROPPING ON GROWTH AND BORON UPTAKE OF WHEAT PLANT (*TRITICUM AESTIVUM* L.) CULTIVATED ON BORON-CONTAMINATED MEDIA

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Abstract

Wheat plant (*Triticum aestivum* L.) was grown as main crop while clover (*Medicago sativa* L.) and sunflower (*Helianthus annuus* L.) were used as intercrops. Boron was applied in the form of Boric Acid (25, 50, & 75 mg L⁻¹). The amount of chlorophyll, carotenoid, proline, B, Ca, K, and P were determined together with % DW and % EC. % DW, total chlorophyll, and carotenoid contents in monocrop and intercrop leaves grown wheat plants decreased in comparison with the control group. However, increases in mentioned parameters were observed in wheat-clover intercrops. Additionally, % EC values and proline contents in the leaves of mono-cultivated wheat plant were higher compared to the control group. Intercropping of wheat plant with clover and sunflower resulted in decreased values. Furthermore, element contents of the mono-cultivated wheat leaves decreased while these values increased when intercropping with clover.

Key words: Boron, Clover, Intercropping, Monocropping, Sunflower, Wheat

Introduction

Intercropping (IC) – a cultivation system where two or more crops grow together on the same field till complete their life cycles (Cecilio Filho, Rezende, & Costa, 2010). Sustainable and productive crops can be obtained using IC – one of the agricultural production systems. Compared to monoculture, IC reduces the amount of soil erosion (Iijima, Izumi, Yuliadi, & Ardjasa, 2004). It has been advocated as an innovative farming approach. However, it has been avoided due to the planting and harvesting complications. It involves a competition for light, water and nutrients. On the other hand, it is beneficial due to higher light interception, more soil root contact, increased microbial activity and deterrent act to pests and weeds on the co-crop (Geren, Avcioglu, Soya, & Kir, 2008).

To a great extent, IC may increase unit/area and unit/time production without affecting the production of main crop when legumes are used as intercrops. They fix atmospheric nitrogen through the leaf litter to improve soil fertility. Further, efficient utilization of nutrients, moisture, space and solar energy is also possible through mixed or IC (Hugar, 2006)]. It can conserve soil and improve soil fertility (Jeranyama, Hesterman, Waddington, & Harwood, 2000) in addition to effective use of natural resources (Horwith, 1985), and pest and disease control (Theunissen, 1997). IC patterns are more effective, with cons and pros, than monocropping to suppress weeds (Girjesh & Patil, 1991).

Nowadays agricultural production systems are focused on how to utilize resources to increase crop production by ICs as compared to the monocropping systems (Li *et al.*, 2001). With few exceptions, literature reflects that intercrops gave higher yields than monocrops (Willey, 1979).

Wheat (*Triticum sativum* L.) is highly consumed food and feed (Pingali, 1999). Sunflower (*Helianthus annuus* L.), the second major crop, has been recognized as one of

the potential substitutes for the traditional vegetable oil sources (oil palm and groundnut) in the tropics (Ogunremi, 2000). Clover (*Medicago sativa* L.) is an important feed for livestock that grows globally (Tekeli, Ates, & Varol, 2005).

Physiological tasks of almost all plants, including economically important cereals, are susceptible to abiotic stresses e.g. high temperature, oxidative stress, drought, salinity, boron etc. All the stated stresses may reduce their biosynthetic capacity and cause certain damage that will result to destroy the plant (Kalefetoğlu & Ekmekçi, 2005). Furthermore, high boron accumulation in leaf may reduce the bio-chemical capacity of plant cells to resist photo-oxidative damage that can further lead to osmotic imbalances that would increase membrane permeability, proline contents, peroxidation of lipids (Reid, 2007).

