

## Foliar Boron Spray for Improved Yield, Oil Quality and Water Use Efficiency in Water Stressed Sunflower

(Semburan Boron Daun untuk Hasil yang Lebih Baik, Kualiti Minyak dan Kecekapan Penggunaan Air dalam Bunga Matahari Bertekanan Air)

MUHAMMAD ASIF SHEHZAD\*, MUHAMMAD MAQSOOD, TASAWER ABBAS & NAVEED AHMAD

### ABSTRACT

*Boron (B) is a mineral considered essential for improving sunflower (*Helianthus annuus L.*) resistance to drought. B supplements (0, 15-, 30- and 45 mg L<sup>-1</sup>) under well-watered and variable water deficit levels (64 and 53 mm irrigation depths) were evaluated for their effects on growth, oil quality and water use efficiency (WUE) in a field study for two consecutive years (i.e. 2011 and 2012). The duration of 50% inflorescence emergence, 50% flowering and 50% maturity stages were reduced with increasing moisture stress. All B application rates improved sunflower growth compared to no B control treatment. The moisture deficit treatments of 64 and 53 mm irrigation depths significantly ( $p < 0.05$ ) reduced the yield-related components. Achenes/head, achenes weight and achene yield under water stress conditions were considerably improved by foliar application of B at 30 mg L<sup>-1</sup>. An increase in protein contents and a decrease in oil contents were observed with B foliar application under moisture deficit treatments. Foliar application of B (30 mg L<sup>-1</sup>) on water stressed plants also resulted in increased WUE. The highest net benefits were achieved with B concentration of 30 mg L<sup>-1</sup> under well-watered and mild deficit water level of 64 mm irrigation depth. The highest application rate of B (45 mg L<sup>-1</sup>) gave the best results at the most severe water deficit level. In conclusion, the B rates of 30 and 41 mg L<sup>-1</sup> performed best for improving drought tolerance in terms of higher sunflower productivity under mild and higher water deficit conditions.*

*Keywords: Boron; fatty acids; Helianthus annuus; water deficit; water use efficiency*

### ABSTRAK

*Boron (B) adalah mineral yang dianggap penting bagi meningkatkan rintangan bunga matahari (*Helianthus annuus L.*) kepada kemarau. B tambahan (0, 15, 30 dan 45 mg L<sup>-1</sup>) paras defisit air baik dan berubah-ubah (64 dan 53 mm kedalaman pengairan) telah dinilai untuk melihat kesannya terhadap pertumbuhan, kualiti minyak dan kecekapan penggunaan air (WUE) dalam kajian lapangan selama dua tahun berturut-turut (2011 dan 2012). Peringkat tempoh 50% kemunculan perbungaan, 50% perbungaan dan 50% matang telah dikurangkan dengan meningkatkan tekanan lembapan. Semua kadar penggunaan B meningkatkan pertumbuhan bunga matahari berbanding rawatan tanpa kawalan B. Rawatan kekurangan lembapan dengan kedalaman pengairan 64 dan 53 mm ( $p < 0.05$ ) dengan ketaranya mengurangkan hasil berkaitan komponen. Aken/kepala, berat aken dan hasil aken dalam keadaan bertekanan air telah bertambah baik dengan penggunaan daun B pada 30 mg L<sup>-1</sup>. Peningkatan kandungan protein dan penurunan kandungan minyak diperhatikan dengan penggunaan daun B pada rawatan defisit lembapan. Penggunaan daun B (30 mg L<sup>-1</sup>) pada tumbuh-tumbuhan bertekanan air telah meningkatkan WUE. Faedah bersih tertinggi dicapai dengan kepekatan B 30 mg L<sup>-1</sup> dengan paras air baik dan sederhana defisit pada kedalaman saliran 64 mm. Kadar aplikasi tertinggi B (45 mg L<sup>-1</sup>) memberikan hasil terbaik pada peringkat defisit air paling teruk. Kesimpulannya, kadar B pada 30 dan 41 mg L<sup>-1</sup> adalah terbaik untuk memperbaiki toleransi kemarau daripada segi produktiviti bunga matahari dalam keadaan defisit air sederhana dan tinggi.*

*Kata kunci: Asid lemak; boron; defisit air; Helianthus annuus; kecekapan penggunaan air*

### INTRODUCTION

Being an essential micronutrient, boron (B) is necessary for plant growth and development. B is important for the support of cell wall synthesis and for structure, protein and carbohydrate metabolism, nucleic acid metabolism, phenol metabolism, indole acetic acid (IAA) metabolism, sugar transport and seed filling, membrane functions and integrity (Dordas & Brown 2005; Rashid et al. 2004). The impact of B on carbohydrates assimilation and transformation was examined by Yan et al. (2003) who

suggested that translocation of soluble sugars was not hindered by B application, but that B deficiency during the reproductive phase lead to poor seed setting. In fact, drought is a major cause leading to B deficiency in many plant species. Extreme water scarcity at different stages of crop development significantly reduces the crop yield (Broschat 2005; Sajedi et al. 2009) by diminishing the leaf area, availability of nutrients, photosynthesis, protein synthesis, respiration, leaf chlorophyll contents, nucleic acid synthesis and by slowing down the rate of expansion

and cell division due to loss of turgor pressure. Lowering the water potential outside the cell by just -0.1 MPa results in a noticeable decline in cellular growth in terms of slow root and shoot growth ultimately affects the synthesis of cell wall (Demir et al. 2006; Jaleel et al. 2007). Inner water status in sunflower plants is considered more detrimental to its productivity in their reproductive stage than in their vegetative phase. Sunflower has great potential in many countries, such as Pakistan, in helping to meet the demand for a supply of edible oil. Its oil contains lecithin, tocopherols, carotenoids, waxes and vitamins that are very helpful in reducing cholesterol levels in the blood (Imran et al. 2011; Mukhtar 2009). However, shortage of irrigation water in Pakistan is a severe environmental constraint to sunflower productivity. In Pakistan, the Indus Basin Irrigation System is no longer meeting the demand for water because of increasing cropped areas and drought over recent years. Per capita water availability has declined from 5260 m<sup>3</sup> to 1066 m<sup>3</sup> and surface accessible water from 103 million acre feet (MAF) at canal heads declined to 34 MAF for normal crop production (Ali et al. 2013). One limitation to growth of sunflower under drought conditions is caused by B deficiency. Boron's mobility and thus the plant's ability to take up B are greatly reduced by drought conditions (Hajiboland & Bastani 2012). To this, the present study was designed to examine the effect of B foliar spray for improving growth, yield and water use efficiency (WUE) of sunflower under water deficit conditions.

