Environment and Ecology 39 (4A) : 1324—1331, October—December 2021
ISSN 0970-0420

Quantitative Study of Enzyme Activities and Protein Content in Wheat under Various Levels of Zn and Fe

Bhupendra Mathpal*, Prakash Chandra Srivastava, Shailesh Chandra Shankhdhar

Received 10 May 2021, Accepted 29 August 2021, Published on 7 October 2021

ABSTRACT

Bioavailability of zinc (Zn) and iron (Fe) is a serious worldwide problem and both these micronutrients plays significant role in regulating activities of antioxidant enzymes in plants. Therefore, to examine the influence of both the micronutrients on biochemical responses of wheat, a greenhouse experiment was planned in the Department of Plant Physiology, G.B. Pant University of Agriculture & Technology, Pantnagar. Two wheat genotypes viz., UP2628 and UP262 were examined in experiment. Tested levels of Zn (0, 0.25 and 0.5% $ZnSO_4$) and Fe (0, 0.5 and 1.0% $FeSO_4$) were sprayed at tillering, flowering and grain filling stage of crop. Maximum (0.135 Units/min) superoxide dismutase (SOD) activity was found at 0.5% Zn alone in UP2628 whereas at the same level of Zn, carbonic anhydrase (CA) activity was also recorded maximum (22.95 Units/mg fr. wt.) in UP2628. Regarding catalase (CAT) activity, maximum (26.87 Units/mg fr. wt.) was found at 1.0% Fe + 0% Zn in UP262 while variety UP2628 expressed maximum (0.211 Units/mg fr. wt.) peroxidase (POD) activity by the use of 1.0% Fe + 0% Zn. A positive correlation was reported between Zn + Fe levels and grain protein content. On comparison of both the varieties, UP2628 was found to be expressed all the enzymes and protein content in more concentration in comparison to UP262, so found to be more efficient in utilization of both the micronutrients.

Keywords SOD, CA, CAT, POD, Protein content in wheat grain.

Bhupendra Mathpal*
Assistant Professor, *School of Agriculture, Lovely Professional University, Phagwara, Punjab 144411, India

Prakash Chandra Srivastava
Department of Soil Science, College of Agriculture, G. B. Pant University of Agriculture and Technology, Pantnagar 263145, India

Shailesh Chandra Shankhdhar
Department of Plant Physiology, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar 263145, India
Email: mathpal.88@gmail.com
*Corresponding author

INTRODUCTION

Among all cereal crops wheat (*Triticum aestivum* L.) act as a staple food crop because of its universal consumption by vast majority of the human population (Bhatt *et al.* 2020). On a global basis, wheat provides more nourishment than any other food crop. It is one of the most popular food crops as a wide variety of food products can be prepared from it (Tiwari and Shoran 2012). Deficiency of micronutrients in the

soil is a severe issue, affecting production of major crops including wheat. Micronutrients plays very important role to regulate the growth and development of both humans as well as crops but still there is a lot to explore pertaining to their availability in soil and translocation to grains (Shiwakoti *et al.* 2019). The main reason behind this deficiency is the low content of Zn and Fe in plant derived food or presence of antinutritional compounds in high concentration which makes them unavailable for humans (Ghandilyan *et al.* 2006).

Both Zn and Fe influence many enzyme activities in plants by being either structural part or act as a cofactor (Tabatabai *et al.* 2015). Among all superoxide dismutases (SODs), carbonic anhydrase (CA), peroxidase (POD) and catalase (CAT) are key enzymes which are Zn and Fe dependent. Superoxide dismutase (SOD) is a key enzyme which is required to fight against environmental stress and scavenges harmful reactive oxygen species (ROS) (Yavas and Unay 2016). This enzyme has a Zn atom that catalyzes CO_2 hydration and the enzyme activity is depends on Zn concentration in the plant (Marschner 1995). There are three isoforms of SOD depending upon type of metal cofactor viz., (Mn-SOD), Fe (Fe-SOD) or Zn and Cu (Cu/Zn-SOD). Cu/Zn-SOD is located in the chloroplast and in cytosol. It has been revealed from studies that Zn plays a catalytic role while Cu has a structural role (Streller *et al.* 1994, Mondola *et al.* 2016).

