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Boron Application in Clay-Loam Soil for Improved Growth, Yield and Protein Contents of Mungbean in Water-Stresses

(Aplikasi Boron dalam Tanah Lom Liat untuk Pertumbuhan yang Lebih Baik, Hasil dan Kandungan Protein Kacang Mung Tegasan-Air)

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ABSTRACT

Boron is considered important to improve the drought resistance, yield and protein contents of pulses. Two years of field experiment was conducted to evaluate the effect of boron application and water stress given at vegetative and flowering stages on growth, yield and protein contents of mungbean during spring 2014 and 2015. The experiment was laid out in randomized complete block design with split-plot arrangement giving more emphasis to boron. The experiment comprised three water stress levels (normal irrigation, water stress at vegetative stage and water stress at reproductive phase) and four boron levels (0, 2, 4 and 6 kg ha^{-1}). Final seed yield was significantly increased by different levels of boron application both under normal and water stressed conditions. The increase in yield was mainly due to greater plant height, number of pods bearing branches, number of pods per plant, number of seeds per pod and 1000-grain weight. Boron application at 4 kg ha^{-1} caused 17%, 10% and 4% increase in grain yield under normal irrigation, stress at vegetative stage and water stress at reproductive phase, respectively. Protein contents were also increased (9-16%) at same boron treatment. Most parameters showed a marked decrease at higher dose (6 kg ha^{-1}) of boron. In conclusion, the boron application at rate of 4 kg ha^{-1} in clay-loam soil performed the best to enhance mungbean growth, yield and seed protein both under normal and water stressed conditions.

Keywords: Climate change; drought stress tolerance; semi-arid conditions; soil boron application; Vigna radiata

ABSTRAK

Boron dianggap penting untuk meningkatkan kemarau rintangan, hasil dan kandungan protein denyutan. Kajian lapangan selama dua tahun telah dijalankan untuk menilai kesan daripada aplikasi boron dan tegasan air diberikan pada peringkat vegetatif dan pembungaan ke atas kandungan pertumbuhan, hasil dan protein kacang mung semasa musim bunga 2014 dan 2015. Kajian telah dibentangkan dalam reka bentuk blok lengkap dengan susunan split-plot yang memberi lebih banyak penekanan kepada boron. Kajian ini terdiri daripada tiga tahap tekanan air (pengairan biasa, tegasan air pada peringkat vegetatif dan tegasan air pada fasa reproduktif) serta empat tahap boron (0, 2, 4 dan 6 kg ha^{-1}). Hasil akhir benih meningkat dengan ketara oleh pelbagai peringkat aplikasi boron kedua-dua di bawah keadaan normal dan tegasan air. Peningkatan hasil adalah disebabkan oleh ketinggian tumbuhan, bilangan lengai berdahan, bilangan lengai setiap tumbuhan, bilangan benih setiap lengai dan berat 1000-bijian. Aplikasi boron pada 4 kg ha^{-1} menyebabkan 17%, 10% dan 4% peningkatan dalam hasil benih di bawah pengairan biasa, tegasan pada peringkat vegetatif dan tegasan air pada fasa reproduktif. Kandungan protein turut meningkat (9-16%) pada rawatan boron yang sama. Kebanyakan parameter menunjukkan penurunan yang ketara pada dos boron lebih tinggi (6 kg ha^{-1}). Kesimpulannya, aplikasi boron pada kadar 4 kg ha^{-1} dalam tanah lom liat melakukan yang terbaik untuk meningkatkan pertumbuhan kacang mung, hasil dan protein benih pada kedua-dua keadaan normal dan tegasan air.

Kata kunci: Aplikasi tanah boron; keadaan separa gersang; perubahan iklim; toleransi tekanan kemarau; Vigna radiata

INTRODUCTION

Changing environmental scenario demands research to cope with the expected water scarcity and drought conditions in future. In addition, pressure of skyrocketing world population requires high crop yield with more nutritious values especially plant based protein contents. Pulse crops are important component of profitable agriculture as pulses are cheap source of protein so they are poor man's meat. Among pulses mungbean (*Vigna radiata* L.) enjoys special place in people's diet due to its high nutritional value, owing to its quick cooking, easy digestibility and anti-flatulent properties (Usman et al. 2007). Mungbean contains 24.5% crude protein, 60% carbohydrate, 1-3% fat, 3.5-4.5% crude fiber, 4.5-5.5% ash, 75 mg calcium, 8.5 mg iron, and 49 mg β-carotene per 100g of grain (Afzal et al. 2004).