Generally plants require low boron content, so a slight increase can affects the plant development adversely; and stops the growth in most situations (Eraslan, Inal, Gunes, & Alpaslan, 2007; Taban & Erdal, 2000). Plants normal nutrition contains 25-100 mg kg⁻¹ boron. Although Robinson *et al.* (1983) have described a 20 mg kg⁻¹ of boron in dry matter content of the plant as critical, there are notable variations between the boron contents in different plants. Boron contents of plants are closely related to the amount of useful boron found in nutritive media of plants. Cereals, such as barley and wheat, are sensitive to boron and monocotyledons contain 20-70 mg kg⁻¹ of boron. Wheat can tolerate boron deficiency up to 2 mg kg⁻¹ in its nutritive environment, while excess boron has a negative influence on its growth (Reid, 2013).

Plants encounter numerous stress factors in the course of their lives. The effects of these stress factors on plants generally take place simultaneously. Abiotic stress is considered the main reason of product losses in vegetative production worldwide. One of the abiotic stress factors is boron toxicity (Yagmur, 2008). Boron toxicity causes oxidative damages in addition to its damages on

the growth, development, and membrane permeability. This can be explained with its direct effect on chloroplast membranes causing stomas to close (Karabal, Yücel, & Öktem, 2003).

The study was aimed to determine the effects of IC on boron uptake of wheat plants that are cultivated on boron-contaminated soils. Clover and sunflower were used as intercrops. In this study, clovers and sunflowers were planted alongside the wheat (intercropping) to reduce the boron uptake of wheat plant cultivated on boron-contaminated soils, and conducted analysis have demonstrated some relative differences in boron uptake of wheat.

Materials and Methods

Culture and treatment: A pot experiment was performed at the Biology Department, Mugla University. All experiments were repeated in triplicates based on trial plot in line with randomly blocks design by growing wheat as a monocrop (MC) and intercropping (IC). Plants were grown for 3 month under controlled conditions at average day (25°C) and night (18°C) temperatures. The pot treatments were wheat/sunflower, wheat/clover, strip intercropping, and sole wheat. Table 1 shows various treatments with different boron concentrations with their code names.

Ten wheat, five sunflower and twenty clover seeds were sowed directly in plastic pots containing 20 L perlite and peat mixture (1:3). After germination, pots were thinned to six wheat, two sunflower and fifteen clover seedlings per pot.

Hoagland and Arnon formulation was used as the basic nutrient mixture that contains (mg L⁻¹): N (270), P (30), K (240), Ca (200), S (60), Mg (50), Fe (3), Mn (0.5), B (0.5), Cu (0.02), and Zn (0.05). Nutrient mixture's pH was adjusted to 6.5 with 0.1 mmol/L potassium hydroxide. During the entire growth period, plants were treated with water and the stated nutrients. Boric acid was applied at three different concentrations i.e., 25, 50 and 75 mg L⁻¹; repeated each 2 week in triplicate where each sample consists of 10 pots (i.e., 30 pots in each treatment). The cultivated plants were harvested after 90 days of seedling.

Dry weight (DW) and macro nutrient analysis: Three random plants were selected from each replicate were grouped as leaves, stems and roots. Dry weight was determined by drying them inside forced air oven at 70°C for 2 days (analyzed on dry weight basis). Dried samples were grinded into powder by pestle & mortar and stored

in plastic bottles. Fresh samples were washed out at 550°C for six hours. Ash was mixed with in 5 mL hot HCl (2 mol/L), filtered and diluted to 50 mL with distilled water, followed by B, Ca, K and P determination by ICP-AES (Inductively Coupled Plasma-Atomic Emission Spectroscopy) (Chapman & Pratt, 1982).

Membrane permeability (EC): After harvest, 1 cm diameter disks were removed from leaves of each plant. Disks were washed with distilled water; twenty of them were put into brown glass bottles containing 10 mL distilled water. After shaken in shaker for 24 h, solutions from the bottles were transferred into tubes to determine C₁ values by EC meters. Solutions were again autoclaved at 120°C for 20 min followed by C₂ measurements at room temperature. Membrane permeability was calculated as (C₁/C₂) × 100 (Lutts, 1996).

