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Assessment of boron toxicity and deficiency on biochemical properties of *Triticum aestivum*

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Abstract

Boron is an essential plant micronutrient taken up via the roots mostly in the form of boric acid. While some plants tolerate high level, phytotoxicity problems are known to occur in soils with naturally elevated boron. The present study was conducted to assess the Boron toxicity and deficiency on biochemical properties of *Triticum aestivum*. The experiment was conducted with six B levels mg/kg, B deficient (B_0), sufficient (B_{10}, B_{20}, B_{30}) and toxic (B_{50} and B_{60}). Fifteen seeds of *Triticum aestivum* L var, siren were sown at 2-cm depth in pots with a height of 30cm and 25cm diameter. The pots were filled with clay loam soil (3kg) of pH 6.9 water holding capacity 50.37%, bulk density 0.96 g/cc, specific gravity 4.03, and organic carbon 0.82%. Biochemical constituents including Chlorophyll a/b, leaf protein, proline, sugar, carotenoid, phenol, and amino acid contents were analyzed. Result show that Chlorophyll a/b protein, proline, sugar, carotenoid, phenol, and amino acid contents increased at B sufficient level (B_{20} and B_{30}) and decrease at B deficient (B_0) and toxic (B_{50} and B_{60}). The present study showed that B deficiency as well as excess concentration affects plant growth and others morpho-physiological processes. Current Studies shows that out of the various concentrations of B, (50mg/kg) was moderately toxic while (60mg/kg) generated high toxicity and induced B stress response to confer tolerance in wheat. Further, a possible mechanism of B toxicity response in wheat is suggested. Conclusively, growing tolerant crops may be the only sustainable solution to improve growth and development of *Triticum aestivum* quantitatively and qualitatively.

Key-words: Boric Acid, Biochemical Parameters, *Triticum aestivum* L.

Introduction

Boron (B) is a necessary micronutrient play a vital role in crop production and crop value (Shah et al., 2017). It occurs in a large variety of metabolic functions. A number of key functions of boron in biochemical process and plant physiology include, sugar transport cell wall structure, lignification and membrane stability. It also regulates the metabolism of protein, carbohydrate, phenol, nucleic acid and indole acetic acid (IAA) (Seth and Aery, 2017). Boron is a fundamental element which is necessary for the development and growth of plant (Tanaka and Fujiwara, 2008). The requirement of boron different in different crops but the optimum assessment of boron for most crops is about 20 mg/kg (Shah et al., 2017). The availability of boron application in proper amount in soil is necessary for growth, development and yield of plants (Tewari et al., 2009). In the ground layer boron availability range from 20 to 200µg/g, while the boron range in soils between 0.4 and 5µg/g (Shah et al., 2017). The boron content in plant tissues normally found about 2µmol/g (20µg/g) of dry mass. Plants absorb the

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boron in the form of boric acid (Hansch and Mendel, 2009). The range of boron among efficiency and deficiency, both is very nearly producing toxic symptoms that affect crop quality and crop production (Cristobal et al., 2008). In early stage the low concentration of boron in soil must show boron shortage symptoms in plants, such as abnormal growth of the apical growing region. Later on, plants show retarded growth, because boron shortage affects physiological, biochemical and molecular processes and result the decreasement in yield (Tewari et al., 2009). When the concentration of boron is more for plant tissues the symptoms of boron toxicity appear in the form of necrotic and chlorotic patches at the apical and margins of older leaves (Seth and Aery, 2017). The optimum requirement of boron for plants is vary throughout the plant kingdom. Graminaceous species require a minor amount of boron than the other monocots and dicots (Seth and Aery, 2014).

