Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

J P P Journal of Pharmacognosy and Phytochemistry

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2018; 7(2): 2979-2982
Received: 11-01-2018
Accepted: 12-02-2018

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Comparative study of Fe and Mn micronutrient accumulation in flag leaf and spike 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 to evaluate the effect of heat stress on micronutrient (Fe & Mn) accumulation in 18 wheat varieties at two phenological stages (Booting and grain filling).Pot experiments were conducted in randomized block design with three replications during *rabbi* 2012-13 at DRPCAU, Pusa, Bihar. Results showed the accumulation of micronutrient under heat stress in booting and grain filling decreased significantly in flag leaf and spike but some varieties enhances the accumulation of micronutrients under heat stress condition significantly. The wheat variety namely AKAW4189-3, MonsAld's, Iepacarabe, PBW343, Pusa gold, AKAW4008 and Halna are good accumulator for Fe in flag leaf and varieties PBW343, HD2888, Kauz-dwarf, HD2733, F5-995, Iepacarabe, and AKAW4008 increased accumulation in spike. Maximum iron accumulation under stress condition recorded in variety AKAW4189-3 (252.33 ppm) in flag leaf and PBW343 (239.73 ppm) in spike. The enhanced Mn accumulation under high temperature was observed in flag leaf of variety namely AKAW4008, Pusa gold, Iepacarabe, F5-995, MonsAld's, and Raj 3765 respectively whereas, AKAW 4008, AKAW4189-3, PBW343, C306, HD2285, HD2888, MonsAld's and Sonalika has good accumulation property of Mn in spike. Variety namely F5-995 (32.57 ppm) and PBW343 (28.46 ppm) show good accumulation of manganese under stress condition in flag leaf and spike respectively. The wheat variety AKAW 4008, F5-995, PBW343,HD2888, MonsAld's and AKAW4189-3 showed overall better micronutrients accumulation in flag leaf and spike under stress condition and appeared as potentially important wheat variety used in plant breeding and biofortification strategy to reduce the problem of malnutrition.

Keywords: wheat, micronutrient, flag leaf, spike and heat stress

Introduction
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 & Christensen 2007; Parent *et al*. 2010) [4, 16]. The optimal mean temperature for the crops growth cycle varies between 15-18 ^{0}C (Chowdhury and Wardlaw, 1978) [7]. Under heat stress the yield performance of wheat genotypes is strongly affected (Spiertz 1977; Wardlaw *et al.*, 2002) [21, 25]. Wardlaw *et al.*, 1989 [24] and You *et al*. 2009 [22] reported that a global reduction in yield of about 3-4% and about 3-10% respectively when the mean temperature rise of just 1 ^{0}C above the optimum value. During grain filling, heat shocks of about 35-40 ^{0}C also have a negative effect on grain quality and dry weight (Ciaffi *et al.*, 1996) [6].
The deficiency of micronutrients in plant is due to their low contents in soil or reduces their availability to plants by biotic and abiotic factors (Sharma and Chaudhary, 2007) [19] Although, micronutrient elements are required in very small quantities for adequate plant growth, development and reproduction and their deficiency may cause great disturbance in the physiological and metabolic processes in plant. Iron is one of the most important elements for plant growth, especially for chloroplast development, photosynthesis, and respiration and DNA synthesis. It also plays a key regulating role in Fe-requiring enzyme reactions and redox systems in plant (Marschner, 1995) [14]. Six micronutrients that is Mn, Fe, Cu, Zn, B and Mo are known to be required for all higher plants (Welch *et al.*, 1991) [27]. Soylu *et al.*, (2005) [20] and Kenbaev & Sade (2002) [13] reported significant increase in number of spikes m^{-2} in wheat with foliar application of different micronutrients individually or in combined iron deficiency induced chlorosis occurs mainly on calcareous soils with high pH and high concentration of bicarbonate. Higher plants have different adaptive mechanisms in response to Fe deficiency. Seed nutrient reserves may be important for an early establishment of crop seedlings under