Micronutrient deficiency and drought are among the key factors of low mungbean yield. Response of mungbean under water stress conditions has been widely considered and it has been considered very sensitive to water stress

(Mahmoodian et al. 2012). Water shortage disrupts different physiological processes including photosynthesis linked with mungbean growth and economic yield (Allahmoradi et al. 2011; Naresh et al. 2013). Liu et al. (2003) found that yield of mungbean was highly reduced by water stress particularly at flowering and early pod setting stage. Nitrogen fixation is also found sensitive to drought. Ashraf and Ibram (2005) reported reduction in number of nodules when the drought overlaps mungbean at 6 weeks after planting. Boron has been found effective in mitigating drought stress (Shehzad et al. 2016; Worbel et al. 2006). The soil application of boron had a significant positive effect on vegetative and reproductive stage in mungbean plants (Kassab 2005; Quddus et al. 2011). Boron supply also increases the uptake and utilization of N, P, K, Na and Ca (Bassil et al. 2004). Boron applications improved growth, yield and protein content of mungbean (Kaisher et al. 2010).

Effect of boron soil application on growth, yield and quality of mungbean under water stress conditions yet has not been considered. We hypothesized that boron application may reduce the effect of water stress at various growth stages on mungbean in term of growth, yield and seed protein contents. Therefore, two year field trail in clay-loam soil was conducted with following objectives: To study the interaction of boron doses and water stress applied at vegetative and reproductive stages of mungbean; to investigate the effect of soil applied boron on growth, yield and protein contents of mungbean; to check the potential of boron in mitigating drought stress in mungbean under clay-loam soil conditions; and to recommend boron dose in different water stress conditions under semi-arid conditions of Faisalabad, Pakistan.

MATERIALS AND METHODS

SITE DESCRIPTION

The study was conducted in 2014 and 2015 at Agronomic Research Area, University of Agriculture, Faisalabad, Pakistan (73.09° East longitude, 31.25° North latitude and altitude 183 m). The soil textural class was clay-loam with slightly alkaline (pH8.5) in nature and 0.71% organic matter. Total nitrogen, available phosphorus and potassium were 0.44%, 5.12 ppm and 127 ppm, respectively. Electrical conductivity was 2.99 $dS m^{-1}$. The climate of the region is semi-arid. Weather data for both years were obtained from Agricultural Metrology Cell, University of Agriculture, Faisalabad (Figure 1).

