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Changes in antioxidants and pungency in response to foliar applications of Mn and Fe in onion

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The aim of the present work was to study the changes in antioxidants and pungency in response to foliar applications of Mn [manganese: Mn_1(0%); Mn_2(0.5%); Mn_3(1.0%)] and Fe [iron: Fe_1(0%); Fe_2(0.5%); Fe_3(1.0%)] in onion genotype Sukhsagar (*Allium cepa* L.); a field experiment in factorial randomized block design with three replications was conducted in research farm of Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India. In this experiment, we observed that the total and free phenol contents were significantly higher than control in both the single (0.5%) and double (1.0%) doses of Fe alone. Again, the activity of enzymatic antioxidant super oxide dismutase (SOD) increased with increasing doses of Fe alone, while the maximum peroxidase (POD) activity was obtained in the treatment combination of double doses (1.0%) of both Fe and Mn. The highest dose of Mn (1%) in combination with all doses of Fe (0, 0.5 and 1.0%) exhibited the highest set of values of catalase (CAT) activity. The activity of 2,2-diphenyl-β-picrylhydrazyl radical scavenging activity (DPPHRAC) assay increased with increasing doses of Fe. The lowest degree of lipid peroxidation was observed in the case of 1% Mn in combination with 1% Fe, which also exhibited the highest pungency. The average values of all the parameters along with the results of PCA, the treatment Mn_3Fe_3 (the double dose or 1.0% both of Fe and Mn) was found to be the most efficient to ensure the antioxidant properties of the genotype Sukhsagar.

Key words: *Allium cepa* L., manganese, iron, antioxidants, pungency.

INTRODUCTION

From time immemorial, onion (*Allium cepa* L.) has been used as common food and also for the treatment of many diseases. The first citation of this plant is found in the Codex Ebers (1550 BC), an Egyptian medical papyrus reporting several therapeutic formulas based on onions as useful remedy for a variety of diseases such as heart problems, headache, bites, worms and tumors (Block, 1985). Egyptians thought onions aided endurance and

assumed large quantities of them. Raw plants were routinely given to asthmatics and to those people suffering from bronchial-pulmonary complaints. Later on, these food plants were known by Greeks and Romans. Who used them as important healing agents and are still being used by most of the people of the Mediterranean area (Griffiths et al., 2002). Processed onions are applied as a poultice to indolent boils, bruises, wounds, to

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relieve hot sensations and applied to the navel for dysentery and fever (Brewster and Rabinowitch, 1990). The different clinical studies have shown the benefit of onions in the reduction of the risk of cardiovascular diseases by inducing lowering of serum cholesterol and blood pressure (Steiner and Lin, 1994). They have liver protective (Dion and Milner, 1996), immune enhancement and anti-infection (Lau, 1989), anti-stress and anti-fatigue (Kawashima, 1986), anti-cancer and cancer preventive effects (Balasenthil et al., 2001; Dion and Milner, 1997; Pinto et al., 1997). There are also several other reports for anti-carcinogenic, antibiotic, antibacterial, antifungal and antioxidant properties of onion (Benkeblia, 2005).

Aerobic organisms are constantly exposed to one or more systems that generate reactive oxygen species (ROS), including the superoxide radical anion ($O_2^{•-}$), hydroxyl radical ($^{•}OH$), hydrogen peroxide (H_2O_2), various peroxyl radicals ($ROO^{•}$), and singlet oxygen ($^1O_2$). These ROS are highly reactive and can damage living cells if formed in significant amounts. To avoid cellular damage by ROS, most biological systems have developed defense mechanisms, antioxidants that convert ROS to unreactive derivatives. Antioxidants are broadly defined as molecules that, when present at low concentrations, as compared to an oxidizable substrate, significantly delay or prevent oxidation of that substrate (Gutteridge, 1994). These include enzymes, frequently known as preventive antioxidants, that is, superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), which prevent or inhibit formation of ROS, and chain-breaking antioxidants, that is, vitamin C and phenolics, which scavenge oxygen radicals and break radical chain sequences.

When cells are exposed to an unusually high load of oxidants and free radicals, natural defense mechanisms may not be sufficient for eradication of free radical-induced deleterious effects. These include oxidation of nucleic acids, proteins, lipids and carbohydrates; cell death; tissue injury; and development of disease processes such as atherosclerosis, carcinogenesis, cirrhosis, fibrosis and inflammation, aging and aging-related disorders in humans (Halliwell and Gutteridge, 1989). Supplementation of antioxidants may ameliorate harmful effects of the oxidative processes in organisms (Logani and Davies, 1980).

Onions (*Allium cepa* L.) have an important status among vegetables because of their particular flavour and ability to enhance the flavour of other foods. Many sulfur compounds contribute to flavour synthesis and the development of flavour precursors. Onion flavour arises from the enzymatic decomposition of *S*-alk(en)yl cysteine sulfoxides (ACSOs) when onion tissue is disrupted. The products of the decomposition process, such as lachrymatory factor and thiosulfinates, present onion's unique flavour (Randle, 1997).

Generally, undamaged onions are odourless but, when onion tissues are cut or disrupted, the enzyme alliinase is released to hydrolyze the *S*-alk(en)yl cysteine sulfoxides to produce pyruvate, ammonia and volatile sulfur compounds. The volatile sulfur compounds play the most important role in the flavour chemistry (Lancaster and Boland, 1990). In 1992, Block pointed out that alliins (*S*-alk(en)yl cysteine sulfoxides) are the major flavour precursors to produce the odour and flavour of the *Allium* species when cells are damaged. Schnug (1993) opined that alliins are the most important sulfur-containing compounds of the secondary metabolism of the genus *Allium*.

