

## Original Research Article

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## Comparative Study of Boron Accumulation in Flag Leaf, Spike and Grain of Wheat (*Triticum aestivum* L.) Grown under Heat Stress

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### ABSTRACT

Micronutrients are essential for human health and crucial for plant survival. Increasing temperature and heat are major abiotic stress which limits yield and nutritional quality of wheat. The objective of study is to evaluate the effect of heat stress on boron (B) micronutrient accumulation in 18 wheat varieties at three phenological stages (Booting and grain filling and maturity). Pot experiments were conducted in randomized block design with three replications during *rabbi* 2012-13 at DRPCA, Pusa, Bihar. Results showed the accumulation of micronutrient under heat stress in booting, grain filling and maturity decreased significantly in flag leaf, spike and grain but some varieties enhances the accumulation of micronutrients under heat stress condition significantly. The wheat variety namely C306 (68.29 ppm), MB4010 (53.66 ppm), HD2888 (52.73 ppm), Iepacarabe (49.71ppm), AKAW4008 (41.16 ppm) and Kauz/AA/Kauz (40.73 ppm) are good accumulator of B in flag leaf and varieties F5-995 (75.50ppm), Sonalika (30.83ppm), MonsAld's (29.93 ppm) and Iepacarabe (21.73ppm) increases boron accumulation in spike under heat stress. The enhanced boron accumulation under high temperature in grain was observed in variety namely AKAW4008 (32.82ppm) and PBW343 (26.94 ppm) respectively. The wheat variety F5-995, PBW343 MonsAld's and AKAW4189-3 showed overall better micronutrients accumulation in flag leaf, spike and grain under stress condition and appeared as potentially important wheat variety used during heat stress.

### Keywords

Wheat, micronutrient, Flag leaf, Spike, Booting and heat stress

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### Introduction

Wheat is one of the most important energy and nutrition sources of over growing world population. Wheat provide 21% food calories and 20% protein to more than 4.5 billion people worldwide and also consumed directly in different forms (Barun *et al.*, 2010). High temperature is a major problem in agricultural cropping systems world-wide, with

unexpected variations in temperature causing reduction in growth, development, metabolism and yield, with great risks for future global food security (Christensen and Christensen 2007; Parent *et al.*, 2010). The optimum temperature for grain development of wheat varies between 15-18 °C (Chowdhury and Wardlaw, 1978; Modhej *et al.*, 2011). Under heat stress Zong-hu and Rajaram (1994) reported that decline in number of spike per

plant, grain number per spike and also reduction in grain weight.

Boron (B) is a member of the subgroup III of metalloids and has intermediate properties between metal and non-metals (Marschne, 1995). Despite its low abundance in nature, boron is widely distributed in lithosphere and hydrosphere, boron concentration ranging from 5-10 mg/kg in rocks (Shorrocks, 1997), 3-30 microgram/kg in rivers (Power and Woods, 1997) and approx.. 4.5mg/L in ocean (Lemarchand *et al.*, 2000). Boron (B) is an essential element for plants and boron deficiency is the most widespread of all the micronutrient deficiencies in many crop regions from tropical to temperate zones (Shorrocks, 1997). Boron is required for flowering, cell wall formation, membrane integrity, carbohydrate utilization and stomatal conductance (Marschner, 1995) but vegetative growth of the plant is apparently unaffected by B deficiency (Mozafar, 1993). However, in many regions in the world B concentration in soil or irrigation water often exceeds plant requirements, thus becoming a threat to crop productivity (Nable *et al.*, 1997). Thus in the field it is commonly observed that plants remained free of B deficiency symptoms until reproductive growth commences when a variety of B deficiency symptoms may appear including abortion of flower buds or flowers, male sterile flowers, aborted fruit and pots with reduced number of seeds and reduced seed size (Dell and Huang 1997; Huang *et al.*, 2000). Under low rainfall conditions boron cannot be sufficiently leached and therefore may be accumulate to leaves that become toxic to plant growth (Reid, 2007a and b) and also induces oxidative stress, leaf chlorosis, necrosis, which reduce photosynthesis and dry matter accumulation. In wheat genotypes due to B deficiency, grain set failure associated with male sterility was observed in Brazil in 1962 (de Silva *et al.*, 1980). Cheng and Rerkasem, 1993; Rerkasem, *et al.*, 1993; Huang *et al.*, 2000 reported that Boron

deficiency cause grain set failure in wheat which is associated with poorly developed pollen and anthers. Jamjod *et al.*, (2004) reported two genes, *Bod1* and *Bod 2* that could account for genotypic variation in response to boron in wheat genotypes. The main goal of this study was to assess whether the greater boron accumulation such as in flag leaf, spike and grain compared with normal and heat stress.