Another Zn containing enzyme is carbonic anhydrase (CA), which is present in dimer, tetramer, hexamer or an octamer form and affects metabolism of carbohydrates in plants. The enzyme is present in chloroplast and dependent on Zn concentration for its activity (Marschner 1995). Zinc concentration modulates the CA activity and therefore the fixation of CO_2 in plants (Alloway 2004). Long known metalloenzymes, CAT and POD are one of the most efficient protein catalysis known. Under Fe-deficiency these showed reduced activity for detoxifying H_2O_2 that is generated during oxidative stress. Deficiency of Fe resulted in oxidative stress in plants due to H_2O_2 accumulation which results reduced activity of peroxidase (Ranieri *et al.* 2001). As both Zn and Fe are important for the activities of major plant enzymes so an experiment was planned to evaluate their impact on important plant enzymes.

MATERIALS AND METHODS

The present investigation was carried out in green house of Plant Physiology Department of G.B. Pant University of Agriculture & Technology, Pantnagar. Seeds of varieties UP 262 and UP 2628 were sown in pots of 4 kg capacity which were filled with Zn deficient soil. Foliar spray of Zn (0, 0.25 and 0.5% $ZnSO_4$) and Fe (0, 0.5 and 1.0% $FeSO_4$) levels was done at tillering, flowering and grain filling stage of crop. Samples were collected from flag leaf of both the varieties at flowering stage and used for the quantification of following enzyme activities while protein content was estimated in grains after harvesting of crop. The experimental design was three factorial completely randomized with three replications of each treatment. Data analysis was done by analysis of variance (ANOVA) using software developed by University and the means were tested at $p \leq 0.05$ level of significance.

SOD activity

Estimation of SOD activity was done in flag leaf at flowering stage of crop by method followed by (Mathpal *et al.* 2015).

CA activity

Quantification of CA activity was done at flowering stage by using a procedure followed by (Mathpal *et al.* 2015).

POD activity

Quantification of peroxidase activity was done at flowering stage of both the varieties according to method followed by (Mathpal *et al.* 2018).

CAT activity

Catalase (CAT) activity was measured at flowering

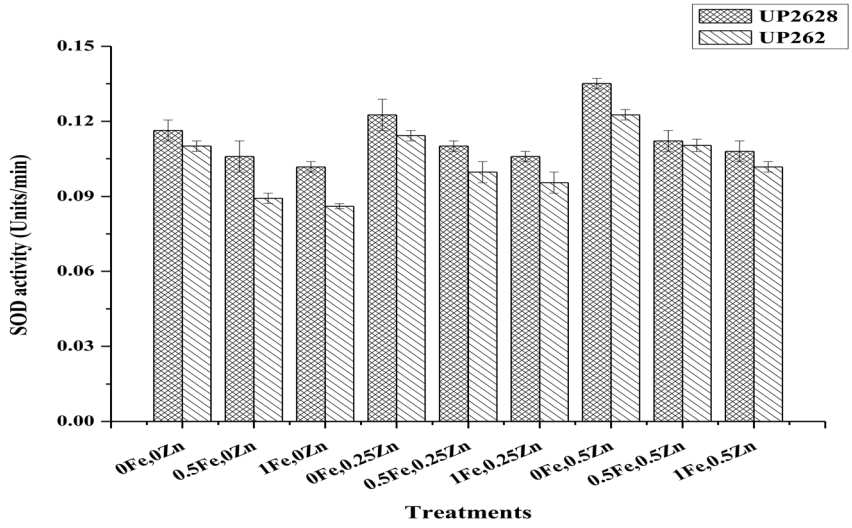

Fig. 1. SOD activity (Units/ min) under various levels of Zn and Fe. Standard deviation (±) is represented by vertical bars on each column.

stage of crop according to the procedure given by (Mathpal *et al.* 2018).

