

Zinc (Zn) and iron (Fe) fertilization for improving the antioxidant enzyme activity and biochemical constituents in capsicum hybrids

Abstract

Micronutrients, particularly iron (Fe) and Zinc (Zn), play a vital role in the growth and development of plants due to their catalytic effect on many metabolic processes. However, the biochemical responses to the applied micronutrients vary with cultivars and their species. A screening experiment was conducted during 2020 to know the antioxidants activities and biochemical reactions in response to iron and zinc fertilization by six capsicum hybrids grown in grow bags under shade net conditions. The experiment consists of three treatments viz., Control (No Fe & Zn), 50 kg FeSO₄ and 37.5 kg ZnSO₄ ha⁻¹ as a basal soil application with six capsicum hybrids viz., Indra, Priyanka, Inspiration, Massilia, Bachata, and Local green. Leaf samples of the capsicum hybrids were collected at fruiting stage and analysed for antioxidant activities. The fruit samples were used for quantifying the biochemical constituents. The results revealed that, application of ferrous sulphate (FeSO₄) and zinc sulphate (ZnSO₄) to capsicum hybrids increased the biochemical constituents in fruits and the antioxidant enzyme activities in leaves. Out of the six hybrids tested, Indra possessed higher ascorbic acid content (9.20 mg 100 g⁻¹ fresh weight), acidity (6.0), and total soluble solids (6.1⁰ Brix) in the fruits, which was followed by Inspiration and Bachata. The superoxide dismutase (6.70 units mg⁻¹ protein) and peroxidase (6.90 units g⁻¹ fresh weight) activities were also higher with the same genotypes. The biochemical and antioxidant response to Zn addition was better than Fe. There was 13.2, 10.9 and 9.5 per cent increase in titratable acidity, total soluble solids, ascorbic acid content in the fruits of Indra due to ZnSO₄ application. The Principal Component Analysis (PCA) and hierarchical clustering revealed that Indra is highly responsive to Zn and Fe fertilization, while the local green showed very less response. The rest of the genotypes such as Inspiration, Bachata, Massilia, and Priyanka, are medium responsive for Zn and Fe fertilization.

Keywords: *Biochemical constituents, antioxidant enzyme activities, Capsicum hybrids, Fe/Zn fertilization, Principal Component Analysis (PCA)*

1. Introduction

Capsicum is one of the most popular vegetables commercially grown under protected cultivation because of its adaptability in different protected structures. Due to its qualitative and quantitative advantages, a hike in demand increased the farmer's interest in cultivating this crop under a protected structure [1]. It is grown on a large scale as an off-season vegetable crop. Protected cultivation of Capsicum for fresh vegetable marketing and processing has recently gained economic importance [2]. Capsicum hybrids are a rich source of vitamin C (ascorbic acid); they also contain antioxidants with important health-related implications [3]. The levels of these compounds are modified by growing conditions and nutrient application and depend on the genotype and fruit maturity [2]. Fertilizers and irrigation are the basic requirements for increasing the fruit yield of capsicum. Capsicum responds well to fertilizer applications and is reported to have a high demand for major nutrients (NPK). Micronutrients are usually required in minute quantities but essential for various activities; particularly iron (Fe) and Zinc (Zn) play vital roles in the growth and development of plants due to their catalytic effect on many metabolic processes. Improvement in growth characters due to the application of micronutrients such as zinc and iron may be due to enhanced photosynthetic and other metabolic activity, leading to an increase in various plant metabolites responsible for cell division and elongation [4]. Iron is an essential element for almost all living organisms as it participates in oxygen transport, DNA synthesis, and electron transport. Many enzymes require iron as a co-factor for their function, which is involved in oxidative phosphorylation, a metabolic pathway that converts nutrients into energy [5]. In plants, iron is involved in the synthesis of chlorophyll and is essential for maintaining chloroplast structure and function [6]. Iron (Fe) also affects fruit quality parameters such as colour, firmness, acidity and affects fruit production, dropping the number of fruits

per plants, fruit size, and yield [7]. Likewise, Zn activates the electrophile and nucleophiles as a component of plant carbonic anhydrase and many other photosynthetic enzymes, which influences the photosynthetic efficiency, chlorophyll structure and content. It is also involved in sucrose and starch formation, protein metabolism, membrane integrity, auxin metabolism, defense mechanism, flowering, and seed production of crop plants [8]. The current study showed that, soils with deficient Zn and Fe could increase the yield by the addition of Zn and Fe fertilizers [9]. It also increases the plant height, number of side branches, and leaf area [10]. Singh *et al.*, [11] found that zinc application at 0.6% was most effective in increasing the total soluble solids and ascorbic acid content. Meena *et al.*, [12] observed that, application of ferrous sulphate and borax at (0.6%) improved the fruit setting time, increased the total soluble solids, total sugar and decreased the acidity in fruits. Hasani, *et al.*, [13] examined the response of capsicum for the foliar spraying of micronutrients on fruit yield and quality and reported that, foliar sprays had positive and significant effects on the fruit yield, total soluble solids, weight of fruit, juice content, anthocyanin index, fruit diameter, and leaf area. Increased fruit yield and quality as well as Nitrogen (N), Phosphorus (P), Potassium (K) intake [14], and water use efficiency [15] were also reported with the foliar spraying of Zn and salicylic acid. The role of these nutrients in various plant metabolic processes was studied by several authors such as Tamilselvi *et al.*, [16]; Hatwar *et al.*, [17] and Shaheen *et al.*, [18] and the results revealed that application of micronutrients as foliar spray caused an improvement in plant growth, fruit yield and its physical and chemical properties of fruits. In the same way, Bhatt *et al.* [19] studied the effect of foliar application of micronutrients and reported significant improvement in yield, which might be attributed to the increased photosynthetic activity and production and accumulation of carbohydrates. Also, Malawadi *et al.* [20] studied the effect of soil application of micronutrients on the yield and quality of capsicum and noticed higher fruit weight, yield, and maximum ascorbic acid content. Likewise, Batra *et al.* [21] and Savitha [22] reported that, foliar application of iron after transplanting resulted in a significant improvement in the ascorbic acid content in fruits due to increased activity of ascorbic acid oxidizing enzyme. Regarding the major quality parameters, Tamilselvi *et al.*, [13] observed maximum total soluble solids (TSS), acidity, and ascorbic acid contents in the fruits with micronutrients application. From the literature, it was perceived that, different forms and levels of Zn and Fe give differential responses to plants' physiology [23], particularly the biochemical responses which vary with cultivars. Hence the present investigation was carried out, to understand the differential biochemical responses of capsicum hybrids in improving fruit biochemical constituents and leaf antioxidant enzyme activities due to zinc and iron fertilisation.