Triticum aestivum L. Wheat *(*siren) is the most broadly grown crop in the earth. In which has long stems and slender leaves that are hollow in most varieties. The arrangement of flower is composed of various numbers of minute flowers, ranging from 20 to 100 (Raza et al., 2007). The flowers are borne in groups of two to six in structures known as Spikelets, which later serve to house the subsequent two or three grains created by the flowers. However grown under a wide range of soil and climates, *Triticum aestivum* is best adapted to temperate regions with rainfall between 30 and 90 cm (12 and 36 inches) (Adiloglu et al., 2007). *Triticum aestivum* is the key species of cereal grasses of the genus *Triticum* (family *Poaceae*) and their edible grains. *Triticum aestivum* is one of the oldest and most vital of the cereal crops of the thousands of varieties known, the most important are common wheat used to make bread. Wheat (*Triticum aestivum*.) is one of the first cultivated food crops and has been the basic staple food of the major civilizations of Europe, North Africa and West Asia for last 8000 years. In Pakistan, wheat being the main faster food cultivated on the largest acreages. Pakistan falls in ten major wheat-producing nation of the world in terms of area under wheat cultivation, total production and yield per hectare (Raza et al., 2007). Wheat is the important diet of population as it constitutes 60% of the daily diet of common man in Pakistan and average per capita utilization is about 125kg and occupies a central position in agricultural policies of the government. The current study aims to screen out different biochemical parameters of *T. aestivum* at different boron levels. Our studies comprised on following objectives:

- To determine the effect of boron on biochemical contents of wheat variety *(*siren).
- To assess the effects of boron on the biochemistry of the test plant.
- The response of *T. aestivum* to boron.

Material and Methods

Experimental design

The experiment was conducted in the botanical garden of Bacha Khan University, Charsadda during the wheat growing season (Winter) of 2019. The experiment was carried out in field using Randomized Complete Block Design (RCBD) during the month of November under natural light conditions, when the average temperature was 22^0C. The seeds of *Triticum aestivum* L *(*siren) were sown at 2 cm depth in pots with a height of 30 cm and 25 cm diameter. The pots were filled with (3kg) of clay loamy soil. The soils were analyzed having sand 27.15%, silt 19.86% and clay 52.98%, pH 6.9 water holding capacity 50.37%, bulk density 0.96 g/cc, specific gravity 4.03 and organic carbon 0.82%. No additional supplement was added to the experimental soil. The pots were treated with boron applied as boric acid at the dose of $B_{10, 20, 30, 50}$ and 60mg/kg. Each treatment was replicated three times in a design. Pots without the addition of boron consider the control (B_0).

Biochemical analysis

- Chlorophyll content of leaves was measured by the method of Arnon (1949).
- Protein content of leaves was calculated by the method of Lowery et al. (1951) using BSA as standard.

- Proline content of leaves was determined by the method of Bates et al. (1973).
- Sugar estimation of fresh leaves was done following the method of Dubois et al. (1956).
- Carotenoid content of leaves was calculated by the method of Aery et al. (2010).
- Phenol content was determined according to the Folin–Ciocalteau method as described by (Mahadevan and Sridhar, 1982).
- Amino acid content was measured by the method of Sparkman et al. (1958) using glycine as standard.

Statistical analysis

All the data collected were determined by using Randomized Complete Block Design (RCBD). (ANOVA) analysis of variance was apply on replicate data using STATISTIX 8.1. When the ANOVA showed a statistical effect, means were separated by least significant differences (LSD) at $P < 0.05$.

Results

The impact of boron application on plant biochemical parameters of wheat were different, sufficient application of boron (B_{10}, B_{20}, B_{30} mg/kg) show promotion in parameters while boron deficient (B_0) and boron excess (B_{50} and B_{60}) showed reduction in biochemical parameters of *T. aestivum*. The occupied results are being follow.

Total Chlorophyll a/b Ratio

Estimation of chlorophyll a/b ratio content (mg/g) of *Triticum aestivum* were recorded during the vegetative phase (Fig1&2). The result showed that maximum chlorophyll a/b ratio was found at sufficient level of boron B_{20} (0.1919,7.004). While minimum chlorophyll a/b ratio was found at highest level of boron B_{60} (0.1216, 2.52) at $P < 0.05$.