## MATERIALS AND METHODS

### WORK PLAN

The proposed field study was conducted at the Agronomic Research Area, Department of Agronomy, University of Agriculture Faisalabad, Pakistan for two consecutive growing seasons of 2011 and 2012. Mean monthly air temperature, rainfall and relative humidity were recorded at the experimental site during crop development (Figure 1). Before planting, soil samples were analyzed in both years from the top soil of 15 cm to determine their physical and chemical characteristics. Physical and chemical properties were not much different between these 2 years. Percent sand (56.42% and 54.28%), silt (20.21% and 21.55%)

and clay (23.37% and 24.17%) values were in 2011 and 2012, respectively. Furthermore, 0.87% and 0.82% organic matter, 1.23 and 1.26 dS m<sup>-1</sup> EC, 8.0 and 7.8 pH, 33.76% and 35.09% SP, 0.059% and 0.054% nitrogen, 6.53 and 6.87 mg kg<sup>-1</sup> phosphorus, 177 and 183 mg kg<sup>-1</sup> potassium and 0.43 and 0.40 mg kg<sup>-1</sup> B values were observed in 2011 and 2012, respectively.

The experiment was arranged in a randomized complete block design with a split plot arrangement. Irrigation levels were randomly assigned to main plots while foliar applied B levels were randomly assigned to sub-plots. An individual plot size was 7.0 × 3.0 m. All treatments were replicated three times. There were three irrigation levels namely WD<sub>0</sub> (having 75 mm irrigation depth, well-watered), WD<sub>1</sub> (having 64 mm irrigation depth) and WD<sub>2</sub> (having 53 mm irrigation depth). Foliar application of B in boric acid (H<sub>3</sub>BO<sub>3</sub>) form, in distilled water, at concentrations of 0, 15, 30 and 45 mg L<sup>-1</sup> were applied at the inflorescence emergence, flowering and seed filling stages of sunflower (*Helianthus annuus* L. 'hybrid Hysun-33'). Tween-20 at 0.01% was used with the spray solution as a surfactant. Rauni and first irrigation was applied without any deficit treatments. Deficit irrigation treatments were applied from the time of inflorescence emergence and then throughout the growing season until maturation of the crop. Total four irrigations except rauni were provided for each growing season to mature the sunflower crop. A measured quantity of water was applied by manual labor with the help of a fountain bucket fitted with a shower head. The required amount of water for 75, 64 and 53 mm depth was calculated as; Plot area = 7 m × 3 m = 21 m<sup>2</sup>, Depth of irrigation = 75 mm = 0.075 m, volume of water required = 21 m<sup>2</sup> × 0.075 m = 1.575 m<sup>3</sup>, volume of bucket = 0.01175 m<sup>3</sup>. Buckets required for 75, 64 and 53 mm depth were 134, 114 and 95 buckets plot<sup>-1</sup>, respectively.

Sunflower seeds were planted on ridges during 1st week of August for both growing seasons using a seed rate of 6 kg ha<sup>-1</sup> and by keeping a specific planting geometry of 75 × 20 cm (rows were 75 cm apart and then plants within a row were 20 cm apart). NPK fertilizer was applied at a rate of 112-57-62 kg ha<sup>-1</sup>, respectively. Urea, di-ammonium phosphate (DAP), potassium sulphate and boric acid were used as a source of nitrogen, phosphorus, potassium and B, respectively.

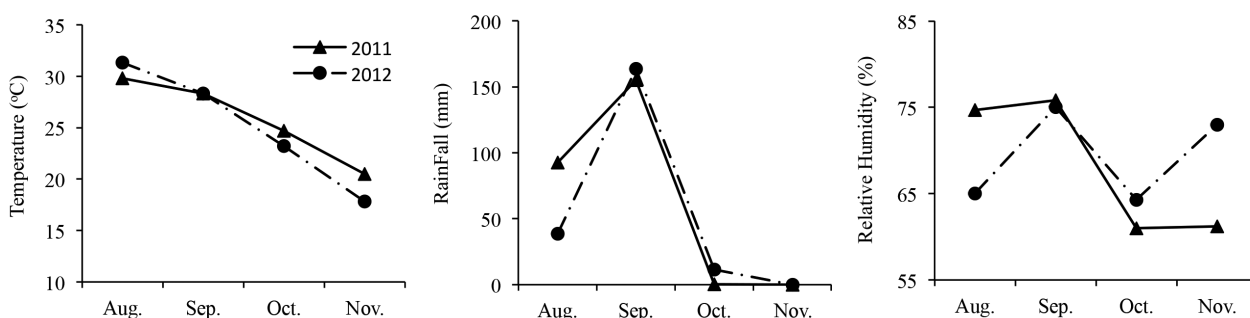


FIGURE 1. Meteorological conditions recorded during sunflower cropping seasons of 2011 and 2012

The entire amount of phosphorus, potassium and one-third of the nitrogen was applied at the time of sowing by band placement. The remaining two-third nitrogen was applied in two splits with one-third at first irrigation and the remaining one-third at flowering stage. Plant protection measures were implemented to keep crop free of insect pests, diseases and parrots. The crop was harvested on 3rd week of November during both cropping seasons.

#### MEASUREMENTS

Attributes regarding sunflower phenology measured were days to 50% inflorescence emergence, 50% flowering and 50% maturity and were determined by counting the days from sowing up to the day when five of ten tagged plants exhibited just visibility of inflorescence among youngest leaves, completely opened flowers and attained full maturity, respectively.

After manual threshing, the heads of 10 randomly chosen plants were separated and total achenes in these heads were counted, then averaged per head. To compute the 1000-achene weight, each of 1000-achenes from the seed lot of five samples per plot was randomly selected and their weight recorded. Weight of five randomly selected plants were recorded after air-drying plants (except achenes) and then converting to  $\text{kg ha}^{-1}$ . The recorded weight was then added to the already calculated achene yield ( $\text{kg ha}^{-1}$ ) to calculate the biological yield. After harvesting the mature plants, the heads were separated, sun-dried and threshed manually which provided the achene yield per plot. The plot yield was calculated at 10% seed moisture content (ASAE 1977) and then converted to  $\text{kg ha}^{-1}$ .

Achene oil contents were determined by Soxhlet extraction method (AOAC 1990). Percent oil content was calculated as follows;

$$\% \text{ oil} = \frac{(\text{weight of flask} + \text{oil}) - \text{weight of flask}}{(\text{weight of flask} + \text{seed}) - \text{weight of flask}} \times 100.$$

Nitrogen in achenes was determined according to Kjeldhal method (Bremner 1964). Percent crude protein was calculated using the formula;

$$\% \text{ crude protein} = (V_1 - V_2) N / 100W \times 14 \times 6.25 \times 100,$$

where  $V_1$  is the sample titration volume (mL);  $V_2$  is the blank titration volume (mL);  $N$  is the normality of standardized  $\text{H}_2\text{SO}_4$ , and  $W$  is the sample weight.

Fatty acid composition (stearic acid, palmitic acid, oleic acid and linoleic acid) in sunflower oil was determined by gas liquid chromatography (GLC) as described by Martin (1979). WUE was calculated by dividing the achene yield by the total water applied.

#### STATISTICAL ANALYSIS

The data collected were analyzed by Fisher's analysis of variance technique (Freed & Eisensmith 1986). Least

significant difference test was used to compare the differences among treatments means when the treatment effect was found to be significant at  $p < 0.05$ . Regression analysis was also done for sunflower yield, using models as a function of B rates with highest level of significance.