2. MATERIALS AND METHODS

2.1 Experimental details

A screening experiment was conducted to understand the antioxidant enzyme activity and biochemical responses of six capsicum hybrids to iron and zinc fertilization grown in grow bags under shade net condition in the farmer's field (110 48' 15.8" N 770 59' 25.3" E) at Thalavadi, Erode district. The experiment consists of three treatments viz., control (No Zn & Fe), 50 kg FeSO₄ and 37.5 kg ZnSO₄ application ha⁻¹ and six capsicum hybrids viz., Indra, Priyanka, Inspiration, Massilia, Bachata, and local green. The experiment was laid out in a Randomised Block Design with three replications. Recommended fertilizer nutrients such as Nitrogen, Phosphorus, and Potassium were applied as per soil test recommendation. About 45 days old seedlings of all the capsicum hybrids were transplanted in each grow bag, and the treatments were imposed. Necessary plant protection measures were carried out as and when needed. The antioxidant enzyme activity in plants and biochemical changes in the fruits of capsicum hybrids for Zn and Fe fertilization was determined and reported.

2.2 Physico-chemical properties of the experimental soil

The experimental soil was sandy loam in texture with neutral pH (7.42) and lesser electrical conductivity (0.33 dS m⁻¹). The organic carbon content of the soil was low (0.40%) and non calcareous in nature (2.50%). The soil had low available nitrogen (157 kg ha⁻¹), low available phosphorus (12.0 kg ha⁻¹), and medium available potassium (280 kg ha⁻¹) status. Regarding the available micronutrient status, zinc was deficient (0.68 mg g⁻¹), and other micronutrients were sufficient (6.50, 3.48 1.00, and 0.52 mg kg⁻¹ for iron, manganese, copper, and boron, respectively) in the soils.

2.3 Estimation of biochemical constituents and antioxidant enzyme activities

The fruit's biochemical constituents, such as acidity, total soluble solids (TSS), and ascorbic acid content were determined after the harvest. The acidity in the fruit juice was estimated by titration with a standard alkali using phenolphthalein as an indicator. From the homogenized fruit juice, 25 ml was taken and titrated against 0.1 N potassium hydroxide in the presence of phenolphthalein indicator till a permanent pink color is obtained. The result was expressed in citric acid per 100 g of fruit sample [24]. Total soluble solids (TSS) content was determined by means of hand held Refractometer, which was calibrated at 20°C and a few drops of fruit juice sample was placed in between the prisms and read at the demarcation line and expressed as Brix [24]. Ascorbic acid content was estimated by titration with an oxidizing agent viz., indophenol dye, and expressed in mg per 100 gram of fresh weight of fruit sample [25].

The Zn and Fe requiring enzymes in the leaf samples at fruiting stage were determined to understand the role of applied Zn and Fe in plant nutrition. Peroxidase activity was estimated using leaf samples homogenized in phosphate buffer. Then, one mL of supernatant was taken and to this 3 mL of 0.05 M pyrogallol and 0.5 mL of 30% H₂O₂ were added. The change in absorbance was measured at 430 nm for every 30 seconds up to 180 seconds. The enzyme activity was calculated and expressed as Units min⁻¹ mg⁻¹ fresh weight of leaf sample [25]. The superoxide dismutase activity was measured using the Nitro blue tetrazolium (NBT) method [26]. Five hundred milligram of leaf sample was macerated using 10 ml HEPES-KOH buffer containing 0.1mM EDTA and centrifuged at 15000 rpm for 15 min. The supernatant was collected and made up to 50 ml volume. One ml of the enzyme extract was mixed with 3 ml of the reaction mixture, and the absorbance was recorded at 560 nm. One unit of SOD activity was defined as the amount of enzyme required for 50% inhibition of NBT activity at 560 nm. The result was expressed in units per gram of fresh leaf weight.

2.4 Statistical analysis

The data obtained from the study were subjected to the analysis of variance to find out the significance as suggested by Panse and Sukhatme [27] using SPSS software. Principal component analysis and hierarchical clustering were plotted using R studio software [28].

3. RESULTS AND DISCUSSION

3.1 Titrable acidity

The characteristic flavor of each horticultural product must not only be associated with the presence of sugars such as sucrose, glucose, galactose, and fructose [29] but also due to the accumulation of some organic acids (citrate and malate) [30]. The acidity data obtained for the fruits expressed as the prevailing organic acid function are shown in Fig. 1. The titrable acidity of the capsicum hybrids increased with zinc (Zn) and iron (Fe) fertilization regardless of capsicum hybrids. The hybrid Indra exhibited higher fruit acidity (6.00, 5.80% citric acid) followed by Inspiration (5.80, 5.60% citric acid) and Bachata (5.70, 5.50% citric acid), respectively, with ZnSO₄ and FeSO₄ application. The increased acidity in the fruit might be due to the metabolic transformation of sugar into organic acids by the addition of Zn and Fe [31].

