Original Research Article

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 vital role in 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 its species. A screening experiment was conducted during 2020 to know the antioxidants and biochemical responses to iron and zinc fertilisation of six capsicum hybrids grown in grow bags under shade net condition. The experiment consists of three Zn/Fe treatments *viz.,* No Fe & Zn, 50 kg $FeSO_4$ and 37.5 kg $ZnSO_4$ ha^{-1} as basal soil application and six capsicum hybrids *viz.,* Indra, Priyanka, Inspiration, Masilia, Bachata and local green. Application of ferrous sulphate and zinc sulphate 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 (6.1^0 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 Principal Component Analysis (PCA) and hierarchical clustering revealed that, Indra is highly responsive to Zn and Fe fertilisation, while the local green showed vey less response. Rest of the genotypes such as Inspiration, Bachata, Massilia and Priyanka are found to be medium responsive for Zn and Fe fertilisation.

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

1. Introduction

Capsicum is one of the most popular vegetables commercially grown under protected cultivation because of its adoptability in different protected structures. Hike in demand increased the farmer's interest in cultivating this crop under protected structure due to its qualitative and quantitative advantages [1]. It is grown on large scale as an off season vegetable crop. Protected cultivation of Capsicum for fresh vegetable marketing as well as for processing has recently gained economic importance [2]. Capsicum hybrids are a rich source of vitamin C (ascorbic acid); it also contains antioxidants which has important health-related implications [3]. The levels of these compounds are modified by growing conditions and nutrient application and also depend on the genotype and fruit maturity [2]. Fertilizers and irrigation are the basic requirement for increasing the fruit yield of capsicum. Capsicum responds well to fertilizer applications and is reported to have high demand for major nutrients (NPK). Micronutrients are usually required in minute quantities but essential for various activiites; particularly iron (Fe) and Zinc (Zn) play vital roles in growth and development of plants due to their catalytic effect on many metabolic processes. Improvement in growth characters as a result of application of micronutrients such as zinc and iron may be due to the enhanced photosynthetic and other metabolic activity which lead 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. A large number of enzymes require iron as a cofactor for their function which is involved in oxidative phosphorylation, metabolic pathway that converts nutrients into energy [5]. In plants, iron is involved in the synthesis of chlorophyll and is essential for the maintenance of chloroplast structure and function [6]. Iron (Fe) also affects fruit quality parameters such as colour, firmness, or acidity and affecting fruit production, dropping the number of fruits per tree, fruit size and hence, yield [7]. Similarly, in plants, Zn activates the electrophile and nucleophiles as a component of plant carbonic anhydrase as well as 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 produce yield with the application of Zn and Fe fertilisers [9]. It is also increase the plant height, number of side branches, and leaf area [10]. Foliar spraying of Zn and salicylic acid increased the fruit yield and quality as well as Nitrogen (N), Phosphorus (P), Potassium (K) intake [11] and water use efficiency [12]. The mechanism behind the role of these nutrients in various plant metabolic processes were studied by several authors such as Tamilselvi *et al.,* [13]; Hatwar *et al.,* [14] and Shaheen *et al*., [15] revealed that, application of micronutrients as foliar spray caused an enhancement in plant growth, fruit yield and its physical and chemical properties of fruits. In the same way, Bhatt *et al.,* [16] studied the effect of foliar application of micronutrients and reported significant improvement in yield which might be attributed to increased photosynthetic activity and increased production and accumulation of carbohydrate. Also, Malawadi *et al.,* [17] studied the effect of soil application of micronutrients on yield and quality of chilli and noticed higher fruit weight, yield and maximum ascorbic acid content in capsicum fruits. Likewise, Batra *et al.,* [18] and Savitha [19] reported that, foliar application of iron after transplanting resulted in significant improvement in the ascorbic acid content in fruits due to the increase in activity of ascorbic acid oxidizising enzyme. As regards the major quality parameters, Tamilselvi *et al.,* [13] observed maximum total soluble solids (TSS), acidity and ascorbic acid contents in the fruits with the application of micronutrients. From the literatures it was perused that different forms and levels of Zn and Fe give differential responses to plants' physiology [20], particularly the biochemical responses which vary with cultivars. Hence this investigation was carried out to understand the differential biochemical responses of capsicum hybrids for zinc and iron fertilization.