Proteins content in grains

Quantification of protein content in grains was done as per procedure used by (Mathpal *et al.* 2015).

RESULTS AND DISCUSSION

SOD activity

Superoxide dismutase (SOD) activity of two wheat varieties namely UP2628 and UP262 is presented in (Fig. 1). The use of 0.5% Fe + 0% Zn and 1.0% Fe +

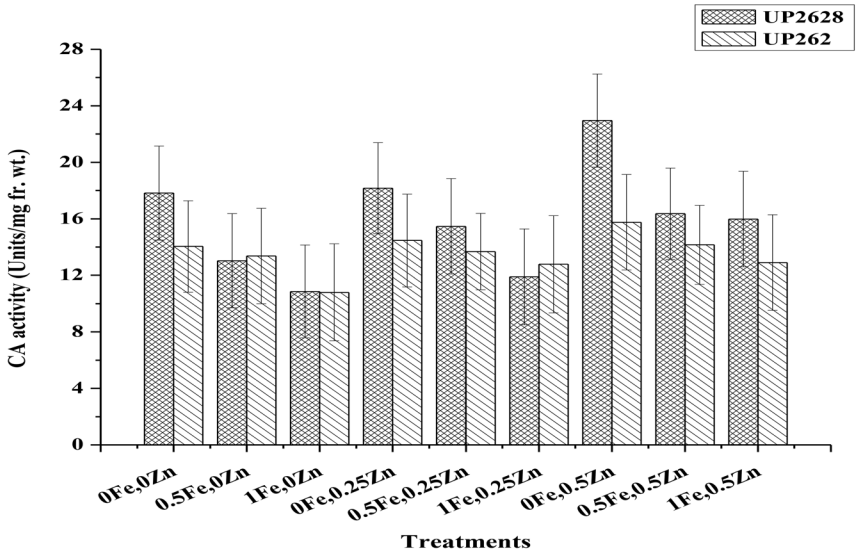

Fig. 2. CA activity (Units/mg fr. wt.) under various levels of Zn and Fe. Standard deviation (±) is represented by vertical bars on each column.

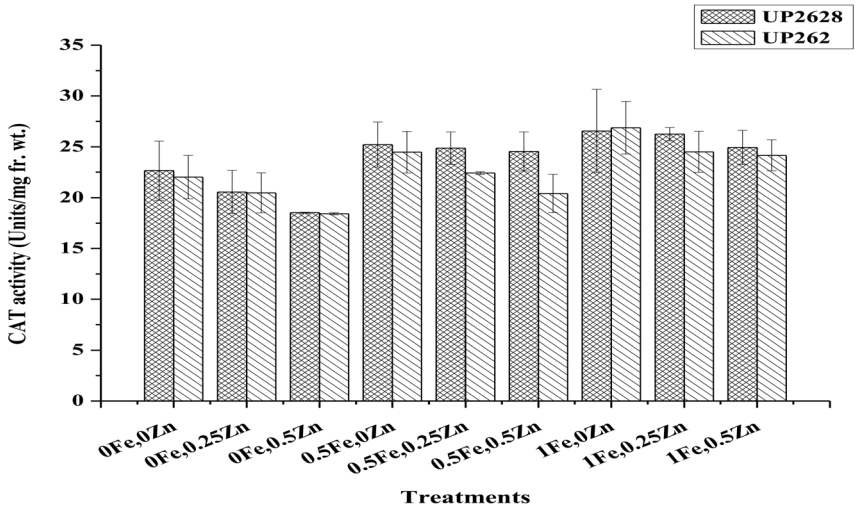

Fig. 3. CAT activity (Units/mg fr. wt.) under various levels of Zn and Fe. Standard deviation (±) is represented by vertical bars on each column.