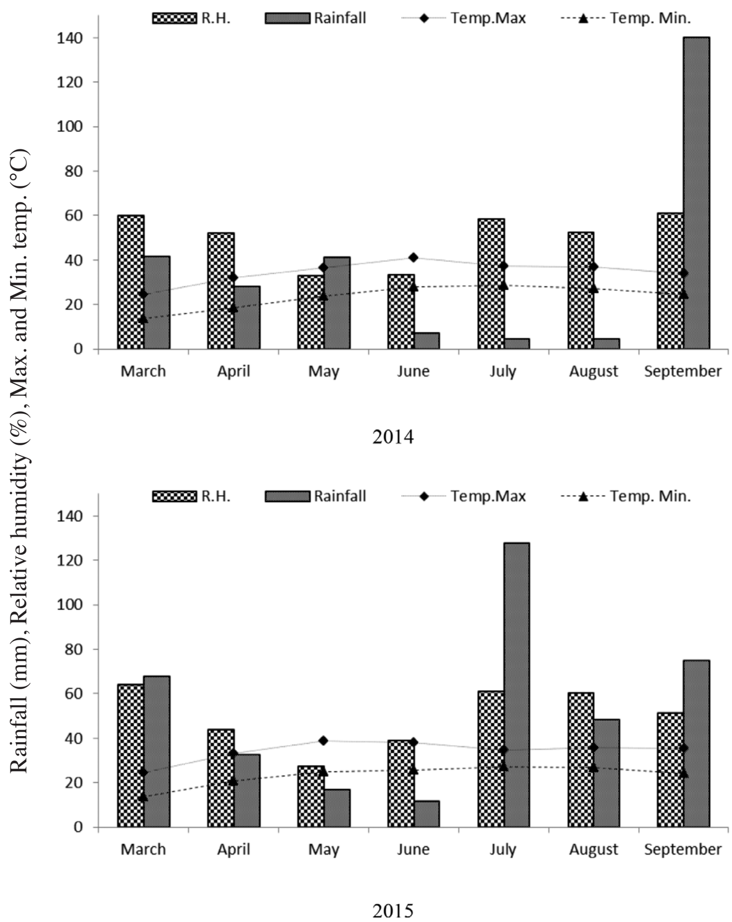

FIGURE 1. Meteorological data during the course of present studies
(Source: Agricultural Metrology Cell, University of Agriculture, Faisalabad)

EXPERIMENTAL DESIGN AND TREATMENTS

Mungbean seeds were collected from Ayub Agricultural Research Institute, Faisalabad. The experiment was laid in a randomized complete block design with split plot arrangement having a net plot size of 5.0 m × 1.8 m. The crop variety AZRI-2006 was sown on 7 March 2014 and 11 March 2015 using a seed rate of 30 kg ha^{-1} in well prepared field maintaining row to row distance of 30 cm and plant to plant distance of 10 cm. Two weeks after emergence, the plants were thinned to a density of 30 plants m^{-2}. Nitrogen phosphorus potassium (NPK) fertilizers were applied at 23-57-62 kg ha^{-1}, respectively. Prescribed doses of phosphorus (P) were applied as single super phosphate (SSP) and potassium (P) was applied as murate of potash. Half dose of nitrogen and all doses of P and K were applied at sowing time, and remaining half nitrogen dose was applied on the first irrigation. Three levels of water stress (I_1= normal irrigation i.e. control, I_2= drought stress at vegetative stage, I_3= drought stress at flowering stage) in main plots and four boron levels (B_1= 0, B_2= 2, B_3= 4 and B_4= 6 kg ha^{-1}) in subplots were applied. Total two irrigations, first at 21 days after sowing (DAS) and second at 42 DAS each having 75 mm irrigation depth, were applied for normal irrigation. The water stress was created by skipping irrigation at vegetative stage (21 DAS) and at reproductive stage (42 DAS) to selected plots. Two stages of hoeing were applied to control the weeds, 1st hoeing was done one month after sowing, and the 2nd hoeing after second irrigation. All other agronomic practices were kept standard and uniform for all the treatments.

STATISTICAL ANALYSES

The data on different crop characteristics such as plant height, number of pods bearing branches, number of pods per plant, number of seeds per pod, 1000-seed weight, protein contents, seed yield, biological yield and harvest index were collected. Plant height was measured from soil to the tip of ten randomly selected plants. Number of leaves, number of umbels counted from ten randomly selected plants and means were calculated. Ten umbels were randomly selected from each plot, seeds were removed and counted. One thousand seeds of main, primary and secondary umbels were counted separately and weighed. The crop harvested at maturity (65 DAS) from each plot was tied into small bundles and these bundles were weighed to plot yield and converted to hectare basis. All plots were harvested separately, threshed with wooden sticks to obtain seed yield. Harvest index was calculated by taking ratio of the seed yield to the biological yield and expressed in percentage. Crude protein contents were determined by Kjeldahl method (AOAC 1990). Percent crude protein was calculated using the formula below:

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

where V_1 is the sample titration volume (mL); V_2 is the blank titration volume (mL); N is the normality of standardized H_2SO_4, and W is the sample weight.

Response values were analyzed as RCBD with three replications using GLM procedure of SAS (SAS Inc.). When the treatment effect was significant at 5% level, the multiple mean comparison treatments were completed and letter groupings generated using least significant difference (LSD) at 5% level of significance. The validity of normal distribution and constant variance assumptions on the error terms was verified by examining the residuals (Steel et al. 1997).