#### RESULTS

The days to achieve 50% inflorescence emergence, 50% flowering and 50% maturity were greater under well-watered conditions than under water stress conditions. The effect of water stress to significantly reduce the length of crop growth stages occurred in both years of the experiment. For all growth stages, the differences between the water stress treatments were significant ( $p < 0.05$ ), i.e. the  $\text{WD}_0$  (well-watered) treatment was different from the  $\text{WD}_1$  treatment (64 mm irrigation depth) that was different from the  $\text{WD}_2$  treatment (53 mm irrigation depth) (Table 1). In 2011, foliar applied B at  $30 \text{ mg L}^{-1}$  rate increased the days to inflorescence emergence compared to simple spray of water. In 2012, all rates of foliar applied B increased days to inflorescence emergence compared to water control. Among the various B rates, the 30 and  $45 \text{ mg L}^{-1}$  concentration rates gave similar results for extending the days to flowering and maturity in both years. The lowest level of  $15 \text{ mg L}^{-1}$  for B was not as affective in improving the time to 50% inflorescence emergence, flowering and maturity of sunflower (Table 1).

Pooled data on achenes per head showed that well-watered plants produced the maximum number of achenes as compared to the water-stressed plants. Under well-watered and 64 mm irrigation depth conditions, B foliar application of  $30 \text{ mg L}^{-1}$  yielded greater achenes for each head compared with water control during both study years. The B level of  $45 \text{ mg L}^{-1}$  was statistically similar with the  $30 \text{ mg L}^{-1}$  B rate for improving the number of achenes per head under irrigation depth of 53 mm in both years (Table 2). A strong positive response to B was observed for the 1000-achene weight of sunflower under water deficit conditions. For the well-watered and 64 mm irrigation depth conditions, plants attained more 1000-achene weights due to the B foliar application of  $30 \text{ mg L}^{-1}$  compared to the water control in 2011 (Table 2). In 2012, all of the B supplement treatments improved 1000-achene weight of sunflower compared to control. Under a higher deficit level of 53 mm irrigation depth, foliar applied B at  $45 \text{ mg L}^{-1}$  along with 15- and  $30 \text{ mg L}^{-1}$  remained effective as well in improving the 1000-achene weight during both years (Table 2).

B fertilization under water deficit improved the biological yield significantly ( $p < 0.05$ ) compared to a water control during both years (Table 3). Plants showed higher potential in providing maximum biological yield under deficit levels of 64 and 53 mm irrigation depths when treated with  $30 \text{ mg L}^{-1}$  of B compared to the water control. As the B concentration was increased to  $45 \text{ mg L}^{-1}$ , biological yield under the least irrigation depth of 53

TABLE 1. Influence of B foliar application on 50% inflorescence emergence, 50% flowering and 50% maturity of sunflower under water deficit conditions

Treatments	50% Inflorescence emergence (days)		50% Flowering (days)		50% Maturity (days)	
	2011	2012	2011	2012	2011	2012
----- Water deficit (WD) -----						
WD <sub>0</sub> <sup>a</sup>	44.00 a <sup>b</sup>	41.58 a	66.42 a	63.75 a	107.32 a	103.58 a
WD <sub>1</sub>	40.50 b	38.16 b	63.41 b	60.67 b	102.33 b	100.66 b
WD <sub>2</sub>	37.58 c	35.17 c	59.75 c	57.08 c	100.08 c	97.42 c
----- B rates (mg L <sup>-1</sup> ) -----						
0	39.00 c	36.55 b	61.33 b	58.22 c	100.67 c	97.89 c
15	40.33 b	38.22 a	63.11 a	60.00 b	102.78 b	100.44 b
30	42.89 a	39.55 a	64.33 a	62.11 a	104.89 a	102.78 a
45	40.55 b	38.89 a	63.99 a	61.66 a	104.66 a	101.11 b
WD × B	NS	NS	NS	NS	NS	NS

<sup>a</sup>WD<sub>0</sub> = 75 mm irrigation depth (well-watered), WD<sub>1</sub> = 64 mm irrigation depth, WD<sub>2</sub> = 53 mm irrigation depth;

<sup>b</sup>Means in a column that are followed by the same letter are not statistically different at the  $p < 0.05$  level of significance; NS = Non-significant

TABLE 2. Influence of B foliar application on number of achenes/head and 1000-achene weight of sunflower under water deficit conditions

B rates (mg L <sup>-1</sup> )	Number of achenes/head			1000-achene weight (g)		
	WD <sub>0</sub> <sup>a</sup>	WD <sub>1</sub>	WD <sub>2</sub>	WD <sub>0</sub> <sup>a</sup>	WD <sub>1</sub>	WD <sub>2</sub>
----- 2011 -----						
0	1144.45 c <sup>b</sup>	951.31 d	781.06 b	50.47 d	40.84 c	33.14 c
15	1167.57 b	1012.91 c	797.21 b	52.74 c	44.91 b	34.58 b
30	1195.74 a	1079.14 a	871.28 a	56.02 a	47.83 a	37.87 a
45	1179.01 ab	1043.38 b	858.73 a	54.34 b	45.96 b	37.61 a
----- 2012 -----						
0	1055.29 c	879.69 c	697.08 c	48.06 c	38.17 c	30.07 b
15	1087.78 b	928.51 b	726.19 b	49.98 b	40.22 b	34.35 a
30	1118.55 a	992.92 a	779.37 a	53.24 a	44.01 a	34.75 a
45	1082.95 b	946.07 b	786.73 a	51.92 a	43.14 a	34.57 a

<sup>a</sup>WD<sub>0</sub> = 75 mm irrigation depth (well-watered), WD<sub>1</sub> = 64 mm irrigation depth, WD<sub>2</sub> = 53 mm irrigation depth.

<sup>b</sup>Means in a column that are followed by the same letter are not statistically different at the  $p < 0.05$  level of significance

TABLE 3. Influence of B foliar application on biological and achene yields of sunflower under water deficit conditions

B rates (mg L <sup>-1</sup> )	Biological yield (kg ha <sup>-1</sup> )			Achene yield (kg ha <sup>-1</sup> )		
	WD <sub>0</sub> <sup>a</sup>	WD <sub>1</sub>	WD <sub>2</sub>	WD <sub>0</sub> <sup>a</sup>	WD <sub>1</sub>	WD <sub>2</sub>
----- 2011 -----						
0	10386 b <sup>b</sup>	9125 c	7630 d	2875 c	2202 d	1638 c
15	10426 b	9307 b	7841 c	2999 b	2299 c	1769 b
30	10681 a	9673 a	8381 a	3153 a	2601 a	1987 a
45	10583 a	9330 b	8057 b	3090 a	2423 b	1831 b
----- 2012 -----						
0	9858 c	8423 c	7095 c	2711 c	2002 d	1503 c
15	9988 b	8751 b	7346 b	2864 b	2145 c	1598 b
30	10617 a	9335 a	7808 a	3137 a	2491 a	1798 a
45	10099 b	8816 b	7777 a	2905 b	2228 b	1746 a

<sup>a</sup>WD<sub>0</sub> = 75 mm irrigation depth (well-watered), WD<sub>1</sub> = 64 mm irrigation depth, WD<sub>2</sub> = 53 mm irrigation depth.