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 fertilisation 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, 50 kg $FeSO_4$ and 37.5 kg $ZnSO_4$ application ha^{-1} and six capsicum hybrids *viz.,* Indra, Priyanka, Inspiration, Masilia, Bachata and local green. The experiment was laid out in a Randomised Block Design with three replications. Recommended fertiliser 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 of capsicum hybrids for Zn and Fe fertilisation were 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 nom 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. As regards the available micronutrients status in soils, 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).

2.3 Estimation of biochemical constituents and antioxidant enzyme activities

The biochemical constituents such as acidity, total soluble solids (TSS) and ascorbic acid content in the fruit was determined after harvest. The acidity in the fruit juice was estimated by titration with a standard alkali using phenolphthalein as 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 colour is obtained. The result was expressed in terms of citric acid per 100 g of fruit sample [21]. Total soluble solid (TSS) content was determined by means of hand Refractometer. Which was calibrated at 20 ^{0}C and few drops of the fruit juice sample was placed in between the prisms and read at the demarcation line and expressed as Brix [21]. 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 [22].

The Zn and Fe requiring enzymes in the leave samples at flowering stage was determined to understand the role of applied Zn and Fe in the plant nutrition. Peroxidase activity was estimated using leaf samples homogenized in phosphate buffer of which one mL of supernatant was taken and 3 mL of 0.05 M pyrogallol and 0.5 mL of 30% H_2O_2 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 [22]. The super-oxide dismutase activity was measured using Nitro blue tetrazolium (NBT) method [23]. 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 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 investigations were subjected to the analysis of variance to find out the significance as suggested by Panse and Sukhatme [24] using SPSS software. Principal component analysis and hierarchical clustering were plotted using R studio software [25].

3. RESULTS AND DISCUSSION

3.1 Titrable acidity

The characteristic flavour of each horticultural product must not only be associated with the presence of sugars such as sucrose, glucose, galactose and fructose [26], but also due to the accumulation of some organic acids (citrate and malate) [27]. The acidity data obtained for the fruits expressed as a function of the prevailing organic acid are shown in Fig. 1. The titrable acidity of the capsicum hybrids increased with zinc (Zn) and iron (Fe) fertilisation 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 $ZnSO_4$ and $FeSO_4$ 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 [28]. Lesser conversion of sugar into organic acid was observed with the local green (5.0 % citric acid) at no Zn and Fe application.

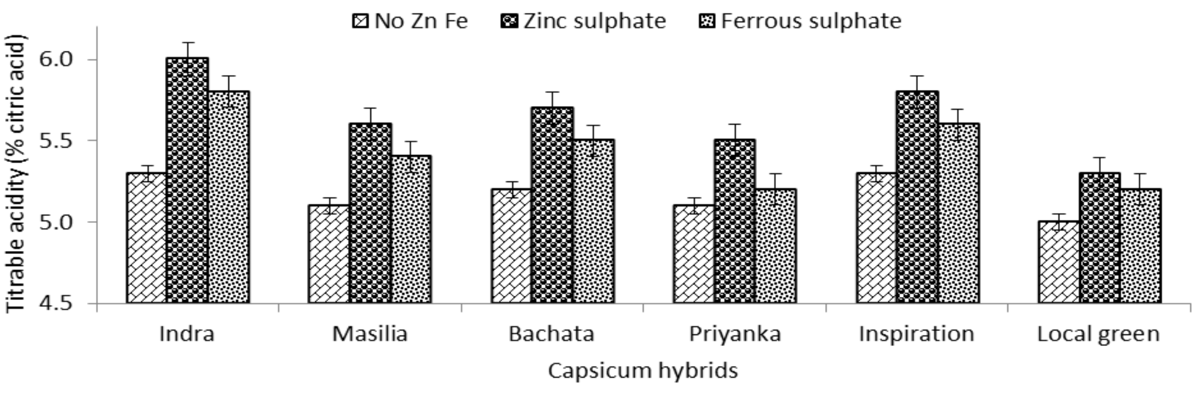

Fig.1. Changes in titrable acidity in the fruits of different capsicum hybrids due to Zn and Fe fertilisation (Error bars represents standard error n=3)