0% Zn combinations reduced enzyme activity by 8.6 and 12.0% in UP2628 while by 19.0 and 21.8% in UP262 respectively than 0% Fe + 0% Zn. The foliar spray of 0.5% Fe + 0.25% Zn and 1.0% Fe + 0.5% Zn resulted in a decrement by 10.5% and 13.8% in UP2628 whereas in UP262 the enzyme activity was reduced by 12.2% and 15.7%, respectively as compared to 0% Fe + 0.25% Zn. Although, spray of 0% Fe + 0.5% Zn increased the SOD activity in both the varieties as compared to 0% Fe + 0% Zn and 0% Fe + 0.25% Zn levels but the application of 0.5% Fe + 0.5% Zn and 1.0% Fe + 0.5% Zn solution decreased the SOD activity by 17.0 and 20.0% in UP2628 and by 10.5 and 17.0% in UP262, respectively than 0% Fe + 0.5% Zn. As a whole, the highest (0.135 Units/min) SOD activity was noted at a combination of 0% Fe + 0.5% Zn in UP2628 while the lowest (0.086 Units/min) in UP262 at 1.0% Fe + 0% Zn.

The above mentioned results clearly indicated that SOD activity improved with increasing Zn levels and showed a reduction with increasing levels of Fe. It might be ascribed to the fact that Cu/Zn-SOD occurs in higher proportion as compared to its other isoforms and iron may inhibit its binding to the enzyme which is essential for its activity. Between the two genotypes, UP2628 showed relatively more activity than UP262 as it was considered a Zn efficient variety. Superoxide dismutase requires Zn for its activity and under Zn deficient conditions a reduction in activity has been reported (Chen *et al.* 2003). Bharti *et al.* (2013) also reported enhanced (19.3% at maximum tillering and 14.7% at flowering) SOD activity in different wheat genotypes under 20 kg Zn ha^{-1} + 0.5 % zinc sulfate in comparison to without Zn application. An enhanced expression of SOD activity under increasing levels of Zn was also reported by Ma *et al.* 2017.

CA activity

The CA activity in two contrasting varieties of wheat is significantly affected by various levels of Zn and Fe (Fig. 2). Application of 0.5% Fe + 0% Zn and 1.0% Fe + 0% Zn decreased the CA activity by 26.8 and 39.0% in UP2628 and by 4.8 and 23.0% in UP262, respectively than 0% Fe + 0% Zn combination. The application of 0.5% Fe + 0.25% Zn and 1.0% Fe + 0.25% Zn reduced the enzyme activity by 14.8 and 34.5% in UP2628 whereas by 5.3 and 11.6% in UP262, respectively than 0% Fe + 0.25% Zn. A reduction of 28.7 and 30.3% in UP2628 while in UP262 a reduction of 10.0 and 18.0% was recorded by the use of 0.5% Fe + 0.5% Zn and 1.0% Fe + 0.5% Zn respectively than 0% Fe + 0.5% Zn. In general, the highest (22.95 Units/mg fr. wt.) CA activity was found at 0% Fe + 0.5% Zn in UP2628 whereas the