<sup>b</sup>Means in a column that are followed by the same letter are not statistically different at the  $p < 0.05$  level of significance

mm was also increased for both years of study (Table 3). Foliar-applied B with 30 mg L<sup>-1</sup> under the mild water stress level of 64 mm irrigation depth significantly ameliorated the drought effects as measured by achene yield in both years. The maximum achene yield response was obtained with 30 mg L<sup>-1</sup> of B in 2011 and with 45 mg L<sup>-1</sup> of B in 2012 (Table 3). The quadratic response of sunflower yield to B application (Figure 2) made it possible to show its maximum efficiency at 35-, 33- and 31 mg L<sup>-1</sup> of B under well-watered and irrigation depths of 64- and 53 mm, respectively, during first year. According to fitted equation, the B rates of 29-, 30- and 41 mg L<sup>-1</sup> corresponded to higher yield in next year under well-watered as well as under water deficit conditions.

Foliar application of B in sunflower generally decreased the achene oil contents with and without exposure to water deficit conditions (Figure 3). B concentration of 30 mg L<sup>-1</sup> resulted in the lowest oil concentration as compared to other application rates in both years of study. The higher values on percent achene oil concentrations occurred with no B when plants were not subjected to water stress. In 2011, under the influence of 64 and 53 mm irrigation depths, the lowest values for oil contents were those plants that treated with B concentrations of 30 mg L<sup>-1</sup> compared to water control. The effect of 45 mg L<sup>-1</sup> B treatment was not significantly different from the 30 mg L<sup>-1</sup> B treatment during 2012 (Figure 3). Achene protein contents were significantly improved with B spray under both stress and non-stress conditions (Figure 4). The maximum percentage of protein contents was measured in seeds from plants amended with all B concentrations compared to water control under well-watered conditions in both 2011 and 2012. Seeds harvested from plants treated with 30 mg L<sup>-1</sup> B and 64 mm irrigation

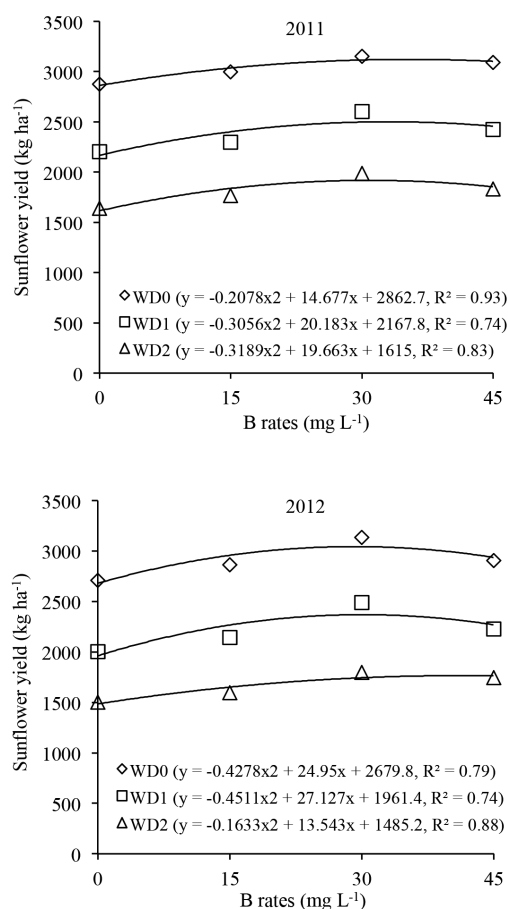


FIGURE 2. Yield of sunflower as a function of B rates under water deficit conditions

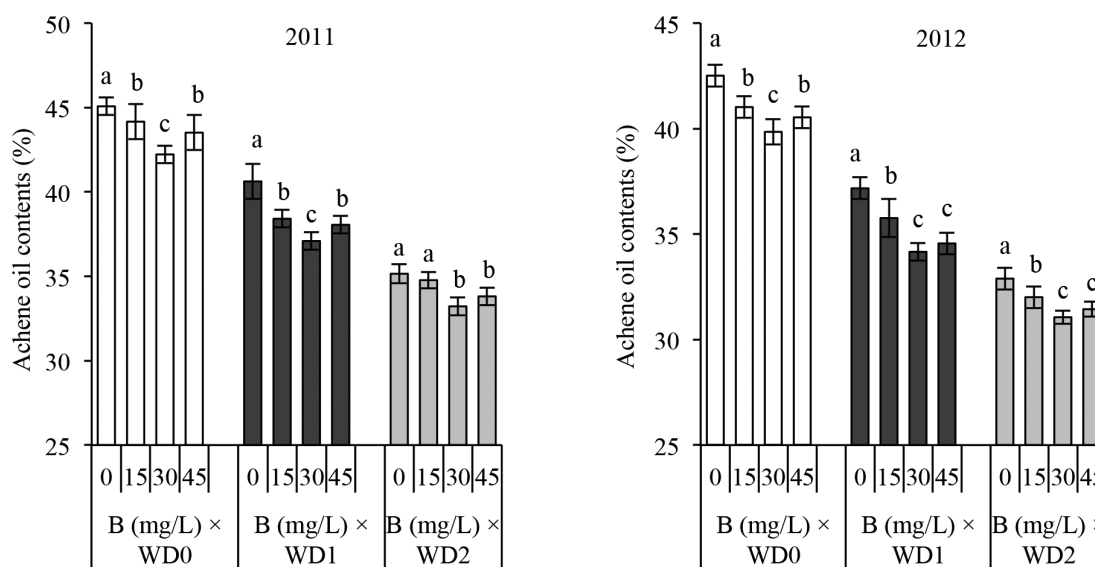


FIGURE 3. Influence of B foliar application on achene oil contents (%) of sunflower under water deficit conditions. Means within a B foliar application and water deficit treatment combination followed by the same letter are not significantly different at the  $p < 0.05$  level of significance

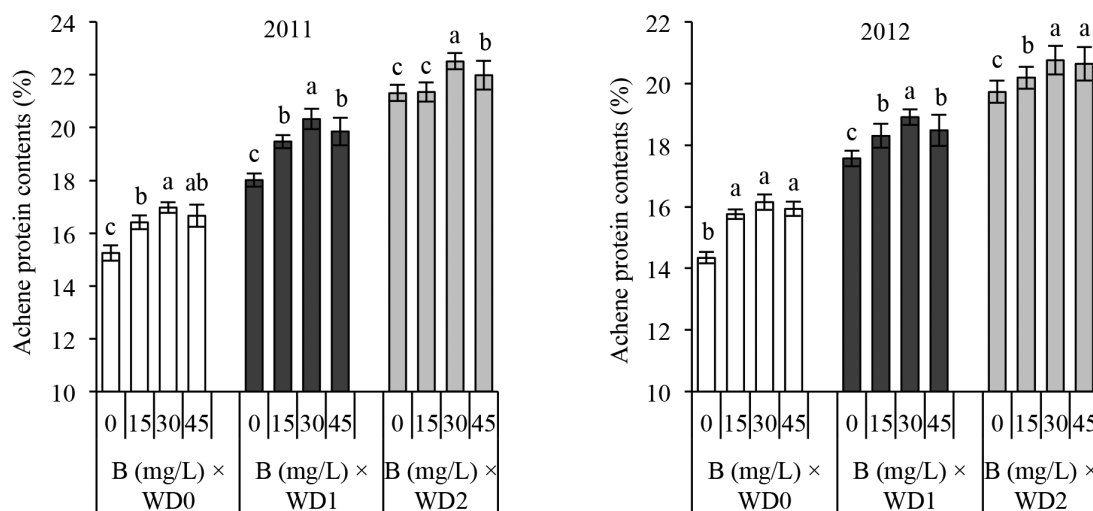


FIGURE 4. Influence of B foliar application on achene protein contents (%) of sunflower under water deficit conditions. Means within a B foliar application and water deficit treatment combination followed by the same letter are not significantly different at the  $p < 0.05$  level of significance

depth provided higher percentage of achene protein contents compared to the seeds harvested from control plots. In both cropping seasons, B application rates of 30- and 45 mg L<sup>-1</sup> under 53 mm irrigation depth resulted in improved seed protein contents, compared to the seeds from plants that did not receive any B rate (Figure 4).