nutrient deficiency (Milberg *et al.*, 1997 and Rengel *et al.*, 1999) [15, 17]. Manganese is necessary in photosynthesis, nitrogen metabolism and to form other compounds required for plant metabolism. Temperature stress (high and low) reduces the nutrient uptake and induces many morphological and physiological disorders in plants. Manganese is also reported to involve in the activation of many enzymes in plant systems, mostly in oxidation-reduction, decarboxylation and hydrolytic reactions (Marschner, 1995) [14] hence may play a role in detoxification of ROS. Recently, it has been reported that Mn has a crucial role production of oxygen free-radicals and increase the anti-oxidative compounds and enzymatic activities under temperature stress.

Wheat (*Triticum aetivum* L.) is one of the most important staple food crops of the family *Poaceace* and second most produced cereal crop worldwide which constitutes about 28% of dietary energy and 20% protein (Braun *et al.*, 2010) [2]. Improving the nutritional quality of wheat is therefore of paramount importance (Velu *et al.*, 2016) [23]. Climate change and abiotic stresses such as drought and heat stress will likely affect nutritional composition of wheat grain. Therefore, this study set out to establish the effect of high temperature on iron and manganese micronutrient and their association with agronomic traits such as booting and grain filling in wheat varieties with varying levels of yield potential under normal and stressed conditions.

Materials and Methods

Plant material and growth conditions

Plant material for Fe and Mn micronutrient 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, DRPCAU, 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 ^{0}C for 24 hours. After that the seeds were grown in greenhouse in 24×21 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 to1/2 Hoagland solution as per schedule and requirement. Maximum and minimum temperatures (^{0}C) as well as relative humidity (%) were recorded during the crop growth period. The minimum (6.5 °C) and maximum (38.9 °C) temperature recorded in open condition while in polyhouse minimum (10.4 °C) and maximum (45.23 °C) temperature recorded.

Sample preparation and nutrient analysis

The concentration of micronutrients (Fe and Mn) was determined in flag leaf and spike at two phenological stages such as booting and grain filling 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, than 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 ^{0}C.Plant samples were digested following the methods given by Hatcher and Wilcox (1950) [11]. 0.5g oven dried sample was digested in diacid mixture (HNO3:HClO4, 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 the volume was ready for Fe and Mn analysis. A double beam Atomic Absorption Spectrophotometer (AAS) (Perkin Elmer USA) was used for the purpose and data was collected. The analysis of micronutrient Fe and Mn was done at 248 and 279 nm respectively.

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 ± standard error (SE) of three replicates in each group. *P*-values ≤0.05 were considered as significant.

Results and Discussion

Temperature and nutrition are two major components of environmental variation that provide significant limitations to a successful crop production. Increasing temperatures during developmental stage disturb metabolism, growth and reproduction in plant. Micronutrients are essential for human health and crucial for plant survival. Elevated temperature affects the micronutrient status in flag leaf, spike and grain of (*Triticum aetivum* L.) variety of two phenological stages (booting and grain filling). The data regarding iron content revealed that iron content significantly decreased under heat stress as compared to normal condition but in severely-stressed plants, levels of Fe and Mn increased in compression to unheated plant**.** Among wheat genotypes AKAW4189-3 (252.33 ppm) recorded significantly highest Fe content in flag leaf followed by MonsAld's (231.36 ppm), Iepacarabe (227.1ppm), PBW343 (177.53ppm),Pusa gold (157.5ppm), AKAW4008(156.53ppm) and Halna (136.63 ppm 17.15) earlier similar pattern was observed by Cakmak *et al.*, (2004) and Dias *et al.*, (2009) [8, 9] in their line of study. Lowest iron content was found in genotype HD2285 (45.86ppm) in flag leaf similar result was reported by Welch and Graham 2004 [26]: Rawashdeh & Sala. (2015) [18]. Dias *et al.*, 2009 [8, 9] where as Velu *et al.*, (2016) [23] and Amarshettiwar *et al*., (2018) [1], reported in wheat grain. As depicted in Figure.2. Fe content significantly increases in spike under heat stress up to (239.73, 239.13, 226.56, 208.96, 198.56, 157.66 and 118.56 ppm) in varieties namely PBW343, HD2888, Kauz-dwarf, HD2733, F5-995, Iepacarabe, and AKAW4008 respectively. Dias *et al.*, 2009 [8, 9] reported similar result in wheat grain filling stage. Iron is an essential mineral as it is needed for hemoglobin synthesis and its deficiency causes iron-deficiency anemia which is a common problem in women and children (U.S. National Library of General Medicine Based on the limited past studies, we know that heat stress can negatively affect plant nutrient relations but the effect of heat stress, chronic or abrupt, on root nutrient uptake rate has been little studied.