It was reported that, application of non-chelated forms of micronutrients (Fe + Zn) produced higher acidic fruits compared to chelated forms [29]. However, the results of the present study are similar to the findings of Dhotra *et al.,* [30], who reported that plants sprayed with $ZnSO_4$ and $FeSO_4$ produced more acidic fruits compared to 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 harvest index for fruits and vegetables [31], because its determination is rapid, relatively low cost and well correlates directly with flavour [27]. The capsicum hybrid Indra had higher TSS (6.10, 5.80 ^{0} Brix) followed by Inspiration (5.90, 5.75 ^{0} Brix) and Bachata (5.80, 5.70 ^{0} Brix) in response to $ZnSO_4$ and $FeSO_4$ application (Fig. 2). Total soluble solid was found to be higher with the $ZnSO_4$ application than $FeSO_4$ with mean of 5.77 and 5.90 ^{0} Brix respectively. Lesser TSS content in the fruit was noticed with local green (5.10 ^{0} Brix) in no Zn and Fe applied control. It was reported that TSS of the fruit was an important quality parameter [32] which could be increased with Zn and Fe application [33] and attributed to increased photosynthesis activity, translocation of sugars from source to sink and conversion of complex form of sugars (polysaccharides) to simple sugars (glucose and fructose) in fruits [34].

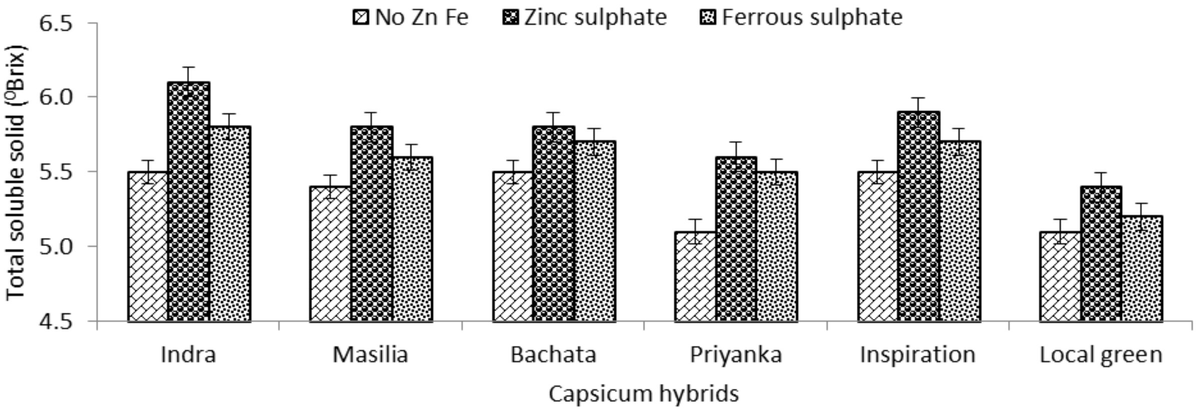

Fig. 2. Changes in total soluble solid content in fruits of different capsicum hybrids due to Zn and Fe fertilisation (Error bars represents standard error n=3)

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 for fruit quality assessment [35]. Ascorbic acid content being highly correlated with antioxidant activity, application of micro nutrients significantly affects its availability in fruits [36]. Among the capsicum hybrids, the fruits of Indra contains higher ascorbic acid t (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 $ZnSO_4$ and $FeSO_4$ application (Fig. 3). The improvement in ascorbic acid content with Zn application was higher than Fe with 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 levels of sugar with the application of micronutrients might be the possible cause for increased ascorbic acid content, which is synthesized from sugar [37]. 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 higher synthesis of auxin [38]. Batra *et al.* [18] 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 increased activity of ascorbic acid oxidase enzyme [19].