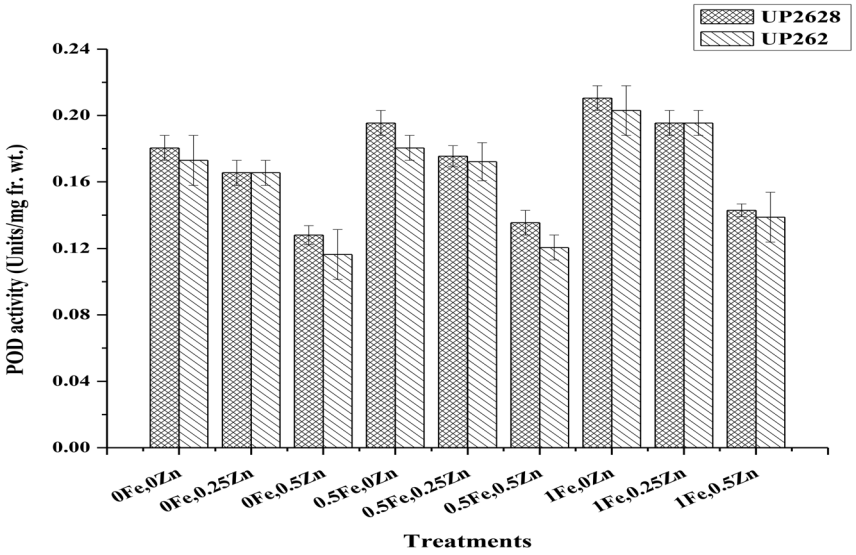

Fig. 4. POD activity (Units/mg fr. wt.) under various levels of Zn and Fe. Standard deviation (±) is represented by vertical bars on each column.

lowest (10.80 Units/mg fr. wt.) was noted for UP262 at 1.0% Fe +0% Zn.

Carbonic anhydrase plays a very important role during dark reaction of photosynthesis as it is required to convert carbon di-oxide into bicarbonate which will be utilized further for CO_2 assimilation. The data clearly showed that a positive correlation was found between CA activity and Zn levels. Variety UP2628 being a Zn efficient variety showed higher activity of enzyme as compared to UP262 which was considered as Zn inefficient variety. A decline in the CA activity has been found in response to reduced Zn supply earlier by (Sasaki *et al.* 1998). A reduction in enzyme activity was found in a Zn inefficient variety when sown in Zn scarce condition (Hacisalihoglu *et al.* 2003). Similar results have also been reported where increasing levels of Zn were found to improve CA activity in leave of rice crop (Mathpal *et al.* 2018).

CAT activity

Effect of Zn and Fe levels on CAT activity is presented in Fig 3. A reduction in enzyme activity of 9.1 and 18.1% in UP2628 and of 7.0 and 16.3% in UP262 was observed by the spray of 0% Fe + 0.25% Zn and 0% Fe + 0.5% Zn, respectively as compared to 0% Fe + 0% Zn. Similarly, foliarly applied solutions of 0.5% Fe + 0.25% Zn and 0.5% Fe + 0.5% Zn resulted in a decrement of 1.3 and 2.6% in UP2628 while in UP262 a decrement of 8.3% and 16.5% was noted than 0.5% Fe + 0% Zn. At 1.0% level of $FeSO_4$, use of 1.0% Fe + 0.25% Zn and 1.0% Fe + 0.5% Zn reduced the CAT activity in UP2628 by 1.2 and 6.1% whereas in UP262 by 8.8 and 10.1%, respectively in comparison to 1.0% Fe + 0% Zn. The highest (26.87 Units/mg fr. wt.). CAT activity was noted in UP262 at 1.0% Fe + 0% Zn while the lowest (18.42 Units/mg fr. wt.) was also found for UP262 under 0% Fe + 0.5% Zn combination of Zn and $FeSO_4$ solutions.

Activity of CAT is directly influenced by the Fe content of the plant cell as Fe is an essential requirement for its proper functioning. Results clearly indicated a direct dependence of CAT activity on applied Fe levels and showed an enhancement as concentration was increased from 0% to 1.0%. Regarding effect of Zn, increasing the zinc sulfate levels (0% to 0.5%) reduced the activity of CAT. The possible reason behind these outcomes may be ascribed to the fact that both Zn and Fe compete for active site of enzyme (Kabata-Pendias 2001, Alloway 2008). On comparing both the varieties, UP2628 reported