## **Materials and Methods**

### **Plant material and growth conditions**

Plant material for micronutrients analysis studies of eighteen wheat variety namely AKAW4008, Halna, Pusa gold, AKAW4189-3, PBW343, HD2733, C306, HD2285, RSP561, Kauz/ AA/Kauz, Iepacarabe, F5-995, HD2888, MonsAld's, Kauz-dwarf, MB4010, Sonalika, and Raj3765 obtain from RKVY Project titled: Enhancement of heat tolerance in locally adapted wheat cultivars of Bihar" in department of Agricultural Biotechnology and Molecular Biology, DRPCA, Pusa, Bihar. Seeds were washed in distilled water and sterilized by immersion in mercury dichloride solution (1:1000) for two minutes. The seed were next washed five times in deionizer water and placed in an oven at 28<sup>0</sup>C for 24 hours. After that the seeds were grown in greenhouse in 24x21 cm pots containing soils. The experiment was conducted using 108 pots. 18 wheat varieties were sown in plastic pot using completely randomized design (CRD) in six replications. During the vegetative growth plant were kept under similar environment conditions. Half of the pots, three replications of each of the 18 wheat varieties were shifted under polyhouse condition to provide heat stress before booting. For each varieties 6 replication were used (Three heat stress and three in natural condition). During the period of experiment the position of the pot was changed weekly, to minimize the effects due to irradiance

variations. Plants were irrigated weekly to 1/2 Hoagland solution as per schedule and requirement. Maximum and minimum temperatures ( $^{\circ}\text{C}$ ) as well as relative humidity (%) were recorded during the crop growth period. The minimum ( $6.5^{\circ}\text{C}$ ) and maximum ( $38.9^{\circ}\text{C}$ ) temperature recorded in open condition. Polyhouse minimum ( $10.4^{\circ}\text{C}$ ) and maximum ( $45.23^{\circ}\text{C}$ ) temperature recorded.

### **Sample preparation and nutrient analysis**

The concentration of Boron was determined at three phenological stages as booting (flag leaf), grain filling (spike) and maturity (grain) of control and stressed plants. The plant samples were washed with 0.2% liquid detergent (The liquid detergent removes waxy coating of the leaf surface and any soil particle) solution, then with 0.1N HCl solution (0.1N HCl removes metallic contaminants) and finally with deionized water (Deionized water washes the previous two solutions). After that the extra moisture was wiped out, the sample was placed in new paper bags and dried in an oven at  $70^{\circ}\text{C}$ . Plant samples were digested following the methods given by Hatcher and Wilcox (1950). 0.5g oven dried sample was digested in diacid mixture ( $\text{HNO}_3:\text{HClO}_4$ , 10:4) on a rectangular hot plate. After the completion of the digestion the colour become milky white. It was filtered through Whatman filter paper and volume was made up to 50 ml by adding double distilled water. After that 2 ml aliquot was taken in conical flask and 1-2 drop NHCl and 10 ml conc.  $\text{H}_2\text{SO}_4$  was added to it after cooling 15 ml 0.05% carmine solution was added to it. After 45 minutes, intensity of colour was measured in photoelectric colorimeter at 585 nm wave length and data were recorded.