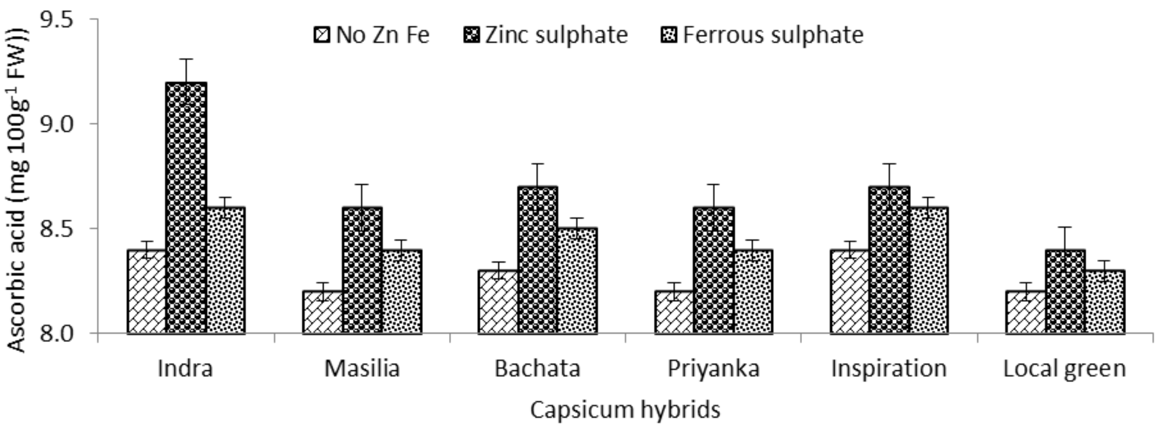

Fig. 3. Changes in ascorbic acid content in the fruits of different capsicum hybrids due to Zn and Fe fertilisation (Error bars represents standard error n=3)

3.4 Superoxide dismutase activity (SOD)

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 in order to defend themselves against oxidants [39]. Application of iron and zinc increased the super oxide dismutase activity in leaves of capsicum hybrids (Fig. 4). All the genotypes exhibited higher super oxide dismutase activity with Zn application than Fe (6.70 to 6.30 Units g^{-1}). 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 observed with Local green hybrid at no Zn and Fe applied (5.10 Units g^{-1}). Zinc has essential role on enzymes including Cu/Zn-SOD [40] and the enhanced enzyme activity in the present study is due to the role of Zn as co-factor for the functioning of SOD. Moreover, activities of SOD depend on the availability of Zn from soil which differs with genotypes [41]. Several studies reported 89% of correlation with the increased SOD activity in various plant species due to Zn fertilisation [42].Kaya *et al.,* [43] reported that pepper plants (*Capsicum annuum* L.) treated with high Zn concentration resulted in decreased H_2O_2 concentrations and MDA and stimulated the SOD activity. Lesser activity of SOD was observed in untreated plants [44]. Wu *et al.,* [45] and Sida-Arreola *et al.,* [46] also reported that the increase in SOD activity with application of Zn fertiliser. Similarly 80% of the studies show increased SOD [42] by evaluating the physiological effect of Fe in plants,. Rout *et al.,* [47] reported that, application of 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**.**