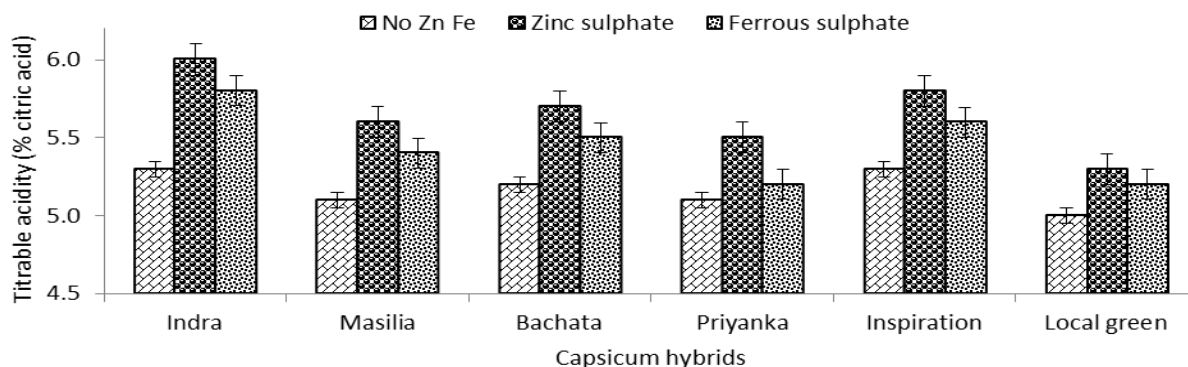


Fig.1. Changes in titrable acidity in fruits of different capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

Lesser **sugar conversion** into organic acid was observed with the local green (5.0 % citric acid) at no Zn and Fe application. It was reported that, application of non-chelated forms of micronutrients (Fe + Zn) produced higher acidic fruits compared to chelated forms [32]. However, the **results of the present study are similar to the findings of Dhotra *et al.*, [33], who reported that plants sprayed with ZnSO₄ and FeSO₄ produced more acidic fruits than control.**

3.2 Total soluble solid (TSS)

The accumulation of solutes during the maturation process is one of the parameters with greater precision and reliability when used as a harvest index for fruits and vegetables [34]. **Its rapid determination, relatively low cost,** correlates directly with flavor [27]. The capsicum hybrid Indra had higher TSS (6.10, 5.80 °Brix) followed by Inspiration (5.90, 5.75 °Brix) and Bachata (5.80, 5.70 °Brix) in response to ZnSO₄ and FeSO₄ application (Fig. 2). Total soluble solids were found to be higher with the ZnSO₄ application than FeSO₄ with a mean of 5.77 and 5.90 °Brix, respectively. Lesser TSS content in the fruit was noticed with local green (5.10 °Brix) in no Zn and Fe applied control. It was reported that TSS of the fruit is an important quality parameter [35], which could be increased with Zn and Fe application [36]. This result in **increasing photosynthesis activity,** translocation of sugars from source to sink and conversion **of sugars from** the complex form (polysaccharides) to **simple form** (glucose and fructose) in fruits [37].

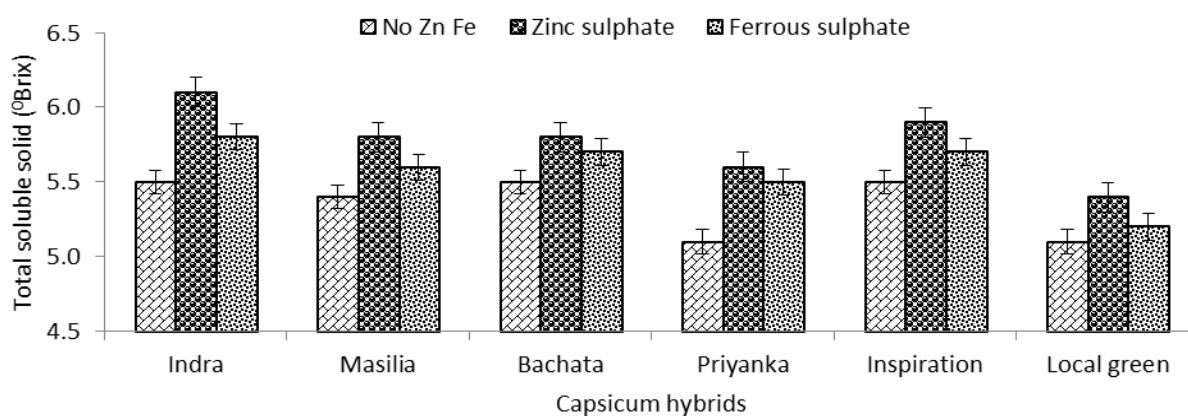


Fig. 2. Changes in total soluble solid content in fruits of different capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