Proline analysis: 500 mg of fresh leaf sample was grinded together with 3 % 5-sulfo-salicylic acid and filtered. 2 mL of filtrate was mixed with 2 mL acetic acid and 2 mL ninhydrin. Ninhydrin was prepared using ninhydrin, acetic acid and orthophosphoric acid. The samples were placed in tubes in a water bath for 1 h at 100°C, then in ice. This fraction was extracted with 4 mL and subjected to UV/visible spectrophotometer at 520 nm. Results were obtained by comparing it with proline standards (Bates, Waldren, & Teare, 1973).

Chlorophyll and carotenoid analysis: Out of each replicate, a plant was selected to determine the contents of chlorophyll and carotenoid. Surface contamination was washed using distilled and deionized water before extraction. Fresh and full expanded leaves were extracted to determine chlorophyll and carotenoid. For this purpose, 1 g leaf sample was grinded in 90% acetone 6. This crude extract was subjected to absorbance determination by UV/visible spectrophotometer.

Statistical analysis: This study used a randomized complete block design. Each pot was considered an experimental unit. Each treatment was repeated three times. Statview-ANOVA test was used for statistical analysis. LSD test ($p \leq 0.05$) was used to compare statistically different groups. The mean values with the \pm SD rates are given in table 2.

Table 1. Wheat intercropped with various co-crops at different amount of boron.

S. No.	Crop combination	Amount of boron (mg kg ⁻¹)	Code name
1.	Wheat only	Control*	W
2.	Wheat only	25**	W25
3.	Wheat and sunflower	25	WS25
4.	Wheat and clover	25	WC25
5.	Wheat only	50	W50
6.	Wheat and sunflower	50	WS50
7.	Wheat and clover	50	WC50
8.	Wheat only	75	W75
9.	Wheat and sunflower	75	WS75
10.	Wheat and clover	75	WC75

*Nutrient solution and irrigation water only

**Boron concentration as (mg L⁻¹) H₃BO₃

Table 2. Boron (B), calcium (Ca), potassium (K) and phosphorus (P) contents in leaves of wheat plants grown under MC and IC systems.

Treatments	Boron (B) (mg L ⁻¹)	Calcium (Ca) (mmol L ⁻¹)	Potassium (K) (mmol L ⁻¹)	Phosphorus (P) (mmol L ⁻¹)
W	10.2 ± 2.02 ⁱ	86.3 ± 0.88 ^c	116.3 ± 0.88 ^h	215.3 ± 1.20 ^e
W25	189.3 ± 2.02 ^f	89.3 ± 1.45 ^{bc}	118.6 ± 1.45 ^g	246.7 ± 0.88 ^b
WC25	120.2 ± 1.76 ^h	57.7 ± 1.45 ^f	126.1 ± 1.45 ^e	134.3 ± 1.20 ^g
WS25	110 ± 2.60 ⁱ	88.7 ± 1.45 ^{bc}	109.8 ± 1.45 ⁱ	232.7 ± 1.45 ^c
W50	230 ± 2.40 ^d	92.7 ± 1.45 ^b	125 ± 0.57 ^f	221 ± 1.52 ^d
WC50	182.7 ± 1.15 ^g	92.3 ± 1.45 ^b	130 ± 1.15 ^d	205.3 ± 2.60 ^f
WS50	196.7 ± 1.15 ^e	103.7 ± 1.45 ^a	141.9 ± 1.45 ^b	247.3 ± 1.45 ^b
W75	502.4 ± 2.33 ^a	73.7 ± 1.45 ^e	114.1 ± 1.20 ⁱ	204.7 ± 0.88 ^f
WC75	293.3 ± 2.64 ^c	86.3 ± 1.20 ^c	151.4 ± 1.76 ^a	247.3 ± 1.76 ^b
WS75	305.7 ± 0.88 ^b	80.3 ± 1.45 ^d	137.3 ± 1.45 ^c	257.3 ± 1.45 ^a

Values followed by different letters in each column differ significantly (LSD test, p<0.05)

Results

% DW content: The treatment carried out with 25 mg L⁻¹ yielded 15, 20, and 15% DW values for monocrop wheat, wheat-clover intercrop and wheat-sunflower intercrop, respectively. % DW values of wheat plants, cultivated on boron-contaminated soils, were found higher in inter-cultivated plant groups (Fig. 1a).