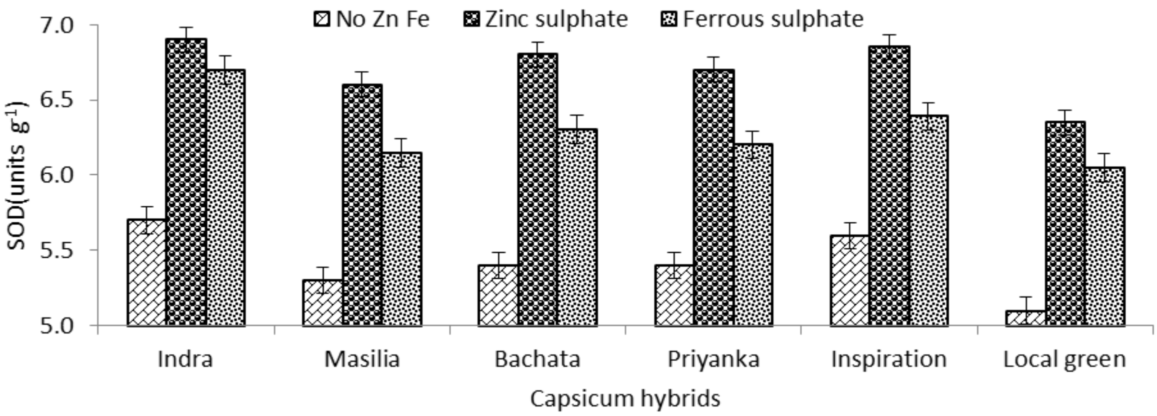

Fig. 4. Changes in leaf superoxide dismutase activity in capsicum hybrids due to Zn and Fe fertilisation (Error bars represents standard error n=3)

3.5 Peroxidase activity (POX)

Peroxidase (POX) enzyme has a protective effect and involved in chlorophyll degradation, ROS generation and membrane lipid peroxidation, which are responsive to injury and a product of senescence [48]. 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 higher impact on peroxidase activity than zinc application with a mean of 6.72 and 6.63 Units min^{-1} g^{-1} Fresh weight respectively. The higher peroxidase activity was observed with Indra (7.00, 6.90 Units min^{-1} 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 Units min^{-1} 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 [49, 50]. Michael *et al.,* [51] also observed increased POX activity in the plants treated with 50 ppm of Zn. Kaya *et al.,* [43] reported decreased H_2O_2 concentrations and stimulation of the enzymatic antioxidants enzyme POD in response to Zn application in pepper plants (*Capsicum annuum* L.). Fe is a constituent of enzymes associated with the cellular antioxidant system such as Fe-SOD hence, plants exposed to Fe application shows variation in POD activities [52]. Tewari *et al.,* [53] and Jucoski *et al.,* [54] also reported similar results with the application of Fe fertilisers.

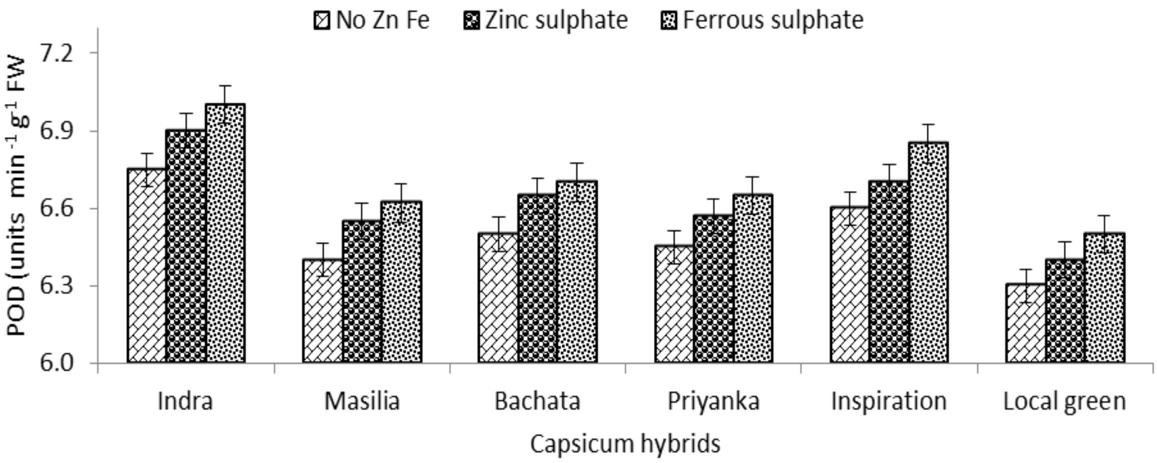

Fig.5. Changes in the leaf peroxidase activity in different capsicum hybrids due to Zn and Fe fertilisation (Error bars represents standard error n=3)