3.3 Ascorbic acid content

The quality of capsicum fruit is defined mainly by the concentration of vitamin C, acidity, soluble solids, and carotenoids. The former being valuable, particularly in the human body, makes it essential to evaluate fruit quality assessment [38]. Ascorbic acid content **is** highly correlated with antioxidant activity, application of micronutrients significantly affects its availability in fruits [39]. Among the capsicum hybrids, the fruits of Indra contains higher ascorbic acid (9.20, 8.60 mg 100 g⁻¹ fresh weight) followed by Inspiration (8.70, 8.60 mg 100 g⁻¹ fresh weight) and Bachata (8.70, 8.50 mg 100 g⁻¹ fresh weight) respectively with ZnSO₄ and FeSO₄ application (Fig. 3). The improvement in ascorbic acid content with Zn application was **greater** than Fe with a mean of 8.70 and 8.47 mg 100 g⁻¹ fresh weight, respectively. Lesser ascorbic acid content was noticed with local green (8.20 mg 100 g⁻¹ fresh weight) at no Zn and Fe applied control. Higher **sugar levels with** micronutrients might be the possible cause for increased ascorbic acid content, which is synthesized from sugar [40]. Zinc plays an active role in the synthesis of Auxins, and increased synthesis of Auxins has been reported to enhance the accumulation of ascorbic acid content. Hence, the increased ascorbic acid content with the application of zinc might be due to the higher synthesis of auxin [41]. Batra *et al.* [21] reported that foliar application of iron at 40, 50, and 60 days after transplantation significantly improved the ascorbic acid content of tomato fruits. The most probable increase in ascorbic acid content might be due to the increased activity of ascorbic acid oxidase enzyme [22].

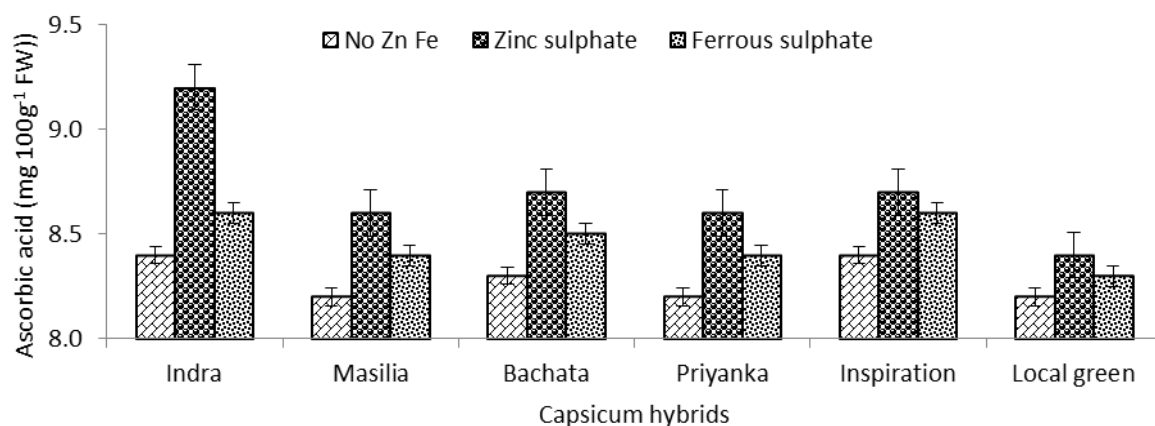


Fig. 3. Changes in ascorbic acid content in the fruits of different capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

3.4 Superoxide dismutase activity (SOD)

The generation of reactive oxygen species such as superoxide radicals, hydroxyl radicals, and hydrogen peroxide causes oxidative damage to plants. Plants with high levels of antioxidants, either constitutive or induced, have been reported to have greater resistance to oxidative damage. Plants have evolved specific protective mechanisms involving antioxidant molecules and enzymes to defend against oxidants [42]. The application of iron and zinc increased the superoxide dismutase activity in the leaves of all capsicum hybrids (Fig. 4). All the genotypes exhibited higher superoxide dismutase activity with Zn application than Fe (6.70 to 6.30 Units g⁻¹) and the highest superoxide dismutase activity was exhibited by Indra (6.90, 6.70 Units g⁻¹), followed by Inspiration and Bachata. Lesser SOD activity was measured with Local green hybrid at no Zn, and Fe applied control (5.10 Units g⁻¹). Zinc has an essential role on enzymes including Cu/Zn-SOD [43], and the enhanced enzyme activity in the present study is due to involvement of Zn as a co-factor for the functioning of SOD.

Moreover, activities of SOD depend on the availability of Zn from soil which differs among the genotypes [44]. Several studies reported an 89% correlation with the increased SOD activity in various plant species due to Zn fertilization [45]. Kaya *et al.* [46] said that pepper plants (*Capsicum annuum* L.) treated with high Zn concentration decreased H₂O₂ concentrations and MDA and stimulated the SOD activity. The lesser activity of SOD was observed in untreated plants [47]. Wu *et al.*, [48] and Sida-Arreola *et al.*, [49] also reported an increase in SOD activity with Zn fertilizer application.

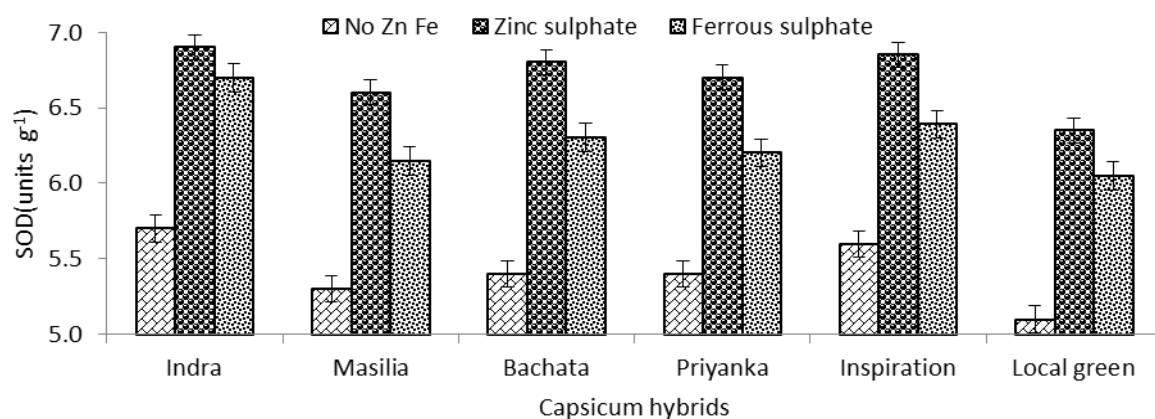


Fig. 4. Changes in leaf superoxide dismutase activity in capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