Achene oil fatty acids i.e. stearic, palmitic, oleic and linoleic acids were significantly affected by B foliar spray under water deficit conditions. The highest concentrations of stearic acid were obtained with the irrigation depth of 53 mm, while minimum stearic acid concentrations were in oil that extracted from untreated seeds. Stearic acid concentration in sunflower seeds was higher in untreated seeds and lowest at a B treatment level of 30 mg L<sup>-1</sup> during both years of 2011 and 2012 (Table 4). Fertilization with 30 mg L<sup>-1</sup> of B provided maximum palmitic acid concentrations during both years of exploration. Palmitic acid concentrations were always greatest with plants under no water stress and these concentrations significantly ( $p < 0.05$ ) decreased with increasing water deficit levels (Table 4). Oleic acid concentrations were gradually increased by imposing increasing levels of water deficits. Foliar-applied B had a negative effect on oleic acid concentrations. Minimum concentrations were recorded for 30 mg L<sup>-1</sup> B treatment in 2011. During 2012, the response to B with 15- and 30 mg L<sup>-1</sup> concentrations was similar for all water conditions, while 45 mg L<sup>-1</sup> of B, of the water deficit of 53 mm depth remained at a low level (Table 4). Linoleic acid concentrations were increased when sunflower plants were sprayed with B concentrations of 30- and 45 mg L<sup>-1</sup> under well-watered conditions. At 53 mm irrigation depth, B level of 30 mg L<sup>-1</sup> improved linoleic acid concentrations over the water control during both years. The lowest linoleic acid

contents were associated with the highest water deficit level of 53 mm irrigation depth (Table 4).

The maximum WUE during first year occurred when sunflower plants were treated with 30 mg L<sup>-1</sup> B; while in following year, the highest WUE occurred when B was applied at 45 mg L<sup>-1</sup> concentration and this efficiency was comparable to 15- and 30 mg L<sup>-1</sup> B concentrations under well-watered conditions (Figure 5). WUE was reduced with decreasing water supply to sunflower plants, but it was improved by a foliar application of B concentrations up to 45 mg L<sup>-1</sup>. Under limited water conditions of 64 and 53 mm irrigation depths, B foliar application of 30 mg L<sup>-1</sup> followed by 45 mg L<sup>-1</sup> resulted in the best WUE during both years (Figure 5). The maximum enhancement in the net income and benefit cost ratio was obtained through foliar spray of B at 30 mg L<sup>-1</sup> concentration under well-watered conditions. At water deficit level of 64 mm irrigation depth, 30 mg L<sup>-1</sup> B level also yielded the highest benefit cost ratio values during both years of study. The lowest benefit cost ratio values were observed under 53 mm irrigation depth during both years (Table 5).

## DISCUSSION

Plants exposed to water deficit appear to shorten the duration needed to achieve a 50% level of various developmental stages. Under unfavorable growth conditions, development stages were shortened to increase the chances of achieving reproductive seed development (Orange & Ebadi 2012). Drought stress conditions distress the stem elongation through delayed cell division of intercalary meristem. Conceivably, to evade these drought effects, plants hasten their development by dropping the time period within growth stages (McMaster et al. 2003). Blum (2005) also

TABLE 4. Influence of B foliar application on achene oil fatty acids of sunflower under water deficit conditions

B rates (mg L <sup>-1</sup> )	Stearic acid (%)				Palmitic acid (%)			
	WD <sub>0</sub> <sup>a</sup>	WD <sub>1</sub>	WD <sub>2</sub>	Means	WD <sub>0</sub>	WD <sub>1</sub>	WD <sub>2</sub>	Means
----- 2011 -----								
0	3.61 <sup>b</sup>	3.85	4.04	3.83 a	6.66	5.96	5.30	5.97 d
15	3.52	3.74	3.94	3.73 b	6.71	6.02	5.52	6.08 c
30	3.46	3.57	3.73	3.59 c	6.90	6.37	5.65	6.31 a
45	3.57	3.67	3.86	3.70 b	6.74	6.23	5.61	6.19 b
Means	3.54 c	3.71 b	3.89 a		6.75 a	6.14 b	5.52 c	
----- 2012 -----								
0	3.19	3.43	3.62	3.41 a	6.48	5.82	5.19	5.83 c
15	3.09	3.30	3.51	3.30 b	6.52	5.84	5.31	5.89 c
30	3.12	3.12	3.25	3.16 c	6.68	6.16	5.43	6.09 a
45	3.05	3.18	3.35	3.19 c	6.55	6.01	5.39	5.98 b
Means	3.11 c	3.26 b	3.43 a		6.55 a	5.96 b	5.33 c	
----- 2011 -----								
Oleic acid (%)								
0	11.40	11.63	11.95	11.66 a	68.12 c	64.09 c	61.24 c	64.48
15	11.32	11.60	11.87	11.59 b	68.53 b	64.18 c	61.32 c	64.67
30	11.21	11.48	11.79	11.49 c	69.13 a	66.15 a	62.96 a	66.08
45	11.30	11.56	11.82	11.56 b	69.06 a	65.92 b	62.79 b	65.92
Means	11.31 c	11.57 b	11.86 a		68.71	65.08	60.07	
----- 2012 -----								
Oleic acid (%)								
0	10.19 a	10.43 a	10.79 a	10.47	63.31 b	60.30 c	57.39 b	60.33
15	10.11 b	10.36 b	10.68 b	10.38	64.02 a	60.46 c	57.53 b	60.67
30	9.97 c	10.26 c	10.58 c	10.27	64.28 a	61.23 a	58.94 a	61.48
45	10.08 b	10.34 b	10.61 c	10.34	64.17 a	60.81 b	58.81 a	61.26
Means	10.09	10.35	10.66		63.95	60.70	58.16	
----- 2012 -----								
Linoleic acid (%)								

<sup>a</sup>WD<sub>0</sub> = 75 mm irrigation depth (well-watered), WD<sub>1</sub> = 64 mm irrigation depth, WD<sub>2</sub> = 53 mm irrigation depth.