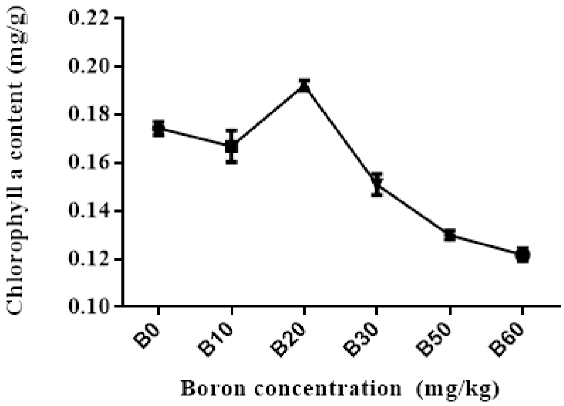

Fig 1. Effect of B on Chlorophyll a content under different boron treatments. Chlorophyll a content was high under normal concentration of B i.e., B_{20}, while under deficient and excess level this parameter was low. Error bars represent ± SD of three biological replicates.

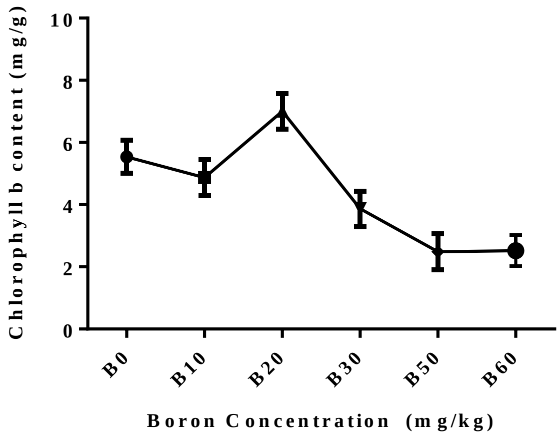

Fig 2. Effect of B on Chlorophyll b content under different boron treatments. Chlorophyll b content was high under normal concentration of B i.e., B_{20}, while under deficient and excess level this parameter was low. Error bars represent ± SD of three biological replicates.

Soluble leaf Protein Contents

Protein content (mg/g) was assessed during the nutrition phase (Fig 3). The result of Protein contents of *Triticum aestivum* showed promotion under sufficient amount of boron B_{30} (6.323). While reduction recorded in excess level of boron B_{60} (2.333) at $P < 0.05$.

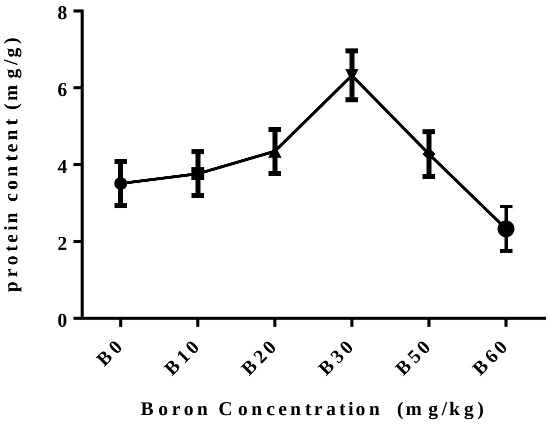

Fig 3. Effect of B on Protein content under different boron treatments. Protein content was high under sufficient concentration of B i.e., B_{30}, while under deficient and toxiclevel this parameter was low. Error bars represent ± SD of three biological replicates.

Proline Contents

Comparison of proline content (mg/g) was determined at the nutrient stage. The proline content of *Triticum aestivum* show significantly increasment under sufficient boron stress (Fig. 4). Maximum increasment showed under boron excess amount B_{30} (26.666) was significantly similar to B_{20} (25.636). While minimum increasment showed under toxic boron B_{60} (21.35) at $P < 0.05$.