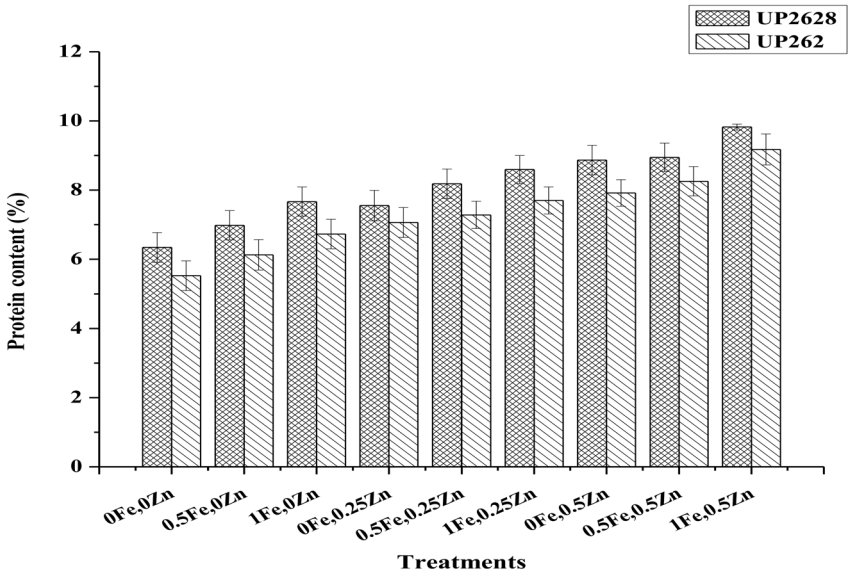

Fig. 5. Protein content (%) in grains under various levels of Zn and Fe. Standard deviation (±) is represented by vertical bars on each column.

better in expressing CAT than UP262. An increment of 33 % was reported with increasing Fe doses from 0 to 200 µM in rice genotypes (Zhang *et al.* 2010). Similarly, an enhanced activity of CAT was also recorded by Babaei *et al.* (2017) under the application of Fe- nano oxides.

POD activity

The results pertaining to influence of Zn and Fe levels on POD activity are represented in Fig 4. The use of 0% Fe + 0.25% Zn and 0% Fe + 0.5% Zn reduced the peroxidase activity by 8.2 and 29.2% in UP2628 and by 4.0 and 32.9% in UP262, respectively than 0% Fe + 0% Zn. The foliar spray of 0.5% Fe + 0.25% Zn and 0.5% Fe + 0.5% Zn solution reduced the POD activity in UP2628 by 10.2 and 30.6% whereas by 4.9 and 33.1% in UP262, respectively in comparison to a combination of 0.5% Fe + 0% Zn. The foliar spray of 1.0% Fe + 0.25% Zn and 1.0% Fe + 0.5% Zn solution reduced the POD activity than 1.0% Fe + 0% Zn by 7.1 and 32.3% in UP2628 whereas in UP262 a decrement of 3.4 and 31.5% was noted, respectively. The variety UP2628 showed maximum POD activity at 1.0% Fe + 0% Zn while the minimum activity was found for UP262 under the 0% Fe + 0.5% Zn combination of Zn and Fe.

Peroxidase is another enzyme that uses Fe as a cofactor. It was found that application of excess Fe induced POD synthesis in wheat. Results showed that an increment in POD activity was reported with increasing levels of Fe while a decrement was recorded in both the varieties at increasing levels of zinc sulfate. Fang and Kao (2000) also reported increased POD activity in rice plant in response to application of Fe. Similarly, it was also found that application of organic fertilizers improved availability and mobilization of Fe that resulted in improved functioning of POD (Denre *et al.* 2013). Application of nano Fe-oxide was also found to be effective in increasing POD activities in leaves of wheat crop (Babaei *et al.* 2017).