<sup>b</sup>Means in a column or row that are followed by the same letter or no letter are not statistically different at the  $p < 0.05$  level of significance

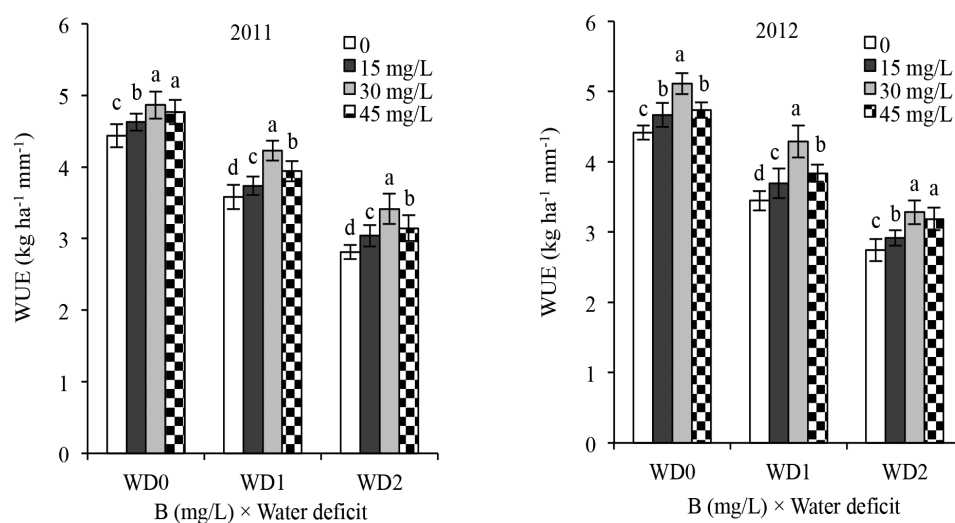


FIGURE 5. Influence of B foliar application on water use efficiency (WUE) of sunflower under water deficit conditions; Means within a B foliar application and water deficit treatment combination followed by the same letter are not significantly different at the  $p < 0.05$  level of significance

TABLE 5. Net income and benefit cost ratio of sunflower as affected by B foliar application under water deficit conditions

Water deficit × B rates	Gross income (Rs. ha <sup>-1</sup> )		Total cost (Rs. ha <sup>-1</sup> )		Net income (Rs. ha <sup>-1</sup> )		Benefit cost ratio		
	2011	2012	2011	2012	2011	2012	2011	2012	
WD <sub>0</sub>	0	131843	118358	76441	74546	55402	43812	1.72	1.59
	15	137451	124946	77970	75975	59481	48971	1.76	1.64
	30	144379	136609	78000	76005	66379	60604	1.85	1.80
	45	141552	126689	78029	76034	63523	50655	1.81	1.67
WD <sub>1</sub>	0	101125	87591	75353	73458	25772	14133	1.34	1.19
	15	105475	93710	76882	74887	28593	18823	1.37	1.25
	30	119117	108573	76912	74917	42205	33656	1.55	1.45
	45	111125	97380	76941	74946	34184	22434	1.44	1.30
WD <sub>2</sub>	0	75313	65855	74266	72371	1047	-6516	1.01	0.91
	15	81243	69930	75795	73800	5448	-3870	1.07	0.95
	30	91095	78549	75825	73830	15270	4719	1.20	1.06
	45	84092	76290	75854	73859	8238	2431	1.11	1.03

showed that reduced water potential caused a decrease in the growth period of plants under drought stress. Indeed, turgor pressure establishes a critical role in cell division and expansion, but its loss resulted in retarded development. Foliar-applied B supplement significantly increased the time to inflorescence emergence, flowering and maturity stages. This was attributed to improvement and recovery in vegetative growth. Such enhancement in growth was accompanied by an increase in B concentration in the plant. The result has improved metabolism, photosynthetic pigments and on biological and enzymatic activity (Jabeen & Ahmad 2011).

Using B under water stress conditions improved measured traits of sunflower crop including the number of achenes per head, 1000-achene weight, biological yield and achene yield. Reduction in the number of achenes/head was recorded when sunflower plants were subjected to water stress due to pollen abortion, sterility, retarded anthers development and pollen fertilization incompatibility (Karim et al. 2012). Furthermore, drought causes reactive oxygen species (ROS) production through energy accumulation in the plant body which increases oxidative stress to biological membranes, proteins, lipids, DNA, ribulose biphosphate activity and stomatal closure (Flexas & Medrano 2002). All these leads affect the respiration and photosynthesis. Under water stress situations, B foliar application considerably increases the achenes/head by maintaining enzyme activation, charge balance, pollen development and overall improved plant growth (Asada 2006). Huang et al. (2000) found depressed pollen viability in B deficient plants caused by a reduction in the development and expansion of pollen cell walls, leading to limited anther elongation due to restricted cell division during initial growth of anthers. A reduced number of cells per anther and constrained cell dimensions thus contribute towards smaller anther length. Another reason for the low number of achenes/head is the reduced chance of pollen maturation through partial impaired

supply of carbohydrates for starch accumulation. Matoh et al. (1998) suggested that B was strongly responsible for the development of pollen tube walls by means of borate diester bonding with rhamno-galacturonan-II. Our findings suggested that photosynthates transport towards achenes was hindered at the higher water deficit of 53 mm compared to 64 mm irrigation depths. This decreased the 1000-achene weight, but this decreased was corrected by supplying foliar B. B application under drought conditions is thought to markedly control carbohydrate metabolism and its transport (Belvins & Lukaszewski 1998). B maintains the proper functioning of certain enzymes as  $\alpha$ -amylase,  $\beta$ -amylase, glucose 6-phosphate dehydrogenase and UDPG-synthesis (Mazher et al. 2006), involved in carbohydrate metabolism. Regarding carbohydrates transport, B promotes the accumulation of starch and sugars in achenes by utilizing complex sugars (polyols) and forming polyol-B-polyol complexes in photosynthetic tissues. These are then transported towards reproductive organs. If these complexes were not formed because B deficiency caused phloem immobility, the end result was to decrease achene weights (Agarwala & Chatterjee 1996). Previous findings of Bellaloui (2012) also suggested that disturbed ratios due to a decrease in sucrose and an increase in stachyose and raffinose in seeds via drought stress was maintained by B application, which contributed towards higher 1000-achene weight.

Application of B provided sufficient recovery against drought and a higher biological yield. A decrease in relative water content, leaf dry biomass and antioxidants as glutathione and ascorbate due to imposed water deficit reduced biological yield. Induction of oxidative stress with increased phenolic contents and decreasing hydrogen peroxide,  $O_2^-$  and lipid peroxidation due to water stress attributed to dry matter yield reduction (Ruiz et al. 2006). Upadhyaya et al. (2012) found that foliar spray of B on rehydrated plants efficiently promoted the recovery of oxidative damage through the stimulation of the enzymatic



activities of superoxide dismutase (SOD), peroxidase (POX), polyphenol oxidase (PPO), catalase (CAT) and glutathione reductase (GR), thus ensuring higher dry matter production. The increase in achene yield of water stressed sunflower due to B was thought to improve photosynthesis, WUE, pollen viability and assimilates partitioning (Wei et al. 2005). Previous studies of Leite et al. (2007) and Silva et al. (2011) also noted that B increased the achene yield as a result of increased in achene numbers and achene weights. B deficiency caused achene yield reduction because of hollow seeds in the central head part and lower achene weights. Likewise, B deficiency in dehydrated plants can affect net photosynthesis that was dependent on well-structured membrane (Han et al. 2008). B deficiencies inhibited photosynthetic rates by reducing protein and chlorophyll contents in leaves and reducing stomatal conductance and CO<sub>2</sub> assimilation that ultimately reduces seed weight and achene yield (Pinho et al. 2010).