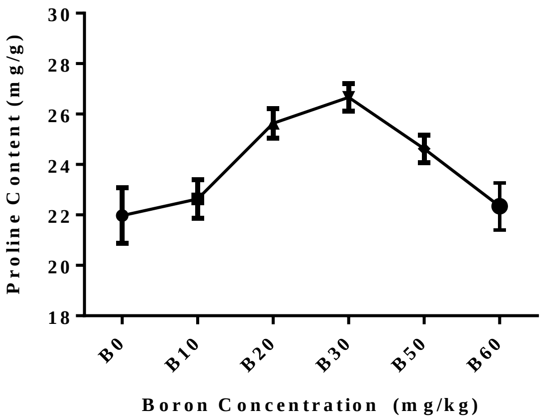

Fig 4. Effect of B on Proline content under different boron treatments. Proline content was high under sufficient concentration of B i.e., B_{30}, while under deficient and excess level this parameter was low. Error bars represent ± SD of three biological replicates.

Soluble Sugar Contents

The assessments of sugar content (mg/g) at the vegetative stage were made. The sugar content of *Triticum aestivum* show significantly increasment under sufficient boron stress (Fig.5). The result demonstrates that maximum sugar content was found for low amount of boron B_{30} (77.333) was significantly similar to B_{20} (76.014). While minimum sugar content was found for toxic boron B_{60} (71.85) at $P < 0.05$.

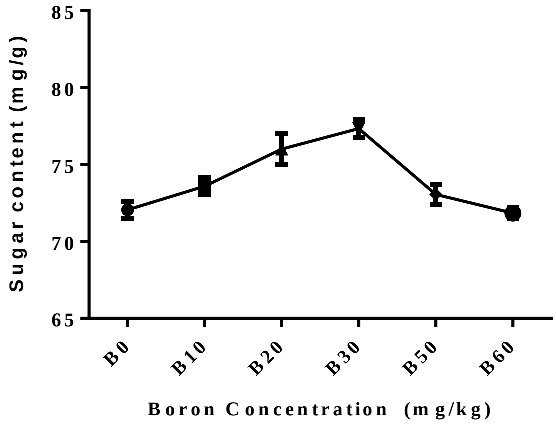

Fig 5. Effect of B on sugar content under different boron treatments. Sugar content was high under sufficient concentration of B i.e., B_{30}, while under deficient and excess B this parameter was low. Error bars represent ± SD of three biological replicates.

Caroteoid Contents

Estimation of carotenoid content (mg/g) was carried out at the nutrient stage (Fig 6). The result of carotenoid contents in *Triticum aestivum* showed reduction under excess concentration of boron. Maximum carotenoid content was observed at sufficient level of boron B_{20} (6.076). While minimum carotenoid content was observed at excess amount of boron B_{60} (1.957) was significantly similar to B_{50} (2.150) at $P < 0.05$.

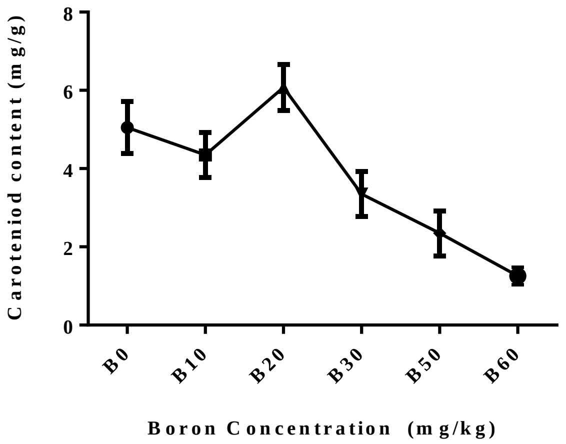

Fig 6. Effect of B on carotenoid content under different boron treatments. Carotenoid content was high under sufficient concentration of B i.e., B_{20}, while under deficient and excess level this parameter was low. Error bars represent ± SD of three biological replicates.