RESULTS AND DISCUSSION

EFFECT ON GROWTH

Effect of boron and water stress levels were significant regarding plant height of mungbean (Table 1). Maximum plant height (53.90 and 54.65 cm) was obtained when boron was applied at 4 kg ha^{-1} and minimum plant height was noted from B_0 (0 kg ha^{-1}) during both years of study. Parallel results were recorded by Nabi et al. (2006) and Quddus et al. (2011) that optimum supply of boron helped the plant to attain the maximum height. Maximum plant height (51.65 cm) was obtained under normal irrigation while drought at vegetative and reproductive stage gave statistically no difference. These results are due to the fact that water stress reduces the plant growth by hampering the process of cell division. Naresh et al. (2013) also observed that water stress negatively affected the plant height. Maximum number of pod bearing branches (8.33) was obtained when boron was applied at 2 kg ha^{-1} but it remained at par with B_2. Further increase in the dose reduced this parameter. Kaisher et al. (2010) also reported that boron application significantly increases the number of pod bearing branches per plant. Our result confirmed that boron has some role in the development of reproductive parts of the plant. This parameter was not affected by water stress. These results are in line with Kassab (2005) and Ranawake et al. (2011). They reported non-significant effect of water stress on number of pods bearing branches per plant of mungbean.

Number of pods per plant also showed the similar trend. Mmaximum value of this parameter (20.56) was obtained at $B_{1,}$ and further increase in dose reduced this parameter but minimum number of pods per plant (13.23) was noted when boron was applied at 0 kg ha^{-1}. Nabi et al. (2006) also reported that application of boron improved the number of pods per plant. Maximum number of pods per plant (18.84) was recorded under normal irrigation. This parameter was reduced under water stress at both vegetative and reproductive stages. These results are quite in line with those of Lalinia et al. (2012). They reported that decrease in the yield of mungbean was mainly due to reduced pod formation and increased pod shedding.

TABLE 1. Effect of boron applications and water stress levels on plant height and yield component of mungbean

Treatments	Plant Height (cm)		No. of pods bearing branches		No. of pods per plant	
	2014	2015	2014	2015	2014	2015
Factor A: Boron levels (kg ha^{-1})						
0 (B_0)	44.53 c	43.45 d	6.46 c	6.43 b	13.23 d	14.62 d
2 (B_1)	49.91 b	48.49 c	8.33 a	7.59 a	19.38 a	20.56 a
4 (B_2)	53.90 a	54.65 a	7.79 ab	7.60 a	17.49 b	18.16 b
6 (B_3)	50.15 b	51.79 b	7.29 b	7.46 a	15.91 c	16.12 c
LSD *≤0.05*	1.97	1.64	0.70	0.63	1.05	1.18
Factor B: Water stress levels						
Normal irrigation (I_1)	51.65 a	50.49 a	7.59	7.14	18.84 a	18.01a
Water stress at vegetative stage (I_2)	48.01 b	47.98 b	7.47	7.43	16.06 b	17.16 a
Water stress at reproductive stage (I_3)	49.21 b	49.90 a	7.35	7.46	14.62 b	15.14 b
LSD *≤0.05*	2.04	1.93	NS	NS	1.98	1.86
Interaction A×B = Non-Significant						

Means within the column and for each treatment represented by same letter are not different according to the $p \leq 0.05$ level of significance; NS = Non-significant

Mung bean plants seem to reduce their various growth and developmental stages in response to water stress. Under stressed conditions, developmental phases were shortened to achieve the reproductive phase (Orange & Ebadi 2012). Water stress caused reduction in stem elongation through reduced cell division of intercalary meristem. Alternatively, to avoid these negative effects, plants complete their developmental stages quickly by reducing the time periods between various developmental phases (McMaster et al. 2003). According to Blum (2005), water deficit push plants to complete their life cycle quickly by reducing growth periods. Undeniably, loss of cell turgor pressure leads to less cell division and less plant growth. Reduction in the number of pods under water stress was due to pollen abortion, sterility and less fertilization (Karim et al. 2012). Additionally, production of reactive oxygen species under water stress caused increase in oxidative stress to various biological membranes and molecules and influenced stomatal conductance (Flexas & Medrano 2002). All these effects resulted to reduced growth of mungbean. Application of boron as soil treatment under water stress condition increases the plant growth and reproductive potential by continuing enzyme activities, increased fertilization and better overall plant growth (Abid et al. 2007; Asada 2006). Under boron deficiency, less pollen viability occurred due to reduced growth and expansion of pollen cell walls that causes less anther growth because of limited cell division during initial development of anther (Huang et al. 2000), leading lesser length of anther. Furthermore, boron has strong role in development of pollen tube walls through bonding rhamno-galacturonan-II monomers by borate cross links (Matoh et al. 1998). Boron also required for the activation of various enzymes including α- and ß-amylases, glucose 6-phosphate dehydrogenase and uridine diphosphate glucose synthesis (Mazher et al. 2006). Literature exhibited that boron application stabilized ratio between sucrose and stachyose/raffinose under water stressed condition (Bellaloui 2012), which led to higher 1000-grain of mungbean.