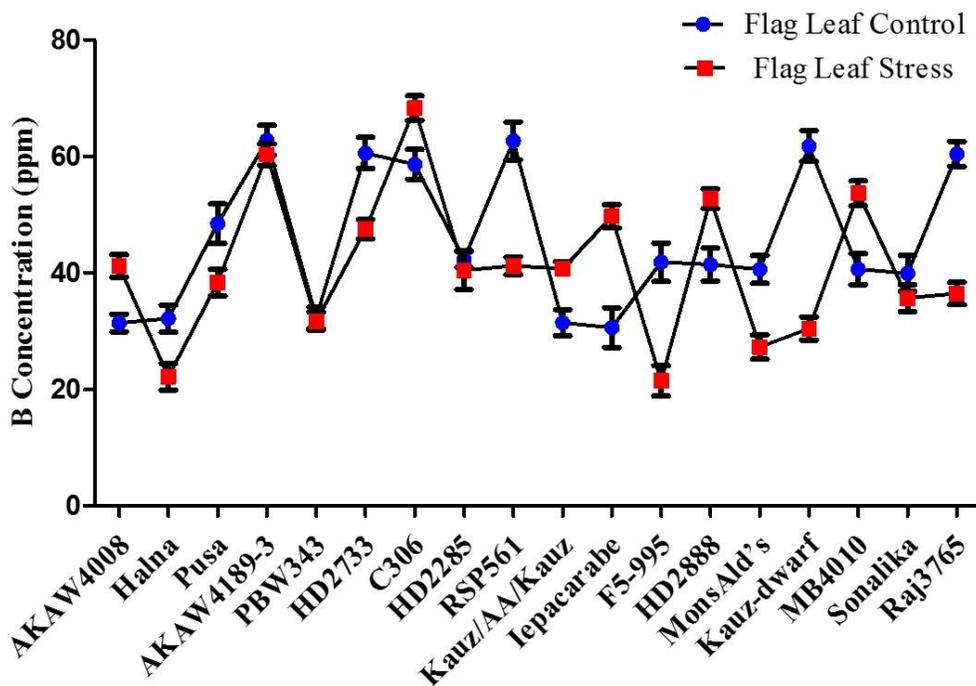
### **Statistical analyses**

Graph and Two-way Analysis of Variance (ANOVA) was carried out using graph pad

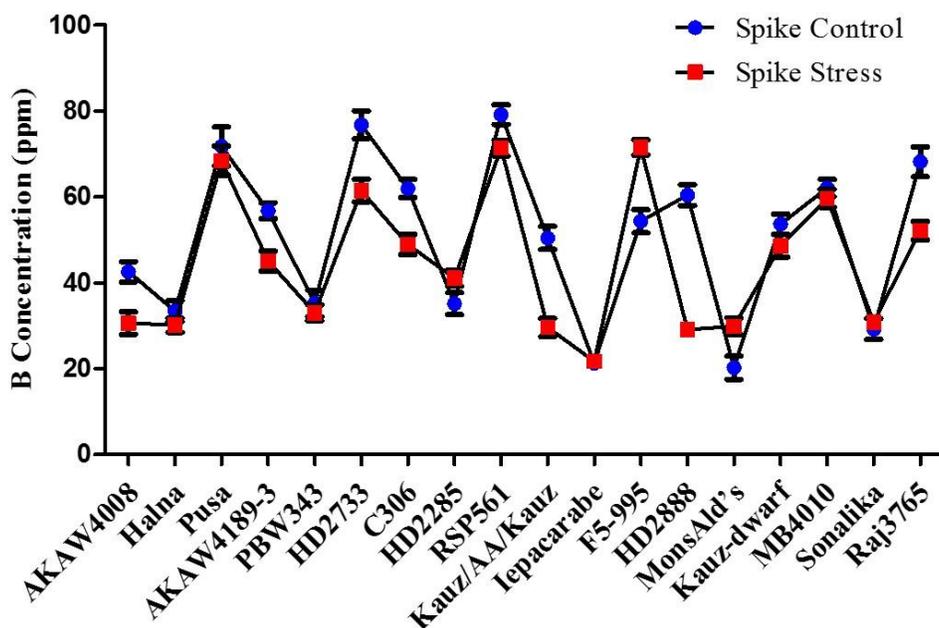
prism statistical software version 5.01. Values presented were means  $\pm$  standard error (SE) of three replicates in each group.  $P$ -values  $\leq 0.05$  were considered as significant.