Manganese is a mineral that is found in several foods including nuts, legumes, seeds and leafy vegetables. Deficiency of manganese leads to osteoporosis and other illnesses. Figure.3 represent the mean value and the SE the accumulation of Mn under heat stress has significantly increased in variety AKAW4008 (21.30 ppm), Pusa gold (24.94 ppm), Iepacarabe (21.83 ppm), F5-995 (32.57 ppm), MonsAld's (17.9 ppm) and Raj3765(23.81) in flag leaf whereas in spike the varieties AKAW4008 (27.49 ppm), AKAW4189-3(15.06 ppm), PBW343 (28.46 ppm), HD2285(27.67 ppm), HD2888(26.76), MonsAld's (28.32 ppm) and Sonalika (23.15ppm) have more Mn accumulated significantly under stress condition similar pattern reported by Dikeman *et al.*, (1982) [10]; Davis *et al.*, (1984). Velu *et al.*, 2016 [23] Amarshettiwar *et al.*, (2018) [1] also reported similar result in grain. The remaining varieties namely, Halna, C306, HD2285 and MB4010 there have been no significant differences in the accumulation of Mn under normal and heat stress condition similar pattern reported by Dias *et al.*, (2009) [8, 9] while in varieties namely C306, Kauz-dwarf and MB4010 the value has significantly decreased similar pattern reported by Dikeman *et al.*, 1982 [10]; Dias *et al.*, 2009 [8, 9] and Chen *et al.*, 2016. The wheat variety AKAW 4008, F5-995, PBW343, HD2888, MonsAld's and AKAW4189-3 showed overall better micronutrients accumulation in flag leaf and spike under stress condition and appeared as potentially important wheat variety used in plant breeding and biofortification strategy to reduce the problem of malnutrition.

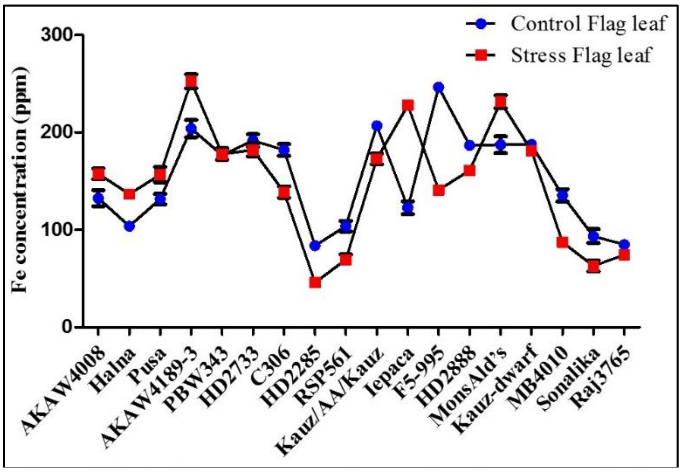

Fig 1: Effect of head stress on micronutrient (Fe) accumulation of 18 wheat varieties under control and stress flag leaf