Total Phenol Contents

The assessments of phenol content (mg/g) at the vegetative stage were made. The phenol content of *Triticum aestivum* show significantly increasment under sufficient boron stress (Fig. 7). The result showed that maximum phenol content was found for sufficient treatments B_{30} (18.315) was significantly similar to B_{20} (17.295). While minimum phenol content was found for toxic boron B_{60} (14.000) at $P < 0.05$.

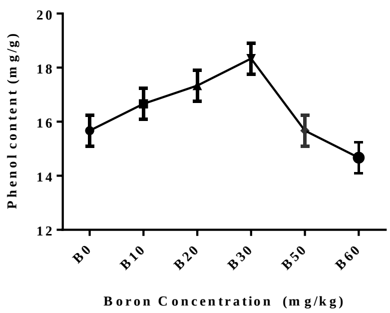

Fig 7. Effect of B on phenol content under different boron treatments. Phenol content was high under sufficient concentration of B i.e., B_{30}, while under deficient and excess level this parameter was low. Error bars represent ± SD of three biological replicates.

Estimation of Amino Acid Contents

The study of amino acid in *Triticum aestivum* during the vegetative phase under boron was determined. The amino acid content of *Triticum aestivum* show significantly increasment under sufficient boron (Fig. 8). The result indicated that total amino acid content in *Tritium aestivum* high under sufficient concentration of boron B_{30} (20.650) was significantly similar to B_{20} (18.990). While low amino acid content was observed at lacking concentration of boron B_0 (15.115) was significantly similar to B_{10} (14.50) at $P < 0.05$.

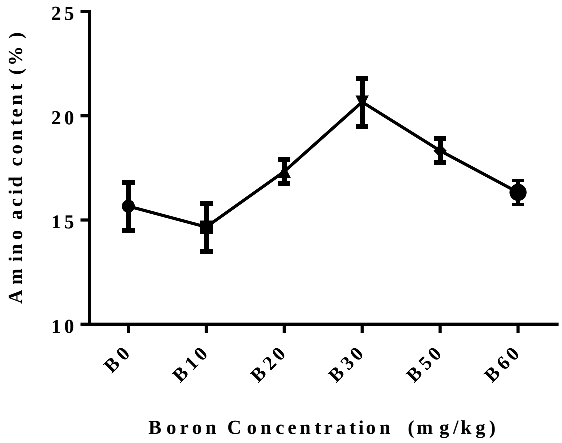

Fig 8. Effect of B on amino acid content under different boron treatments. Amino acidcontent was high under sufficient concentration of B i.e., B_{30}, while under deficient and toxic level this parameter was low. Error bars represent ± SD of three biological replicates.

Discussion

Boron fertilizer is a basic factor that plays a vital role in growth and yield responses in all the crops. Boron is supplied as a fertilizer in those areas where boron is lower concentration but high concentration of boron can also be poisonous to plants. Current Studies shows that out of the various concentrations of B, (50mg/kg) was moderately toxic while (60mg/kg) generated high toxicity and induced B stress response to confer tolerance in wheat. Further, a possible mechanism of B toxicity response in wheat is suggested. Biochemical result showed that the Chlorophyll "a" and chlorophyll "b" contents were increased with sufficient amount of boron (20mg/kg) as compared to excess and deficient amount of Boron.