Protein content

Result pertaining to protein content is presented in Fig 5. The use of 0.5% Fe + 0% Zn and 1.0% Fe + 0% Zn enhanced the protein concentration by 10.1 and 20.8% in UP2628 and by 10.8 and 21.7% in UP262, respectively over 0% Fe + 0% Zn. The use of 0.5% Fe + 0.25% Zn and 1.0% Fe + 0.25% Zn combinations of Zn and Fe improved the protein concentration by 8.3 and 13.7% in UP2628 and by 3.1 and 9.0% in

UP262, respectively than 0% Fe + 0.25% Zn level of zinc and ferrous sulfate. A further increment in protein content was recorded with the application of a combination of 0.5% Fe + 0.5% Zn and 1.0% Fe + 0.5% Zn, a percent increment of 0.9 and 10.8 in UP2628 and of 4.2 and 15.9% in UP262 was recorded as compared to 0% Fe + 0.5% Zn, respectively. The highest protein concentration was noted in UP2628 under the 1.0% Fe + 0.5% Zn combination of zinc and ferrous sulfate while the minimum was noted at 0% Fe + 0% Zn in UP262.

The results clearly depicted that a positive correlation was observed between Zn and Fe levels and grain protein content. It might be due to the involvement of Zn in gene expression by virtue of being an important part of Zn finger motifs (Roy *et al.* 2012). Besides this, both Zn and Fe are integral component of many proteins involved in plant metabolism. Irrespective of the varying Zn and Fe levels, higher protein content was recorded in UP2628 which showed a better transport of Zn and Fe from vegetative parts to reproductive parts as compared to UP262. A percent increase of 4.40 in the wheat grain protein was reported under 40 kg Zn ha^{-1} than to zero use of Zn (Morshedi and Farahbakhshb 2010). A positive correlation between applied Zn + Fe levels and grain protein content was also reported by Mathpal *et al.* 2018.

CONCLUSION

Results of study clearly indicated that variety UP2628 was performed best under all applied concentrations of Zn and Fe and expressed more protein content in grains than UP262. On the basis of findings of study it can be concluded that UP2628 (Zn efficient) can be used for crop improvement program for the development of more Zn efficient varieties.

ACKNOWLEDGEMENT

We acknowledge the financial support provided by Indian Council of Agricultural Research (ICAR), New Delhi, India for successful completion of study.

REFERENCES

Alloway BJ (2004) In zinc in soil and crop nutrition. International Zinc Association. Brussels, Belgium and Paris, pp 135.

Alloway BJ (2008) Micronutrients and crop production. In micronutrient deficiencies in global crop production, pp 1—39.

Babaei K, Sharifi RS, Pirzad A, Khalilzadeh R (2017) Effects of bio fertilizer and nano Zn-Fe oxide on physiological traits, antioxidant enzymes activity and yield of wheat (*Triticum aestivum* L.) under salinity stress. *J Pl Int* 12 (1) : 381—389.

Bharti K, Pandey N, Shankhdhar D, Srivastava PC, Shankhdhar SC (2013) Effect of different zinc levels on activity of superoxide dismutases and acid phosphatases and organic acid exudation on wheat genotypes. *Physiol Mol Biol Pl* Doi. 10.1007/s12298-013-0201-7.

Bhatt R, Hossain A, Sharma P (2020) Zinc biofortification as an innovative technology to alleviate the zinc deficiency in human health : A review. *Open Agric* 5 : 176—187.

Chen G, Wang R, Quampah A, Yang X (2003) Physiological characterization of zinc efficient rice (*Oryza sativa* L.) genotype at different Zn^{2} + levels. *Rice Sci* 11 : 47—51.

Denre M, Bandopadhyay PK, Chakravarty A, Pal S, Bhattacharya A (2013) Changes in some biochemical characteristics in response to foliar applications of chelator and micronutrients in green pungent pepper. *Int J Pl Physiol Biochem* 5 (2) 25—35.