Effect of boron on membrane permeability:

Evaluation of wheat (cultivated under boron toxicity) leaves % EC revealed a clear difference between MC and IC plants. For all three boron treatments (25, 50, and 75 mg L⁻¹), % EC values of monocrop wheat leaves were higher than that of clover and sunflower wheat IC (Fig. 1b). % EC values of W75, WC75, and WS75 ICs were 60, 55, and 52 %, respectively. It indicates that the % EC decreased significantly when IC method was applied –a positive correlation between % EC values and boron. However, % EC values obtained from MC wheat leaves were higher than that of wheat intercropped with both clover (WC25, WC50, WC75) and sunflower (WS25, WS50, WS75) (Fig. 1b).

Proline concentrations: Proline content showed an increase in MC wheat when boron concentration was increased. However, intercropping of wheat has led to a significant decrease in proline contents (49 nmol/g FW) in leaves in of W25. This ratio declined by 23 % to 38 (nmol/g FW) in case of WC25. Similar results were also obtained at 50 and 75 (mg L⁻¹) boron concentrations (Fig. 1c).

Total chlorophyll (Total Chl) and carotenoid (Car) content:

Highest decrease was observed in W75 i.e., 47%, followed by 45% in in WS75 (Fig. 1d). Unlike higher boron concentrations i.e. 50 and 75 mg L⁻¹, low boron concentrations (25 mg L⁻¹) were not significant in all of the intercropped samples in case of Car. Car content in W50 and WC50 were found as 7.2 and 12.6 (µg/g FW) in the leaves of MC (Fig. 1e). This difference represents an increase of 75%.

Boron, calcium, potassium and phosphorus concentrations:

As boron concentrations applied in our study increased, boron uptake of MC wheat plants increased accordingly. A correlated increase in B contents has been observed on leaves of the plant (Table 1). In W50, Ca contents were higher compared to W25; although Ca content of W75 was also lower. High concentration of boron affected the Ca uptake of monocrop W75. However, WC75 Ca uptake was more by 17%. A similar situation was also observed with K uptake. In this case, Ca uptake of WC75 increased by 33% (Table 1).

In this study, we assessed the effects of three different boron treatments on certain nutrients (Table 2) in wheat leaves and observed decreases in Ca, K and P contents of monocrop wheat leaves. However, the contents of these nutrients increased when the wheat plants were intercropped with clover (Table 1).

Discussion

% DW content: Applied boron treatments of 25, 50, 75 mg L⁻¹ decreased the % DW values of all groups compared to control. Nonetheless, % DW ratio in the monocrop wheat leaves was lower compared to the % DW ratio of clover-wheat and sunflower-wheat intercrops at all three boron concentrations. These results reflect an important difference between dry matter contents of mono-cultivated and poly-cultivated wheat plants, depending on the growing technique. According to Baykal & Oncel (2007), dry matter contents of the wheat shoots, grown under boron toxicity, decreased when boron content was increased. Habtamu *et al.* (2014) has reported that the length of root and shoot, root and shoot (fresh and dry weight) and seedling vigor index can decrease boron beyond 0.25 mg/L. Phytotoxicity increased significantly with increase in the concentration of boron in the germinating medium. For two years, Song *et al.* (2007) has studied monocrop wheat and wheat-corn intercrop. They found per annum 24-26 % production increase after IC. Inal *et al.* (2007) found that the dry matter content of peanut-maize intercrop is lower compared to the dry matter of monocrop. On the other hand, they also found that inter-cultivated plants are healthier and did not show iron deficiency symptoms. According to the studies mentioned above, the data obtained from our study are consistent with the literature.