### **Results and Discussion**

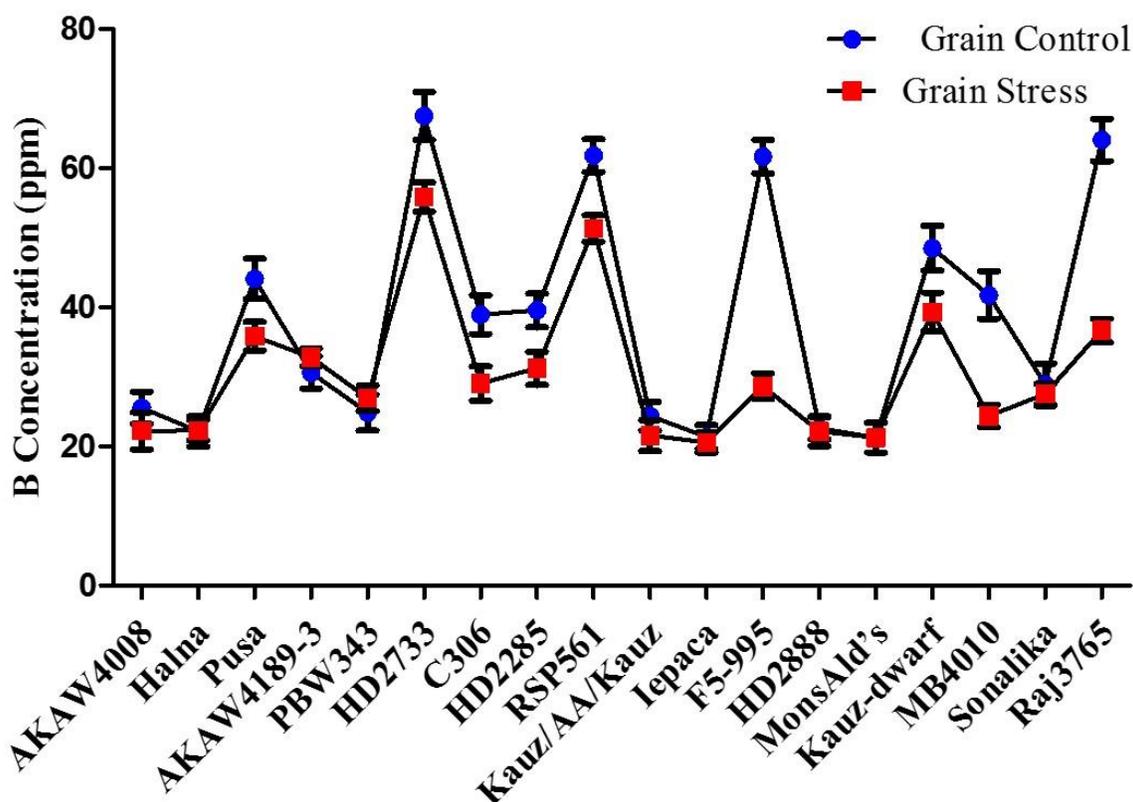
Temperature and nutrition are two of the major components of environmental variation, and together provide significant limitations to successful crop production. Increasing temperatures during developmental stage disturb metabolism, growth and reproduction in plant. Elevated temperature affects the micronutrient accumulation in flag leaf, spike and grain of (*Triticum aestivum* L.) variety at three phenological stages (booting, grain filling and maturity). Analysis of Boron was done in flag leaf, spike and seeds obtained from three phenological stages respectively of 18 varieties of wheat grown under two conditions i.e. normal and heat stress. The data regarding boron content revealed that boron content significantly decreased under heat stress as compared to normal condition but in severely-stressed plants, levels of B increased in comparison to unheated plant. Among wheat genotypes C306 (68.29 ppm) recorded significantly highest B content in flag leaf under high temperature followed by MB4010 (53.66 ppm), HD2888 (52.73 ppm), Iepacarabe (49.71 ppm), AKAW4008 (41.16 ppm) and KAUZ/AA/KAUZ (40.73 ppm). Marschner (1995) observed that the amounts of B required for seed formation are generally greater than those required for vegetative growth. Varieties namely Halna, Pusa gold, AKAW4189-3, HD2733, HD2285, RSP561, Iepacarabe, F5-995, MonsAld's, Sonalika, and Raj3765 have accumulated significantly more boron in flag leaf under normal condition. Abdel-Motagally and El-Zohri, (2016) also reported that improvement of wheat yield grown under drought stress by boron foliar application at different growth stages such as booting and grain filling stage (Fig. 1).



**Fig.1.** Effect of heat stress on micronutrient (B) accumulate of 18 wheat varieties under control and stress flag leaf



**Fig.2.** Effect of heat stress on micronutrient (B) accumulate of 18 wheat varieties under control and stress spike



**Fig.3.** Effect of heat stress on micronutrient (B) accumulate of 18 wheat varieties under control and stress grain

Despite the fact that wheat is grown as a winter season crop in India, the occurrence of heat stress (maximum daily temperatures above 35°C) is a frequent phenomenon across the India wheat belt during the growing season, particularly during the grain filling period (Blumenthal *et al.*, 1995).

As depicted in figure 2, boron content significantly increases in spike under heat stress up to (75.50, 30.83, 29.93, 21.73 ppm) in varieties namely F5-995 Sonalika, MonsAld's and Iepacarabe respectively. The remaining varieties namely AKAW4008, Halna, Pusa gold, AKAW4189-3, PBW343, HD2733, C306, HD2285 RSP561, Kauz/AA/Kauz, HD2888, Kauz-dwarf, Raj3765 and MB4010 there have been reduced B

accumulation significantly under high temperature in comparison to normal condition.

Deficiency of boron leads to reduced flowering and grain filling stage produce less number of seeds in spike. Figure 3 represents the mean value and SE the accumulation of B under heat stress significantly increased in seeds in two variety AKAW4008 (32.82ppm) and PBW343 (26.94 ppm). Arif *et al.*, (2006) observed that the enhanced wheat crop yield by B spraying at booting stage may be due to provision of B at initial stages which might have enhanced the accumulation of assimilate in the grains. Velu *et al.*, (2016) also reported the amount of Fe and Zn increased in grain of PBW 343 wheat variety under elevated

temperature. Not more studies were carried out to examine the effect of boron and climatic changes in wheat genotypes. The overall results showed that boron accumulation in flag leaves faster than the spike and grain under heat stress. The wheat variety F5-995, PBW343 MonsAld's and AKAW4189-3 showed overall better micronutrients accumulation in flag leaf, spike and grain under stress condition and appeared as potentially important wheat variety used in plant breeding for production of heat stress variety.

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