EFFECT ON YIELD TRAITS AND PROTEIN CONTENTS

Boron application at of 4 kg ha^{-1} gave the maximum number of seeds 10.63 and 11.46 per pod during 2014 and 2015, respectively (Table 2). These results are due to the fact that boron has its role in pollen tube development which helps in the process of fertilization. Kaisher et al. (2010) and Nabi et al. (2006) reported that boron application helps in increasing the number of seeds per pod. Number of seeds per pod was affected by water stress at vegetative stage, where minimum number of seeds per pod (9.04) was recorded. Water stress at some critical stage hampered that process. Kumaga et al. (2003) stated that water stress reduced the number of seeds per pod in legume crops. Reduction in number of seeds only at I_2 in this experiment might be due to the fact that at this stage water stress reduced the pod length which caused the reduction in number of seeds per pods. Final seed yield was also affected by 1000-seed weight and boron application markedly affected this parameter. Maximum 1000-seed weight (47.63 and 46.59 g) was recorded at B_2 during both the years and minimum value (41.96 g) of this parameter was recorded at B_0. Boron might affect the sugar and starch formation which increases the seed weight and size and thereby 1000-seed weight. Our results are in line with those of Patil et al. (2006). They reported that application of boron increased the grain weight. 1000-seed weight was also affected by water stress during both the years with water stress at reproductive stage yielded minimum seed weight (42.35 and 43.45 g). Our findings confirmed the results reported by Lalinia et al. (2012) and Ranawake et al. (2011). They observed significant reduction in 1000-seed weight by imposing water stress. Protein contents were also significantly affected by boron

TABLE 2. Effect of boron applications and water stress levels on yield components and protein contents of mungbean

Treatments	No. of seeds per pod		1000 Seed weight (g)		Protein contents (%)	
	2014	2015	2014	2015	2014	2015
Factor A: Boron levels (kg ha^{-1})						
0 (B_0)	8.98 b	9.15 c	41.96 c	42.56 b	18.41 b	18.16 d
2 (B_1)	10.15 a	10.89 b	45.19 b	46.15 a	19.82 a	20.26 b
4 (B_2)	10.63 a	11.46 a	47.63 a	46.59 a	20.10 a	21.13 a
6 (B_3)	9.44 b	9.65 c	46.14 ab	45.49 a	18.96 b	19.15 c
LSD *≤0.05*	0.61	0.85	1.67	1.81	0.63	0.76
Factor B: Water stress levels						
Normal irrigation (I_1)	10.29 a	10.89	47.46 a	47.89	19.01	19.65
Water stress at vegetative stage (I_2)	9.04 b	10.02	45.89 a	46.15	19.54	19.14
Water stress at reproductive stage (I_3)	10.06 a	10.16	42.35 b	43.45	19.42	19.06
LSD *≤0.05*	0.79	NS	2.29	2.45	NS	NS
Interaction A×B = Non-significant						

Means within the column and for each treatment represented by same letter are not different according to the p<0.05 level of significance; NS = Non-significant

application. Maximum protein contents (20.10% and 21.16% during 2014 and 2015, respectively) were recorded at B_2. Kaisher et al. (2010) and Patil et al. (2006) reported that the application of boron increased protein contents. Water stress at different growth stages did not affect the protein contents of seed. Non-significant effect of water stress on protein contents was reported by Oranki et al. (2011). The interaction between boron and water stress at different levels was found non-significant for all these parameters.