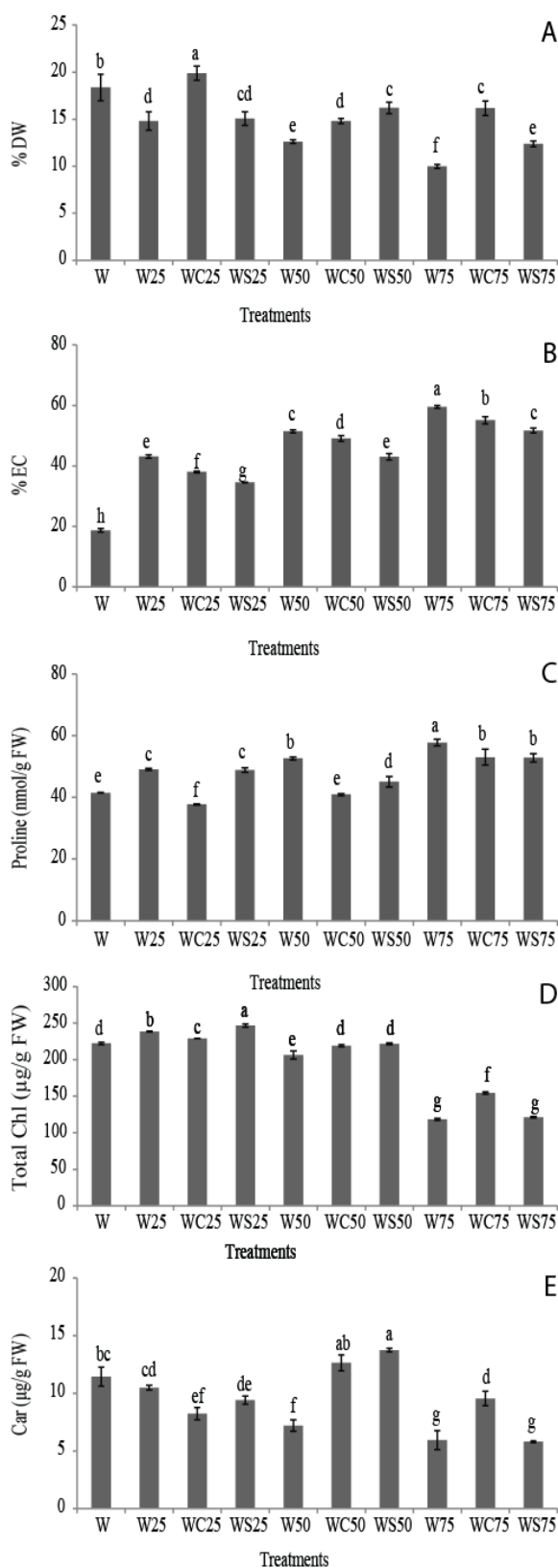


Fig. 1. Effects of boron treatments on: a) % DW; b) % EC; c) Proline contents; d) Total chlorophyll contents; and e) Total carotenoid contents of wheat plants grown under MC and IC system.

Effect of boron on membrane permeability: One of the parameters examined in this study, % EC also known as membrane permeability, can be defined as ion instability caused by the intracellular and extracellular osmotic inconsistency developed under salt and water stresses. This test provides also valuable information about membrane stability and relative ion contents in apoplastic regions (Ghoulam, Foursy, & Fares, 2002; Munns, 2002).

According to Karabal *et al.* (2003), % EC values tend to increase after boron treatments. Gunes *et al.* (2006) also found an increase in % EC when boron was applied to grape plants. The stated analysis reveals a positive correlation between the % EC and an increase in MDA (Malondialdehyde) due to an increase in membrane permeability in the leaves of plants under stress. MDA ratio of melon leaves intercropped with rice was also found lower compared to that of monocrop melon by almost 300%.

Proline concentrations: Proline accumulation in plants under biotic and abiotic stress is an indication of adaptation. Increase in proline concentration is one of the most common responses of plant metabolism to dehydration and cellular osmotic regulation (Cramer *et al.*, 2007; Delauney & Verma, 1993).