Similarly 80% of the studies shows increased SOD activity [45] by evaluating the physiological effect of Fe in plants. Rout *et al.* [50] reported that applying Fe at different concentrations (0, 25, 50, 100, and 200 μM of FeSO_4) increased the SOD activities to mitigate the oxidative damage caused by ROS in plants.

3.5 Peroxidase activity (POX)

Peroxidase (POX) enzyme has a protective effect and is involved in chlorophyll degradation, ROS generation and membrane lipid peroxidation which are responsive to injury and a product of senescence [51]. With the application of iron and zinc, peroxidase activity in leaves of capsicum hybrids increased substantially irrespective of genotypes (Fig. 5). The observation also depicted that iron application had a higher impact on peroxidase activity than zinc application with a mean of 6.72 and 6.63 $\text{Units min}^{-1} \text{g}^{-1}$ Fresh weight, respectively. The higher peroxidase activity was observed with Indra (7.00, 6.90 $\text{Units min}^{-1} \text{g}^{-1}$ Fresh weight) followed by Inspiration and Bachata. Lesser POX activity was observed with Local green hybrid at no Zn and Fe applied control (6.30 $\text{Units min}^{-1} \text{g}^{-1}$ Fresh weight). This might be due to the de novo biosynthesis of enzyme by Fe^{+2} activities in response to the presence or absence of Fe in leaves [52, 53]. Michael *et al.* [54] also observed increased POX activity in the plants treated with 50 mg kg^{-1} of Zn. Kaya *et al.* [46] reported decreased H_2O_2 concentrations and stimulation of enzymatic antioxidants enzyme POD in response to Zn application in pepper plants (*Capsicum annum* L.). Iron (Fe) is a constituent of enzymes associated with the cellular antioxidant system, such as Fe-SOD; hence, plants exposed to Fe application show variation in POD activities [55]. Tewari *et al.*, [56] and Jucoski *et al.*, [57] also reported similar results with the application of Fe fertilizers.

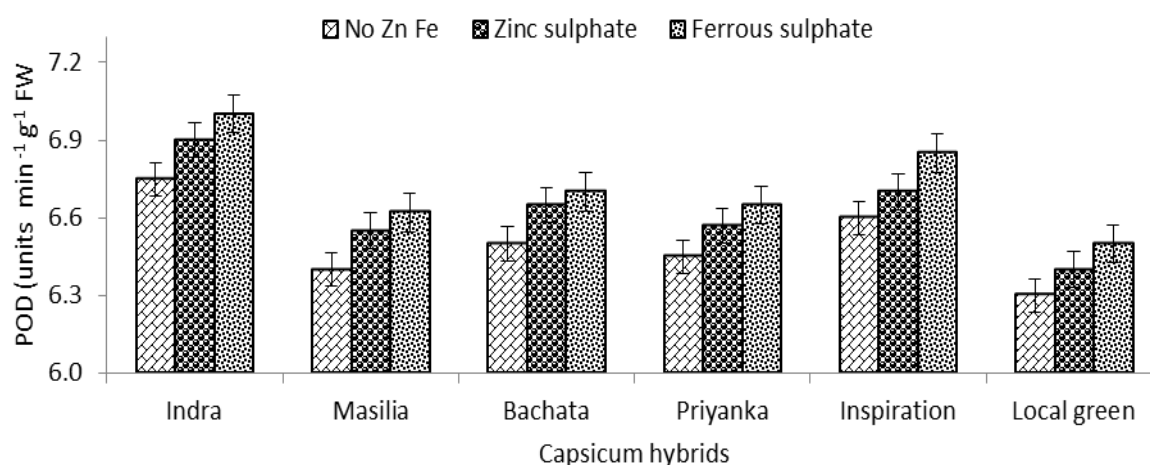


Fig.5. Changes in the leaf peroxidase activity in different capsicum hybrids due to Zn and Fe fertilization (Error bars represents standard error n=3)

3.6 Principal component analysis and hierarchical clustering

The principal component scatter plots for individuals' capsicum hybrids were studied, and found that the individuals lying closer to each other seemed to be similar based on the variables studied. Indra and Inspiration were most distant from the origin in the direction of variable vectors, making it the most responsive hybrids to Zn and Fe fertilization. Individuals falling far from the origin and opposite the variable vectors were less responsive. Further cluster analysis (Fig. 6) was studied for grouping the capsicum hybrids based on their behaviors. The results revealed that the genotype Indra was a highly responsive hybrid, whereas local green was less responsive to Zn and Fe fertilization. The rest of the genotypes, viz., Inspiration, Bachata, Massilia, and Priyanka were grouped as medium responsive to antioxidant enzyme activity and biochemical changes due to zinc and iron fertilization.