This results are parallel with (Seth and Aery., 2014) observed that photosynthetic pigments (Chlorophyll "a" and chlorophyll "b") contents were increased with lower concentration of boron (4µg/g) as compared to higher applied doses of boron. Inbaraj and Muthuchelian (2011) observed decreasement in carotenoid, chlorophyll a, b, and total chlorophyll contents in boron deficient and boron excess treatments in comparison to boron sufficient treatments. Wang et al. (2011) observed reduction in carotenoid and chlorophyll contents at higher doses of boron (100, 300 and 500µmol/l) in pear plants. High concentration of boron decreased the levels of chlorophyll a, chlorophyll b and carotenoid contents were observed (Seth and Aery 2017). Present studies indicate that reduction in protein contents was observed at deficient and excess of boron concentrations (B0, B50 and B60) and increasement at sufficient boron concentration (B30) Similar result is reported by Seth and Aery (2017) and noted the minimum protein contents at lower dose of boron (4µg/g) and maximum protein contents at sufficient applied boron (32µg/g) in wheat plant. Mahboobi et al. (2000) study those protein profiles of barley in response to higher concentration of boron. Proline is synthesized during nitrogen metabolism and functions as protectant and maintains cellular homeostasis (Costa et al., 2011; Szabados and Savoure, 2010). Varshney et al. (2015) explored the boron induced impacts on photosynthesis, growth and antioxidant system under various boron levels (0, 10, 20, 30, 40, 50, and 60mg/kg) in two varieties of *Brassica juncea*. The B treatments (20, 30, 40, 50 or 60 mg/kg) significantly increased the proline and activity of antioxidant enzymes (CAT, SOD, and POX) in both the varieties. The studies of Varshney et al. (2015) support and are in accord with the present investigation. In our study, accumulation of proline content increase at sufficient boron concentration (B_{20} and B_{30} mg/kg) may be due to increased proline biosynthesis and protein breakdown and or decreased breakdown of proline. Current studies indicated that the variation in the sugar content at various concentration of boron. Decreasement in sugar content was observed at toxic boron concentration (B_{60}) and increasement at sufficient boron concentration (30mg/kg). These results are similar with the views of (Qasim et al., 2003; Tumova et al., 2015; Hellal et al., 2009). In present studies increasement in the soluble sugar content at sufficient soil boron applications indicates plants adaptive response to boron stress for efficient translocation of boron for their active exclusion from the roots to soil (Reid, 2014). Maximum increasement in carotenoid contents was observed at sufficient level of boron (20mg/kg) beyond this concentration carotenoid contents started decreasing on higher concentration of boron decreasement in carotenoid contents has been reported by (Wang et al., 2011) observed a decreasement in chlorophyll and carotenoid contents at higher doses of boron (100, 300 and 500µmol/l) in pear plants. Decreased levels of chlorophyll a, chlorophyll b and carotenoid contents were observed as boron concentration increased Landi et al. (2013) observed a reduction in carotenoid contents under boron stress conditions (10 and 20 mg L^{-1} nutrient solution) in *Cucurbita pepo* while there is no significant difference in carotenoid contents was observed in *Cucumis sativus.* Qasim et al. (2013) studied the effects of boron toxicity on two genotypes of tomato (cv. *Kosaco* and cv. *Josefina*) under 0.5 and 2 mM application of boron, the leaves of cv. *Josefina* showed a maximum carotenoid concentration, whereas did not occur in cv. *Kosaco*. Phenolic compound are the markers of stress in plants. In present investigations, phenolic accumulation was observed at sufficient amount of boron concentrations (30mg/kg) the same result is reported by Pardossi et al. (2015) and Shah et al. (2017) who noticed a remarkable increase in leaf total phenolic concentration as tolerance measure to cope with boron sufficient. Our result showed that maximum concentration of amino acid content was reported at sufficient amount of boron (20 and 30mg/kg) was significantly similarly to (Szabados and Savoure, 2010) he indicate that the boron application had a positive effect on the amino acid content of wheat plant.

Conclusion

We concluded that both B deficiency and toxicity alter physiology and biochemistry of plants. It causes inhibition of root growth, plant height, plant biomass and finally decrease plant growth. Furthermore, biochemical content also decreased under B deficiency and excess level. The biochemical contents are maximum at sufficient concentration B_{20} and B_{30} treatment and the plant grow well and healthy at this level. Therefore, growing B tolerant crops may be the only possible solution to boron toxicity and deficiency.

Conflict of interest: All authors declare no conflict of interest

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