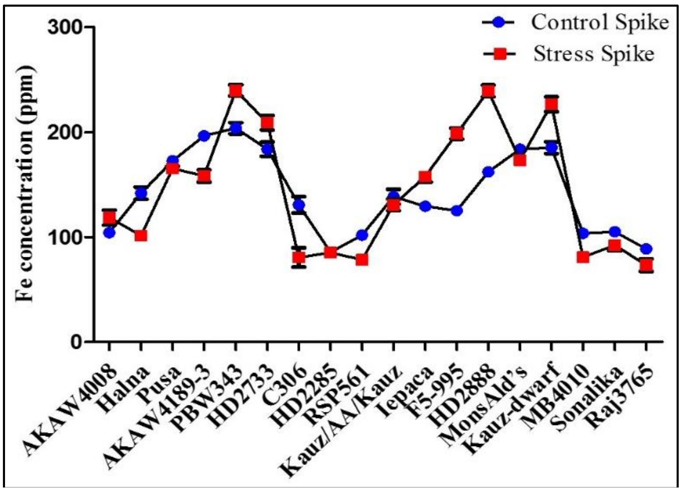

Fig 2: Effect of head stress on micronutrient (Fe) accumulation of 18 wheat varieties under control and stress spike

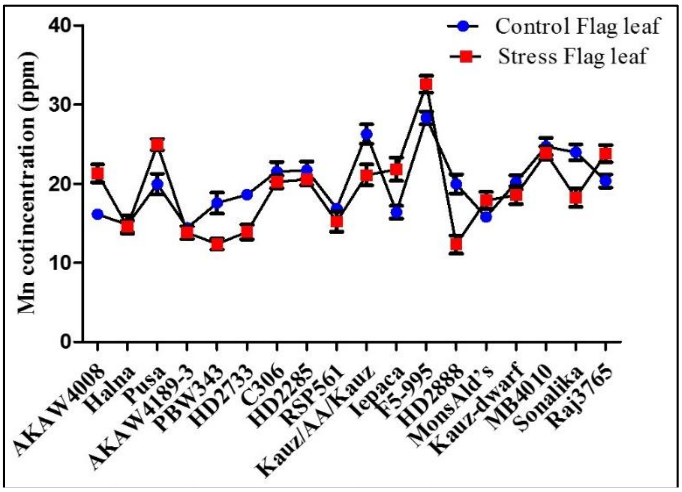

Fig 3: Effect of head stress on micronutrient (Mn) accumulation of 18 wheat varieties under control and stress flag leaf

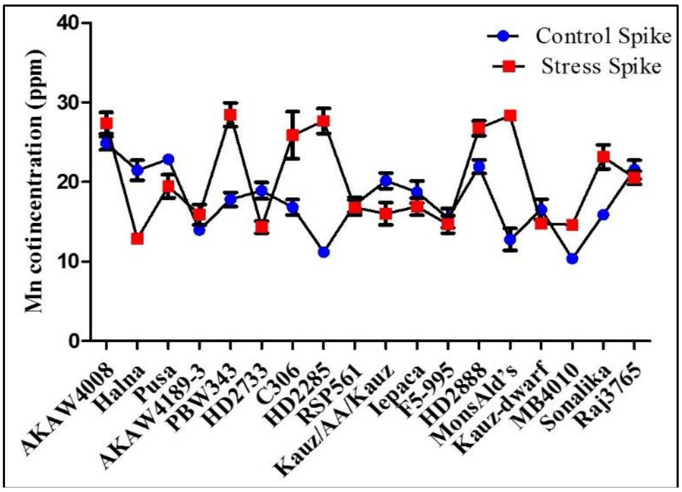

Fig 4: Effect of head stress on micronutrient (Mn) accumulation of 18 wheat varieties under control and stress spike

Acknowledgment

The authors are thankful to DBT for providing financial support in the form of Research Fellowship during the course of my study and provide fund for my research work. We would like to thanks Dr. V. K. Shahi Dean of FBS&H PUSA and all faculty members of AB & MB for their support during this research work.

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