The decrease in oil concentration in achene was due to death of cells via cell membrane shrinkage, loss of water through more transpiration rate, inhibition of photosynthesis by stomata closure and by damaging the photosynthetic apparatus and chlorophyll pigments. Also, water stress due to accumulation of constituents like proline, H<sub>2</sub>O<sub>2</sub> and melondialdehyde (MDA) affects the ratio of protein and oil in seeds (Hajiboland & Bastani 2012; Hassan et al. 2011). Reduced oil contents in heavier achenes under added B nutrition was due to dilution of oil. There was thus an inverse relationship between protein and oil production. Accumulation of higher protein concentrations in sunflower seeds under water deficit situations was due to production of late embryogenesis abundant proteins (Christensen 2005), which provided a defense mechanism of plants against water stress damage. In support of our results, Bellaloui (2011) also observed that B deficiency in water-stressed plants affected Boron's association with pectin, glycolipids, glycoproteins and o-diphenols. Moreover, its deficiency augmented the phenolic concentrations and polyphenoloxidase activity that led to phenols oxidation, ion leakage and production of reactive quinones and peroxidative damage to membrane proteins and lipids. However, foliar-applied B improved protein contents in seeds under water stress because of an increase of proteolytic activities or formation of new proteins (Amutha et al. 2007).

Treating sunflower with foliar-applied B increased palmitic and linoleic acids and decreased stearic and oleic acid concentrations. Increase stearic acid concentration, while in contrast to reductions in palmitic acid under water stress situation conditions; agree with previous results of Flagella et al. (2002). Increases in oleic acid and decrease in linoleic acid concentrations under water stress might be due to enzymatic activity of  $\Delta 12$  desaturase, which is responsible for conversion of oleic acid to linoleic acid and explains the inverse association of oleic acid with linoleic acid. Under prevailing conditions of water deficit, B alleviates drought effects by aiding in maintaining proper

water status, finally increased un-saturated fatty acids concentrations in plants (Ali et al. 2009; Waraich et al. 2011). B foliar application thus is a management option to oil quality and protein content in sunflower seeds produced under water stressed conditions.

WUE of sunflower plants was substantially improved with B application under water deficit conditions. Previous studies also reported higher water contents in B treated seedlings in contrast to B deficient or no B treated seedlings. Adequate B application affects the water balance by encouraging more water absorption through improved roots and mycorrhizas production (Mottonen et al. 2005). Karim et al. (2012) also found maximum WUE and leaf water contents with foliar-applied B under limited water conditions.

## CONCLUSION

B foliar application provided positive response in alleviating the drought stress effects in sunflower. A foliar application of B at a concentration of 30 mg L<sup>-1</sup> provided the best results for sunflower growth in well-watered as well as in the mild water deficit level of 64 mm irrigation depth. At higher water deficit level of 53 mm irrigation depth, B concentration of 41 mg L<sup>-1</sup> in foliar sprays performed better than lower B concentrations for improving drought tolerance in a sunflower crop.

## ACKNOWLEDGEMENTS

Financial support of Higher Education Commission (HEC), Pakistan for this field study is highly acknowledged. Also thanks to Prof. Dr. Warren A. Dick from School of Environment and Natural Resources, Ohio Agricultural Research and Development Center (OARDC), Ohio State University, USA for their valuable edits and comments.

## REFERENCES

- Agarwala, S.C. & Chatterjee, C. 1996. Physiology and biochemistry of micronutrient elements. In *Advancements in Micronutrients*, edited by Hemantaranjan, A. Jodhpur, India: Scientific Publishers. pp. 205-366.
- Ali, H., Iqbal, N., Ahmad, S., Shahzad, A.N. & Sarwar, N. 2013. Performance of late sown wheat crop under different planting geometries and irrigation regimes in arid climate. *Soil & Tillage Research* 130: 109-119.
- Ali, Q., Ashraf, M. & Anwar, F. 2009. Physico-chemical attributes of seed oil from drought stressed sunflower (*Helianthus annuus* L.) plants. *Grasas Y Aceites* 60: 475-481.
- Amutha, R., Muthulaksmi, S., Baby-Rani, W., Indira, K. & Mareeswari, P. 2007. Studies on biochemical basis of heat tolerance in sunflower (*Helianthus annuus* L.). *Research Journal of Agriculture and Biological Sciences* 3: 234-238.
- AOAC. 1990. *Official Methods of Analysis*. 15th ed. Virginia: Association of Official Analytical Chemists, Inc. pp: 770-771.
- Asada, K. 2006. Production and scavenging of reactive oxygen species in chloroplasts and their functions. *Plant Physiology* 141: 391-396.