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. The Indra and Inspiration were most distant from the origin in direction of variable vectors thereby making it most responsive hybrids to Zn and Fe fertilization. Individuals falling far from the origin and in opposite to the variable vectors were less responsive. Further cluster analysis based on agglomerative hierarchical clustering and the resultant dendrogram (Fig. 6) were studied for grouping the capsicum hybrids based on their behaviours and the reuslts revealed that, the genotype Indra was highly responsive hybrid where as local green was less responsive to Zn and Fe fertilisation. Rest of the genotypes viz., Inspiration, Bachata, Massilia and Priyanka was grouped as medium responsive to antioxidant enzyme activity and biochemical changes due to zinc and iron fertilisation.

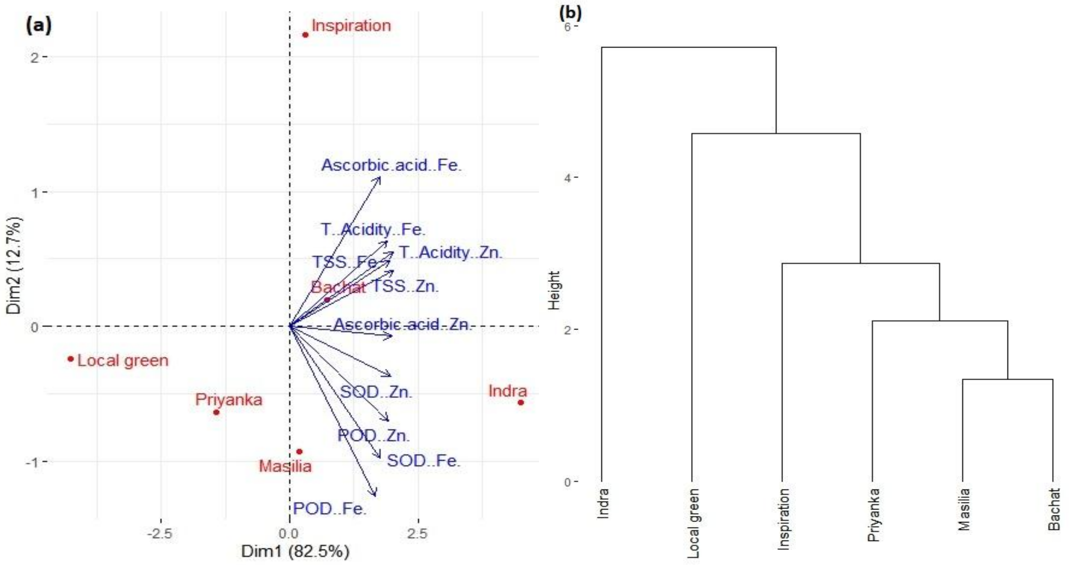

Fig.6. (a) Principal Component Analysis and (b) Hierarchical clustering for grouping the capsicum hybrids based on their antioxidant enzymes and biochemical responses to Zn and Fe fertilization

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 mg 100 g^{-1} fresh weight), acidity (6.0% citric acid) and total soluble solids (6.1 ^{0}Brix) in the fruits was 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. Based on the principal component analysis and hierarchical clustering, the capsicum hybrid Indra was highly responsive, while Inspiration, Bachata, Massilia, Priyana were medium responsive to antioxidant enzyme and biochemical changes upon zinc and iron fertilisation. However, the local green genotype was poor responsive to Zn & Fe fertilisation 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 research and country. There is absolutely 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 any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely 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 any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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