10 mmol of boric acid on roots and leaves of durum wheat and bread wheat has led to an increase in proline concentration (Selcuk, 1999). In our previous study, we have also found that tomato leaves treated with different boron concentrations and pesticide increased proline amounts compared to control samples. These findings also support our present study.

Total chlorophyll (Total Chl) and carotenoid (Car) content: Carotenoid contents of MC wheat were found inversely proportional to boron concentrations. Unlike higher boron concentrations i.e. 50 and 75 mg L⁻¹, low boron concentrations (25 mg L⁻¹) were not significant in all of the intercropped samples in case of Car. Car content in W50 and WC50 were found as 7.2 and 12.6 (µg/g FW) in the leaves of MC (Fig. 1e). This difference represents an increase of 75%.

A reduction has been observed in total Chl and Car amounts in the leaves of MC and IC wheat while a distinct increase has occurred in chlorophyll content in WC75 (Fig. 1d and 1e). It means at high boron concentration; IC has a positive influence on Chl content of wheat; as it reduces plant boron uptake. We found an adverse effect of boron on photosynthetic metabolism of plants when it exceeded the limits required for the growth and development of the plant. Similar to our results, Wang *et al.* (2014) have also detected an important decrease in photosynthetic pigments of pear plant exposed to boron stress. Zuo *et al.* (2000) studied Fe nutrition and Chl content of peanut IC with corn and found that Chl content of the young peanut leaves increased significantly in comparison with MC of peanut.

Boron, calcium, potassium and phosphorus concentrations: Boron contents in leaves of MC wheat plants increased with increasing boron concentrations. The comparison of boron concentrations in leaves of MC and IC plants revealed remarkable results. For all three

boron concentrations, boron contents in leaves of inter-cultivated plants showed important decrease. As boron concentrations applied in our study increased, boron uptake of MC wheat plants increased accordingly. Furthermore, a correlated increase in B contents has been observed on leaves of the plant (Table 1). Meanwhile, boron uptake of intercrop wheat plant decreased substantially. According to Atalay (2003), an increase in boron content in the root and stem occurred when amount of boron was increased in the fertilizer. Similar results were also reported by Nable & Paull (1990) during the study of wheat and barley plant tissues.

In W50, Ca contents were higher compared to W25; although Ca content of W75 was also lower. High concentration of boron affected the Ca uptake of monocrop W75. However, WC75 Ca uptake was more by 17%. A similar situation was also observed with K uptake. In this case, Ca uptake of WC75 increased by 33% (Table 1).

P contents in leaves of W25 was increased, but decreased at high boron concentrations. In this study, notable results were produced by WC, again. Compared to W, P contents decreased significantly in WC25 and W50. However, WC75 produced strange results. Compared to W, an increase of P contents was observed in IC wheat leaves (Table 1).

In this study, we assessed the effects of three different boron treatments on certain nutrients (Table 2) in wheat leaves and observed decreases in Ca, K and P contents of monocrop wheat leaves. However, the contents of these nutrients increased when the wheat plants were intercropped with clover (Table 1). Inal *et al.* (2007) compared the P, K, and Ca contents of MC corn and corn-peanut IC and reported that leaves of the corn intercropped with peanut contain more P and K, although their Ca content was slightly lower. Li *et al.* (2001) studied mono and inter-cultivated wheat and maize and evaluated P and K contents in leaves. Researchers asserted that intercropping of maize and wheat increased their P and K uptakes by 50 % compared to mono-cultivated wheat and maize.

Conclusion

This is notable intercropping study that involves boron as a stress factor in growth media. One of the important finding of this study is inverse behavior of boron uptake by wheat plants in respect to macro element uptake. Wheat plants grown under IC system uptake less boron while more macro elements. Growth of the wheat plant and other biochemical parameters also reflects a connection with the stated finding. Moreover, the data from this study suggest that intercropping can enhance growth, development, and nutrition of plants, regardless of whether any stress factor is present.

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