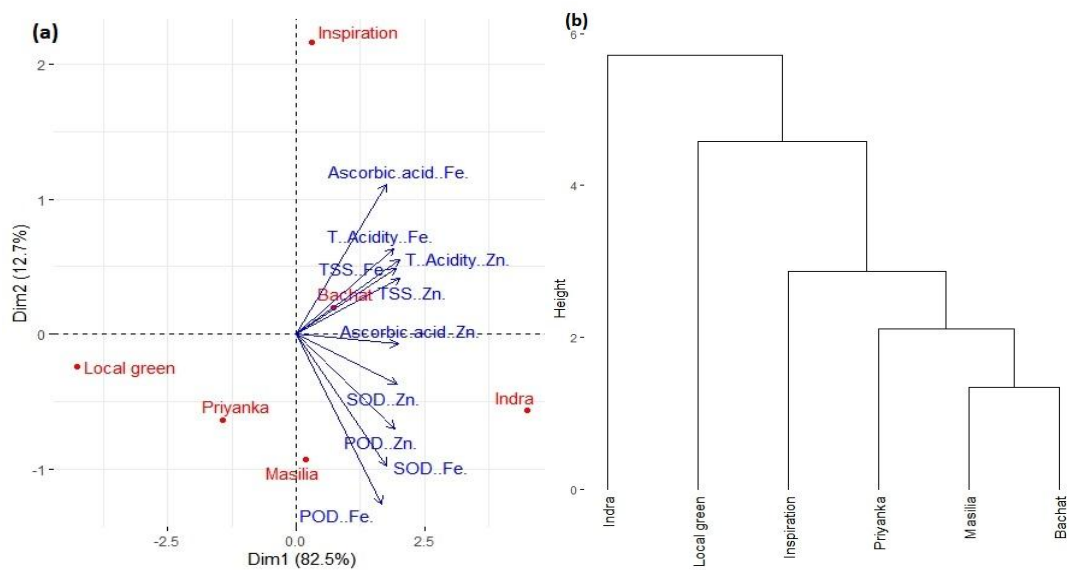


Fig.6. (a) Principal Component Analysis and (b) Hierarchical clustering for grouping the capsicum hybrids based on their antioxidant enzymes and biochemical responses to Zn and Fe fertilization

4. CONCLUSION

It can be concluded from the study that, application of ferrous sulphate and zinc sulphate to capsicum hybrids increased the biochemical constituents in fruits and antioxidant enzyme activities in the leaves. Out of the six capsicum hybrids, higher ascorbic acid content ($9.20 \text{ mg } 100 \text{ g}^{-1}$ fresh weight), acidity (6.0% citric acid), and total soluble solids (6.1°Brix) in the fruits were observed with Indra followed by Inspiration and Bachata. Lesser biochemical and antioxidant enzyme activity for the Zn and Fe application was registered with local green. **The capsicum hybrid Indra was highly responsive based on the principal component analysis and hierarchical clustering. At the same time, Inspiration, Bachata, Massilia, and Priyana were responsive to the antioxidant enzyme and biochemical changes upon zinc and iron fertilization.** However, the local green genotype was lesser responsive to Zn & Fe fertilization, which was evident from the lesser antioxidant enzyme activity in plants and biochemical constituents in fruits.

DISCLAIMER

The products used for this research are commonly and predominantly used in our area of study and country. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for **litigation but knowledge advancement.** Also, the research was not funded by the producing company rather, it was **financed** by the personal efforts of the authors.

COMPETING INTERESTS DISCLAIMER

Authors have declared that no competing interests exist. There is no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for litigation but the advancement of knowledge. Also, the research was not funded by the producing company rather, it was funded by the personal efforts of the authors.

REFERENCES

- [1] Kumar P, Chauhan RS, Grover RK. Economic analysis of capsicum cultivation under polyhouse and open field conditions in Haryana. *Int. J. Farm Sci.* 2016;6(1):96-100.
- [2] Buczkowska H, Michałojć Z, Nurzyńska -Wierdak R. Yield and fruit quality of sweet pepper depending on foliar application of calcium Turk. *J. Agric.* 2016; 40: 222-228.
- [3] Ramana-Rao TV, Gol NB, Shah KK. Effect of postharvest treatments and storage temperatures on the quality and shelf life of sweet pepper (*Capsicum annum L.*). *Sci. Hort.* 2011;132:18-26.
- [4] Mondal S, Bose B. Impact of micronutrient seed priming on germination, growth, development, nutritional status and yield aspects of plants. *J. Plant Nutr.* 2019;42(19):2577-2599.
- [5] Rout GR, Sahoo S. Role of iron in plant growth and metabolism. *Rev. Agric. Sci.* 2015;3:1-24.
- [6] Tardy AL, Pouteau E, Marquez D, Yilmaz C, Scholey A. Vitamins and minerals for energy, fatigue and cognition: A narrative review of the biochemical and clinical evidence. *Nutrients.* 2020;12(1):.228.
- [7] Alvarez-Fernandez A, Paniagua MP, Abadia J, Abadia A. Effects of Fe deficiency-chlorosis on yield and fruit quality in peach (*Prunus persica L. Batsch*). *J. Agric. Food Chem.* 2003;51(19):5738-5744
- [8] Suganya A, Saravanan A, Manivannan N. Role of zinc nutrition for increasing zinc availability, uptake, yield, and quality of maize (*Zea mays L.*) grains: An overview. *Commun. Soil Sci. Plant Anal.* 2020;51(15): 2001-2021.
- [9] Pahlavan-Rad MR, Pessarakli M. Response of wheat plants to zinc, iron, and manganese applications and uptake and concentration of zinc, iron, and manganese in wheat grains. *Commun. Soil Sci. Plant Anal.* 2009;40(7-8): 1322-1332.
- [10] Dodd J, Krikun J, Haas J. Relative effectiveness of indigenous populations of vesicular-arbuscular mycorrhizal fungi from four sites in the Negev. *Isr. J. Plant Sci.* 1983;32(1): 10-21.
- [11] Singh PC, Chaturvedi OP, Mishra CN. Effect of zinc boron and copper on physicochemical characteristics and shelf life of aonla. National Seminar on Management of Medicinal and Aromatic Plants in Farming Systems Prospective 20-22 March, 2007. Directorate of extension C.S. Azad Uni, Agric, and Tech., 2007;161- 162.
- [12] Meena D, Tiwari R, Singh OP. Effect of nutrient spray on growth, fruit yield and quality of aonla. *Ann. Plant Soil Res.* 2014;16 (3): 242-245.
- [13] Hasani M, Zamani Z, Savaghebi G, Fatahi R. Effects of zinc and manganese as foliar spray on pomegranate yield, fruit quality and leaf minerals *J Soil Sci. Plant Nutr.* 2012;12 (3), 471-480.
- [14] Abou El-Yazied A. Effect of foliar application of salicylic acid and chelated zinc on growth and productivity of sweet pepper (*Capsicum annum L.*) under Autumn planting. *Res. J. of Agric. and Biol. Sci.* 2011;7(6): 423–433.
- [15] Yadav D, Shivay YS, Singh YV, Sharma VK, Bhatia A. Enhancing nutrient translocation, yields and water productivity of wheat under rice–wheat cropping system through zinc nutrition and residual effect of green manuring. *J. Plant Nutr.* 2020;43(19): 2845-2856.