- ASAE. 1977. *Moisture Measurements in Grains and Seeds*. Standard 353. American Society of Agricultural Engineers Yearbook. p. 417.
- Bellaloui, N. 2012. Soybean seed phenol, lignin, and isoflavones and sugars composition altered by foliar boron application in soybean under water stress. *Food and Nutrition Sciences* 3: 579-590.
- Bellaloui, N. 2011. Effect of water stress and foliar boron application on seed protein, oil, fatty acids, and nitrogen metabolism in soybean. *American Journal of Plant Sciences* 2: 692-701.
- Blum, A. 2005. Drought resistance, water-use efficiency, and yield potential-are they compatible, dissonant, or mutually exclusive? *Australian Journal of Agricultural Research* 56: 1159-1168.
- Bremner, J. 1964. Organic forms of nitrogen. In *Methods of Soil Analysis, Part-2. Am. Soc. of Agron., Inc.*, edited by Black, C.A. Madison, Wisconsin. 9: 1235-1255.
- Broschat, T.K. 2005. Boron deficiency in palms. University of Florida, Environmental Horticultural Department Circular ENH1012. Gainesville, FL: University of Florida.
- Christensen, P. 2005. Foliar fertilization in vine mineral nutrient management programs. In *Proceedings of the Soil Environment and Vine Mineral Nutrition Symposium*, edited by Christensen, P. & Smart, D.R. CA: American Society of Enology and Viticulture Davis. pp. 83-90.
- Demir, A.O., Goksoy, A.T., Buyukcangaz, H., Turan, Z.M. & Koksall, E.S. 2006. Deficit irrigation of sunflower (*Helianthus annuus* L.) in a sub-humid climate. *Irrigation Science* 24: 279-289.
- Dordas, C. & Brown, P.H. 2005. Boron deficiency affects cell viability, phenolic leakage and oxidative burst in rose cell cultures. *Plant and Soil* 268: 293-301.
- Flagella, Z., Rotunno, T., Tarantito, E., Caterina, R.D. & Caro, A.D. 2002. Changes in seed yield and oil fatty acid composition of high oleic sunflower (*Helianthus annuus* L.) hybrids in relation to the sowing date and the water regime. *European Journal of Agronomy* 17: 221-230.
- Flexas, J. & Medrano, H. 2002. Drought-inhibition of photosynthesis in C<sub>3</sub> plants: Stomatal and non-stomatal limitations revisited. *Annals of Botany* 89: 183-189.
- Freed, R.D. & Eisensmith, S.P. 1986. M. Stat. Micro-Computer Programme, Michigan State Univ. Agric. Michigan, Lansing, USA (Unpublished).
- Hajiboland, R. & Bastani, S. 2012. Tolerance to water stress in boron-deficient tea (*Camellia sinensis*) plants. *Folia Horticulturae* 24: 41-51.
- Han, S., Chen, L.S., Jiang, H.X., Smith, B.R., Yang, L.T. & Xie, C.Y. 2008. Boron deficiency decreases growth and photosynthesis, and increases starch and hexoses in leaves of citrus seedlings. *Journal of Plant Physiology* 165: 1331-1341.
- Hassan, N.M., El-Sayed, A.K.A., Ebeid, H.T. & Nemat Alla, M.M. 2011. Molecular aspects in elevation of sunflower tolerance to drought by boron and calcium foliar sprays. *Acta Physiologiae Plantarum* 33: 593-600.
- Huang, L., Pant, J., Dell, B. & Bell, R.W. 2000. Effects of boron deficiency on another development and floret fertility in wheat (*Triticum aestivum* L. 'Wilgoyne'). *Annals of Botany* 85: 493-500.
- Imran, M., Ali, A., Waseem, M., Tahir, M., Mohsin, A., Shehzad, M., Ghaffari, A. & Rehman, H. 2011. Bio-economic assessment of sunflower-mungbean intercropping system at different planting geometry. *International Research Journal of Agricultural Science and Soil Science* 1: 126-136.
- Jabeen, N. & Ahmad, R. 2011. Effect of foliar applied boron and manganese on growth and biochemical activities in sunflower under saline conditions. *Pakistan Journal of Botany* 43: 1271-1282.
- Jaleel, C.A., Manivannan, P., Kishorekumar, A., Sankar, B., Gopi, R., Somasundaram, R. & Panneerselvam, R. 2007. Alterations in osmoregulation, antioxidant enzymes and indole alkaloid levels in *Catharanthus roseus* exposed to water deficit. *Colloids Surf B Biointerfaces* 59: 150-157.
- Karim, M.R., Zhang, Y.Q., Zhao, R.R., Chen, X.P., Zhang, F.S. & Zou, C.Q. 2012. Alleviation of drought stress in winter wheat by late foliar application of zinc, boron, and manganese. *Journal of Plant Nutrition and Soil Science* 175: 142-151.
- Leite, R.M.V.B.C., Castro, C. & Brighenti, A.M. 2007. Indications for the sunflower crop on Rio Grande do Sul, Parana, Mato Grosso do Sul, Mato Grosso, Goias and Roraima States. *Pesquisa Agropecuaria Brasileira* 42: 1517-1752.
- Martin, P.G. 1979. *Manuals of Food Quality Control 3. Commodities*. Food and Agriculture Organization of the United Nations, Rome. pp. 313-320.
- Matoh, T., Takasaki, M., Takabe, K. & Kobayashi, M. 1998. Immuno-cytochemistry of rhamnolacturoan II in cell walls of higher plants. *Plant and Cell Physiology* 39: 483-491.
- Mazher, A.A.M., Zaghoul, S.M. & Yassen, A.A. 2006. Impact of boron fertilizer on growth and chemical constituents of *Taxodium distichum* grown under water regime. *World Journal of Agricultural Sciences* 2: 412-420.
- McMaster, G.S., Wilhelm, W.W., Palic, D.B., Porter, J.R. & Jamieson, P.D. 2003. Spring wheat leaf appearance and temperature: Extending the paradigm. *Annals of Botany* 91: 697-705.
- Mottonen, M., Lehto, T., Rita, H. & Aphalo, P.J. 2005. Recovery of Norway spruce (*Picea abies*) seedlings from repeated drought as affected by boron nutrition. *Trees* 19: 213-223.
- Mukhtar, I. 2009. Sunflower disease and insect pests in Pakistan: A Review. *African Crop Science Journal* 17: 109-118.
- Orange, M.J. & Ebadi, A. 2012. Responses of phenological and physiological stages of spring safflower to complementary irrigation. *African Journal of Biotechnology* 11: 2465-2471.
- Pinho, L.G.R., Campostrini, E., Monnerat, P.H., Netto, A.T., Pires, A.A., Marciano, C.R. & Soares, Y.J.B. 2010. Boron deficiency affects gas exchange and photochemical efficiency (JPI test parameters) in green dwarf coconut. *Journal of Plant Nutrition* 33: 439-451.
- Rashid, A., Yasin, M., Ashraf, M. & Mann, R.A. 2004. Boron deficiency in calcareous soil reduces rice yield and impairs grain quality. *International Rice Research Notes* 29: 58-60.
- Ruiz, J.M., Rivero, R.M. & Romero, L. 2006. Boron increases synthesis of glutathione in sunflower plants subjected to aluminum stress. *Plant and Soil* 279: 25-30.
- Sajedi, M.A., Arakani, M.R. & Boojar, M.A. 2009. Response of maize to nutrients foliar application under water deficit stress conditions. *American Journal of Agricultural and Biological Sciences* 4: 242-248.
- Silva, C.A.T.D., Cagol, A., Silva, T.R.B.D. & Nobrega, L.H.P. 2011. Boron application before sowing of sunflower hybrids. *Journal of Food, Agriculture and Environment* 9: 580-583.
- Upadhyaya, H., Dutta, B.K., Sahoo, L. & Panda, S.K. 2012. Comparative effect of Ca, K, Mn and B on post-drought stress recovery in tea (*Camellia sinensis* (L.) O Kuntze). *American Journal of Plant Sciences* 3: 443-460.

Waraich, E.A., Ahmad, R., Saifullah, Ashraf, M.Y. & Ehsanullah. 2011. Role of mineral nutrition in alleviation of drought stress in plants. *Australian Journal of Crop Science* 5: 764-777.

Wei, X.R., Hao, M.D., Zhang, C.X. & Wang, X.G. 2005. Effects of zinc and manganese fertilizers on maize photosynthetic performance under soil drought condition (in Chinese). *Acta Agronomica Sinica* 31: 1101-1104.

Yan, H., Li, W.X., Guo, Y.F. & Liu, D.S. 2003. Effect of boron on carbohydrate assimilation and translocation in wheat. *Acta Pedologica Sinica* 40: 440-445.

Naveed Ahmad  
Edible Fats & Oils Laboratory, NIFSAT  
University of Agriculture, Faisalabad-38040  
Pakistan

\*Corresponding author; email: asifbukhari01@gmail.com

Received: 12 May 2015

Accepted: 10 March 2016

Muhammad Asif Shehzad\* Muhammad Maqsood &  
Tasawer Abbas  
Department of Agronomy  
University of Agriculture, Faisalabad-38040  
Pakistan