EFFECT ON YIELD

Data presented in Table 3 and Figure 2 indicate that final seed yield was the main concern in this experiment. Interactive effect of boron and irrigation treatments on seed yield was significant (Figure 2). Maximum seed yield (1190.67 kg ha^{-1}) was recorded where boron applied at the rate of 4 kg ha^{-1} under normal irrigation (I_1), and minimum seed yield (943.00 kg ha^{-1}) was recorded at B_0 where water stress was applied at vegetative stage (I_2). Our findings are in line with the results reported by Ranawake et al. (2011). Interactive effect of boron and irrigation was also significant on biological yield (Figure 2). They reported that water stress decreased the seed yield by negatively affecting the yield contributing parameters. Maximum biological yield (7269 kg ha^{-1}) was recorded where boron applied at the rate of 4 kg ha^{-1} under normal irrigation (I_1) which remain at par with B_1 at same level of water stress. Minimum biological yield (943 kg ha^{-1}) was recorded at B_0 when water stress was applied at vegetative stage (I_2). These results were supported by Ercoli et al. (2008) and they concluded that water stress reduced the dry matter accumulation in cereals. Wrobel et al. (2006) explained that boron can mitigate the drought effect and its application to soil improved the parameters of the main yield components, thus increasing yield level and enriching the chemical composition of crops. Maximum harvest index 16.35% and 16.31% was recorded at B2 and I_1, respectively. The interaction of these parameters was found to be non-significant (Table 3).

Boron application provides recovery to water stress and thus increase in yield. Introduction of oxidative pressure under water deficit with more concentration of phenolic and reduced H_2O_2, O^{2-} and lipid peroxidation credited to decrease in biological yield (Ruiz et al. 2006). Boron facilitated to recover the oxidative damage caused due to water deficit by motivating activity of various enzymes including superoxide dismutase, peroxidase, polyphenol oxidase, catalase and glutathione reductase, thus guaranteeing more dry matter production (Upadhyaya et al. 2012). More grain yield of mungbean under water deficit might be due to the role of boron in photosynthesis, pollen viability and source sink relationship (Wei et al. 2005). Scientific literature in the past exhibited that boron enhanced grain yield by increasing number of pods and 1000-grain weight (Leite et al. 2007; Silva et al. 2011). Indirectly boron deficiency may influence the net photosynthesis as it has strong role in membrane development and photosynthesis depending on well-structured membrane (Han et al. 2008). In addition, boron scarcity reduced photosynthetic potential by decreasing leaf protein and chlorophyll, stomatal activity and eventually decreases mungbean yield (Pinho et al. 2010).

Boron availability was influenced by various soil and climate factors, including soil pH, type of soil texture, rainfall, temperature, carbonate contents and soil organic matter (Goldberg et al. 2000). High soil pH reduced the availability of boron, as Wear and Patterson (1962) reported that increasing soil pH caused significant reduction in boron availability at any level of water. Furthermore, semi-arid weather condition also caused boron deficiency due to hot and dry soils (Rerkasem & Jamjod 2004). Therefore, in clay-loam with slightly alkaline (pH8.5)

TABLE 3. Effect of boron applications and water stress levels on seed yield, biological yield and harvest index of mungbean

Treatments	Seed yield (kg ha^{-1})		Biological yield (kg ha^{-1})		Harvest index (%)	
	2014	2015	2014	2015	2014	2015
Factor A: Boron levels (kg ha^{-1})						
0 (B_0)	995.56 d	1034.97 c	6306.67 b	6297.98 c	15.78 c	16.43
2 (B_1)	1073.44 b	1054.66 b	6811.11 a	6784.11 b	15.76 c	15.56
4 (B_2)	1095.67 a	1093.26 a	6863.67 a	6826.77 a	15.96 b	15.96
6 (B_3)	1023.11 c	1003.39 d	6256.11 b	6196.79 d	16.35 a	16.19
LSD ≤*0.05*	12.51	11.96	63.62	58.84	0.15	0.22
Factor B: Water stress levels						
Normal irrigation (I_1)	1112.00 a	1090.91 a	6819.42 a	6790.25 a	16.31 a	16.10
Water stress at vegetative stage (I_2)	961.25 c	956.93 b	6198.25 c	6171.25 b	15.51 c	15.91
Water stress at reproductive stage (I_3)	1067.58 b	1091.87 a	6660.50 b	6617.32 a	16.03 b	16.49
LSD ≤*0.05*	11.39	18.23	70.36	55.87	0.06	NS