- [16] Tamilselvi P, Vijayakumar RM, Nainar P. Studies on the effect of foliar application of micronutrients on growth and yield of tomato (*Lycopersicon esculentum* Mill). Cv. PKM-1. South Ind. Hort. 2002;53(1-6): 46-51.
- [17] Hatwar GP, Gondane SU, Urkude SM, Gahukar OV. Effect of micronutrients on growth and yield of chilli. J. Soils and Crops 2003; 13(1): 123-125.
- [18] Shahean AM, Fatma AR, Singer SM. Growing onion plants without chemical fertilization. Research J. of Agri. And Biolo. Sci. 2007; 3(2): 95-104.
- [19] Bhatt L, Srivastava BK, Singh MP. Studies on the effect of foliar application of micronutrients on growth, yield and economics of tomato. Prog. Hort. 2004;36(2): 331-334.
- [20] Malawadi MN, Shashidhara GB, Palled YB. Effect of secondary and micronutrients on yield, nutrient uptake and quality of chilli. Karnataka J. Agric. Sci. 2004;17(3): 553-556.
- [21] Batra VK, Kamboj M, Arora SK, Mange RS. Effect of foliar application of micronutrients on the quality and shelf-life of tomato. Haryana J. Hort. Sci., 2006; 35(1-2): 140-142.
- [22] Savitha HR. Effect of iron on yield and quality of red chilli (*capicum annum* L.) in a calcareous vertisol of Zone-8 of Karnataka. M.Sc. Soil Sci. and Agri. Chem. Univ. of Agri. Sci. 2008.
- [23] Avalos-Llano KR, Martin-Belloso O, Soliva-Fortuny R. Effect of pulsed light treatments on quality and antioxidant properties of fresh-cut strawberries. Food chem. 2018;264: 393-400.
- [24] AOAC. Official Methods of Analysis Association of Official Analytical Chemists. 20th ed. 111 North 19th street, suite. 16th, Arlington, Virginia, USA; 1994.
- [25] Sadasivam S, Manikkam A. Biochemical methods for agricultural sciences, Wiely Estern Ltd., Madras; 1992.
- [26] Beauchamp C, Fridovich I. Superoxide dismutase: improved assays and an assay applicable to acrylamide gels. Anal. Biochem. 1971;44(1): 276-287.
- [27] Panse VC, Sukhatme PV. Statistical methods for Agricultural workers. 3rd ed. ICAR, New Delhi; 1978
- [28] Kassambara A. Practical guide to cluster analysis in R: Unsupervised machine learning. vol. 1. Sthda; 2017.
- [29] Giovannoni J, Nguyen C, Ampofo B, Zhong SL, Fei ZJ. The epigenome and transcriptional dynamics of fruit ripening. *Annu. Rev. Plant Biol.* 2017;68: 61–84. doi: 10.1146/annurev-arplant-042916-040906
- [30] Beckles DM. Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. Postharvest Biol. Tech. 2012;63(1): 129-140.
- [31] Brahmachari VS, Kumar N, Kumar R. Effect of foliar feeding of Calcium, Potassium and Growth substances on yield and quality of guava (*Psidium guajava* L.). Haryana J. hort. Sci. 1997;26(3-4): 169-173.
- [32] El-Sheikh MH, Khafagy SAA, Zaied NS. Effect of foliar application with some micronutrients on leaf mineral content, yield and fruit quality of "Florida Prince and Desert Red" peach trees. Res. J. Agri. Biol. Sci. 2007;3(4):309-315.
- [33] Dhotra B, Bakshi P, Jeelani MI, Vikas V. Influence of foliar application of micronutrients on fruit growth, yield and quality of peach cv. Shan-e-Punjab. Indian Res. J. Genet. & Biotech. 2018;10(1):105-112.