Fang WC, Kao CH (2000) Enhanced peroxidase activity in rice leaves in response to excess iron, copper and zinc. *Pl Sci* 158 : 71—76.

Ghandilyan A, Vreugdenhil D, Aarts MGM (2006) Progress in genetic understanding of plant iron and zinc nutrition. *Physiol Planta* 126 : 407—417.

Hacisalihoglu G, Hart JJ, Wang YH, Cakmak I, Kochian LV (2003) Zinc efficiency is correlated with enhanced expression and activity of zinc-requiring enzymes in wheat. *Pl Physiol* 131 : 595—602.

Kabata-Pendias A, Pendias H (2001) Trace elements in soils and plants, CRC Press, Boca Raton-London-New York-Washington DC.

Ma D, Sun D, Wang C, Ding H, Qin H, Hou J, Huang X, Xie Y, Guo T (2017) Physiological responses and yield of wheat plants in zinc-mediated alleviation of drought stress. *Front Pl Sci* Doi: 10.3389/fpls.2017.00860.

Marschner H (1995) Mineral nutrition of high plant. Academic Press, pp 330—355.

Mathpal B, Srivastava PC, Shankhdhar D, Shankhdhar SC (2015) Improving key enzyme activities and quality of rice under various methods of zinc application. *Physiol Mol Biol Pl* 21 4 : 567—572.

Mathpal B, Srivastava PC, Shankhdhar SC (2018) Impact of zinc and iron levels on key enzyme activities and grain protein content of rice. *Pl Arch* 18 2 : 1291—1296.

Mondola P, Damiano S, Sassoand A, Santillo M (2016) The Cu, Zn superoxide dismutase : Not only a dismutase enzyme. *Front Physiol* doi: 10.3389/fphys.2016.00594.

Morshedi A , Farahbakhshb H (2010) Effects of potassium and zinc on grain protein contents and yield of two wheat genotypes under soil and water salinity and alkalinity stresses. *Pl Ecophysiol* 2 : 67—72.

Ranieri A, Castagna A, Baldan B, Soldatini GF (2001) Iron deficiency differently affects peroxidase isoforms in sunflower. *J Exp Bot* 52 : 25—35.

Roy S, Duttay S, Khanna K, Singla S, Sundar D (2012) Prediction of DNA-binding specificity in zinc finger proteins. *J Biosci* 37 (3) : 483—491.

Sasaki H, Hirose T, Watanabe Y, Ohsugi R (1998) Carbonic anhydrase activity and CO_2-transfer resistance in Zn-deficient rice leaves. *Pl Physiol* 118 : 929—934.

Shiwakoti S, Zheljazkov VD, Gollany HT, Kleber M, Xing B, Astatkie T (2019) Micronutrients in the soil and wheat: Impact of 84 years of organic or synthetic fertilization and crop residue management. *Agronomy* (9) : 1—13.

Streller S, Karpinski S, Hallgren JE, Wingsle G (1994) Four cytosolic Cu Zn-superoxide dismutases in germinating seeds of *Pinus sylvestris* (L.). *Physiol Planta* 92 : 443—450.

Tabatabai SMR, Oveysi M, Honarnejad R (2015) Evaluation of some characteristics of corn under water stress and zinc foliar application. *GMP Rev* 16 : 34—38.

Tiwari V, Shoran J (2012) Growth and production of wheat. Soils, plant growth and crop production. Annual Report, 2012-2013. Crop Improvement Division, Directorate of Wheat Research, (ICAR), Karnal, India, pp. 5.

Yavas I, Unay A (2016) Effects of zinc and salicylic acid on wheat under drought stress. *J Ani Pl Sci* 26 : 1012—1018.

Zhang Y, Zheng GH, Liu P, Song JM, Xu GD, Cai MZ (2010) Morphological and physiological responses of root tip cells to Fe^{2+} toxicity in rice. *Acta Physiol Planta Doi* 10.1007/s11738-010-0590-y.