Means within the column and for each treatment represented by same letter are not different according to the p<0.05 level of significance; NS = Non-significant

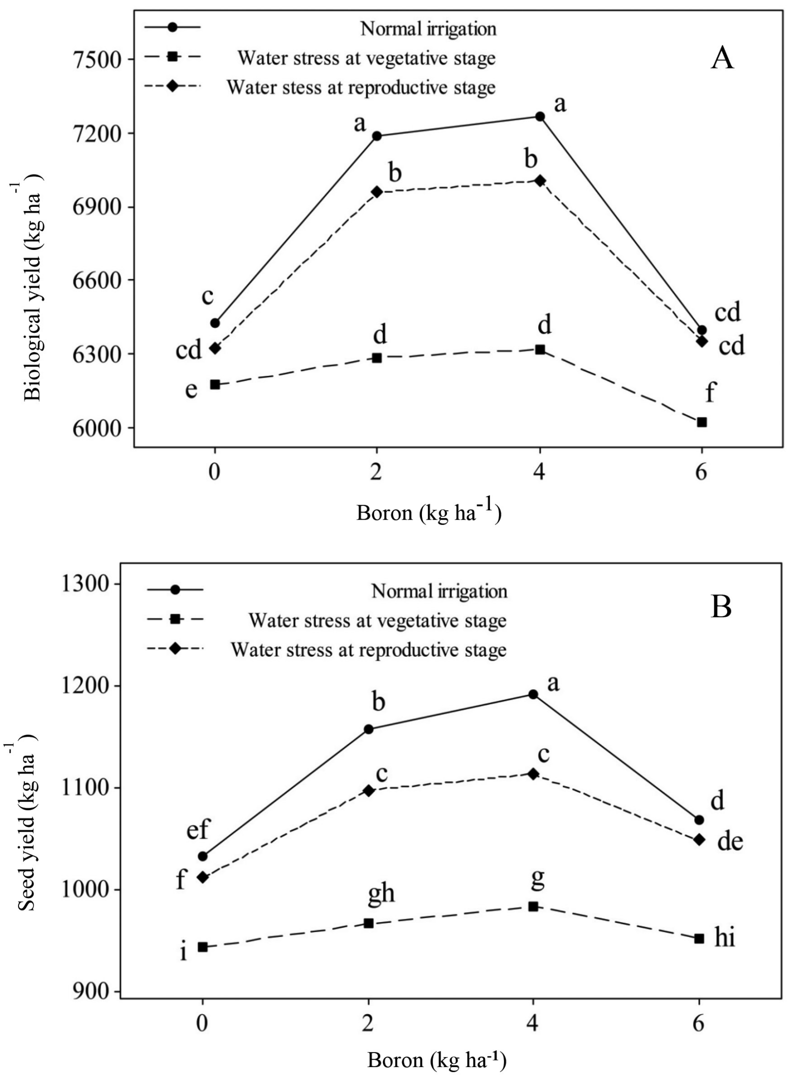

FIGURE 2. Least squares means (average of both years) of boron application (kg ha^{-1}) on biological yield (A) and seed yield (B) of mungbean for normal irrigation, water stress at vegetative stage and water stress at reproductive stage. Within each graph, means sharing the same letter are not significantly different at the 0.05 level

nature soil of Faisalabad and semi-arid hot condition, it is crucial to optimize the dose of boron application and water availability to achieve good crop yield.

Conclusion

Water stress at both vegetative and reproductive stages hampered the growth, yield component, yield and protein contents of mungbean. Boron application helped to mitigate the effect of water stress. Boron application at 4 kg ha^{-1} caused 17%, 10% and 4% increase in grain yield under normal irrigation, stress at vegetative stage and water stress at reproductive phase, respectively. Protein contents were also increased (9-16%) at the same boron treatment. However, boron at 6 kg ha^{-1} showed negative effects on mungbean. Thus, the boron application at rate of 4 kg ha^{-1} in clay-loam soil performed the best to enhance mungbean growth, yield and seed protein both under normal and water stressed conditions.

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