- [34] Sánchez Sánchez-Cañete FJ, Pontes Pedrajas A. La comprensión de conceptos de ecología y sus implicaciones para la educación ambiental. *Revista Eureka sobre enseñanza y divulgación de las ciencias*, 2010;7:271-285
- [35] Ali S, Javed HU, Rehman URN, Sabir IA, Naeem MS, Siddiqui MZ, Saeed DA, Nawaz MA. Foliar application of some macro and micronutrients improves tomato growth, flowering and yield. *Int. J. Biosci.* 2013;3(10):280–87. doi:10.12692/ijb/3.10.280-287.
- [36] Aydin S, Yagmur B, Hakerlerler H, Coban H. Effects of different types and levels of zinc sulphate application in vineyards (*Vitis vinifera* L.) in a semiarid environment. *Chem. Asian J.* 2007; 19 (1):555–63.
- [37] Eman AA, El-Migeed MMMA, El-Moneim A, Omayma AMMI. GA3 and zinc sprays for improving yield and fruit quality of Washington Navel orange trees grown under sandy soil conditions. *Res. J. Agric. & Biol. Sci* · 2007;3(5):498-503.
- [38] Hacisevki A. An overview of ascorbic acid biochemistry. *J. Fac. Pharm. Ankara*, 2009;38(3): 233-255.
- [39] Kim JS, Ahn J, Lee SJ, Moon B, Ha TY, Kim S. Phytochemicals and antioxidant activity of fruits and leaves of paprika (*Capsicum Annuum* L., var. special) cultivated in Korea. *J. Food Sci.* 2011;76(2):193-198.
- [40] Mengel K, Kirkby EA. Principles of plant nutrition. *Ann. Bot.* 2004;93(4):479–480.
- [41] Nawaz MA, Ahmad W, Ahmad S, Khan MM. Role of growth regulators on preharvest fruit drop, yield and quality in Kinnow mandarin. *Pak. J. Bot.* 2008; 40:1971- 1981.
- [42] Devi EL, Kumar S, Singh TB, Sharma SK, Beemrote A, Devi CP, Wani SH. Adaptation strategies and defense mechanisms of plants during environmental stress. In *Medicinal plants and environmental challenges*. Springer, Cham; 2017.
- [43] Broadley MR, White PJ, Hammond JP, Zelko I, Lux A. Zinc in plants. *New Phytol.* 2007;173: 677–702.
- [44] Singh P, Shukla AK, Behera SK, Tiwari PK. Zinc application enhances superoxide dismutase and carbonic anhydrase activities in zinc-efficient and zinc-inefficient wheat genotypes. *J. Soil Sci. Plant Nutr.* 2019;19(3):477-487.
- [45] Tavanti TR, de Melo AAR, Moreira LDK, Sanchez DEJ, dos Santos Silva R, da Silva RM, Dos Reis AR. Micronutrient fertilization enhances ROS scavenging system for alleviation of abiotic stresses in plants. *Plant Physiol. Biochem.* 2021;160:386–396.
- [46] Kaya C, Ashraf M, Akram NA. Hydrogen sulfide regulates the levels of key metabolites and antioxidant defense system to counteract oxidative stress in pepper (*Capsicum annuum* L.) plants exposed to high zinc regime. *Environ. Sci. Pollut. Control Ser.* 2018;25 (13):12612–12618.
- [47] Uday B, Saini M, Kumar P. Effect of zinc oxide nanoparticles on growth and antioxidant system of chickpea seedlings. *Toxicol. Environ. Chem.* 2013;95 (4): 605–612.
- [48] Wu S, Hu C, Tan Q, Li L, Shi K, Zheng Y, Sun X. Drought stress tolerance mediated by zinc-induced antioxidative defense and osmotic adjustment in cotton (*Gossypium Hirsutum*). *Acta Physiol. Plant.* 2015;37(8):167.
- [49] Sida-Arreola JP, Sánchez E, Preciado-Rangel P, Márquez-Quiroz C. Does zinc biofortification affect the antioxidant activity in common bean? *Cogent Food Agric.* 2017;3(1):1283725.

- [50] Rout JR, Behera S, Keshari N, Ram SS, Bhar S, Chakraborty A, Sudarshan M, Sahoo SL, Effect of iron stress on *Withania somnifera* L.: antioxidant enzyme response and nutrient elemental uptake of in vitro grown plants. *ecotoxicology* 2015;24 (2): 401–413.
- [51] Cao X, Cai C, Wang Y, Zheng X. The inactivation kinetics of polyphenol oxidase and peroxidase in bayberry juice during thermal and ultrasound treatments. *Innovative food science & emerging technologies*. 2018; 45, 169-178.
- [52] Peng XX, Yu XL, Li MQ, Yamauchi M. Induction of prooxidase by Fe²⁺ in detached rice leaves, *Plant Soil*. 1996; 180:159–163.
- [53] Li X, Ma H, Jia P, Wang J, Jia L, Zhang T, Wei X. Responses of seedling growth and antioxidant activity to excess iron and copper in *Triticum aestivum* L. *Ecotoxicol. Environ. Saf.* 2012;86:47-53.
- [54] Michael P.I, Krishnaswamy M. Oxidative stress and antioxidants in cowpea plants subjected to boron and high irradiance stresses. *J. Plant Nutr.* 2012;35 (14): 2180–2197.
- [55] Sun C, Wu T, Zhai L, Li D, Zhang X, Xu X, Ma H, Wang Y, Han Z. Reactive oxygen species function to mediate the Fe deficiency response in an Fe-efficient apple genotype: an early response mechanism for enhancing reactive oxygen production. *Front. Plant Sci.* 2016;7:1726.
- [56] Tewari R.K, Hadacek F, Sassmann S, Lang I. Iron deprivation-induced reactive oxygen species generation leads to non-autolytic PCD in *Brassica napus* leaves. *Environ. Exp. Bot.* 2013; 91: 74–83.
- [57] Jucoski G, Cambraia J, Ribeiro C, de Oliveira J.A, de Paula S.O, Oliva, M.A. Impact of iron toxicity on oxidative metabolism in young *Eugenia uniflora* L. plants. *Acta Physiol. Plant.* 2013;35 (5):1645–1657.