Although, fragmentary reports are there on the content of the antioxidants but information on the activity of antioxidative enzymes is lacking. Therefore it is important to have detailed information about the content of non-enzymatic antioxidants and the activity of enzymatic antioxidants in onion. The incidences of micronutrients have received a great deal of importance in crop production during recent years because of the widespread occurrences of their deficiencies from different parts of the country. Researchers from almost all the states in the country have also reported significant responses of many crops to micronutrient fertilization.

Iron is an essential element for plant growth and development, since it is fundamental for the proper functioning of numerous metabolic and enzymatic processes. Some studies estimates indicate that a large number of diverse materials can serve as sources of plant nutrients. The majority of nutrient input to agriculture comes from commercial mineral fertilizers. Mineral fertilizers are considered to play a significant but lesser role in nutrient contribution, leaving aside their beneficial effects on soil physicochemical and biological properties. Foliar feeding is a relatively new and controversial technique of feeding plants by applying liquid fertilizer directly to their leaves.

Iron is the component of iron-sulfur (Fe-S) proteins and also non-heme iron proteins which are required during photosynthesis, respiration and N_2 fixation (Taiz and Zeiger, 1991). It is also reported that, it regulates the chlorophyll biosynthesis (Marschner, 1995). About 80% leaf iron is localized in chloroplast and 60% of iron is involved in regular functioning of electron transport chain (Terry and Abadía, 1986). Thus under iron deficiency, the level of chlorophyll fluorescence (Morales et al., 1991) and photosynthetic activity of the plants are decreased markedly (Iturbe-Ormaexte et al., 1995). Iron is a component of several metalloenzymes, including catalase and peroxidase (Valenzuela et al., 1992).

Enzymatic activities of certain antioxidant enzymes like superoxide dismutase (SOD) and peroxidase (POD) are altered under such conditions. Superoxide dismutase is the major scavenger of superoxide ($O_2^{•-}$) in plant system and its enzymatic reaction leads to formation of H_2O_2 and O_2 (Fridovich, 1995). The hydrogen peroxide (H_2O_2) is decomposed in the presence of peroxidase (Minotti and Aust, 1987). Hence, under iron deficient condition normal

physiological and biochemical processes in the plant system are severely hampered.

Divalent manganese ions (Mn^{2+}) is converted to Mn^{3+} or Mn^{4+} easily, therefore in the plant manganese plays an important role on oxidation and reduction processes, as electron transport in photosynthesis. Moreover manganese acts as an activator of several enzymes (more than 35 different enzymes), which involve oxidation reactions, carboxylation, carbohydrates metabolism, phosphorus reactions and citric acid cycle. Of the most important enzymes, protein-manganese in Photosystem II and superoxide dismutase can be pointed. There is more than 90% of superoxide dismutase in chloroplasts with about 4 to 5% of it in mitochondria (Jackson et al., 1978; Millaleo et al., 2010; Mukhopadhyay and Sharma, 1991; Uehara et al., 1974). Manganese also activates several enzymes of the shikimic acids pathway and subsequent pathways, leading to the biosynthesis of aromatic amino acids, such as tyrosine, various secondary products, such as lignin, flavonoids, as well as indol acetic acid (IAA) (Burnell, 1988; Hughes and Williams, 1988). For example, Mn^{2+} affects phenylalanine ammonia-lyase (PAL) and stimulates peroxidases required for lignin biosynthesis.

The literature on Mn and Fe fertilization of onion is scarce. Bar-Akiva (1971) reported that the peroxidase and catalase activities have been distinguished by Fe and Mn deficiency in Citrus, as peroxidase activity is lowered by Fe deficiency and increased by Mn deficiency in cucumber and tomato (Valenzuela and Romero, 1968). Therios et al. (2005) also reported that peroxidase levels are positively influenced by the increment of Fe and the decline of Mn, in young and old leaves of citrus. The aim of this present study was to assess the changes in antioxidants (non-enzymatic antioxidants: ascorbic acid, phenolics; enzymatic antioxidants: antioxidant super oxide dismutase (SOD), peroxidase (POD), catalase (CAT); antioxidant activities: 2,2-diphenyl-β-picrylhydrazyl radical scavenging activity (DPPHRAC), molybdate reducing antioxidant potential (MRAP), lipid peroxidation (LP) and pungency (PAD) in response to foliar applications of Mn and Fe in onion.

MATERIALS AND METHODS

Field experiment

The seedling was grown in nursery beds prepared in a sandy loam soil. The beds were 1.5 m long and 1.0 m wide. Weathered cow dung manure, 4 kg•m^{-2}, was mixed into the beds. Beds were drenched with formaldehyde (4%) and covered with polythene sheets for ten days to avoid damping off disease. Seedling was treated with Thiram (3 g•kg^{-1} of seed) (Gujarat Pesticides Private Limited, Gandhinagar, Gujarat, India) prior to sowing. Onion seeds of the genotype Sukhsagar the most popular cultivar in West Bengal was obtained from AICRP on Vegetable Crops and were sown at 0.5 cm depth and 5 cm apart and covered with finely sieved leaf mold. After sowing, beds were covered with straw until germination and hand watered regularly. Seedlings were raised as

before and hardened by withholding water 4 days before transplanting.

The main field was of sandy loam soil. Land preparation was done with 3-4 ploughings and beds were prepared. Fertilizer at the rate of 60N-60P-60K kg•ha^{-1} was applied before transplanting and incorporated well into the soil. The source of nitrogen (N) was urea, that of phosphate (P) was single superphosphate and potash (K) was muriate of potash. Additional nitrogen fertilizer at the rate of 60 N kg•ha^{-1} was applied 21 days after transplanting. The 40 days old seedlings were transplanted on muddy flat surface soil in 5 cm deep furrows with 15 cm spacing between rows and 10 cm within plants in plots. The plot size was 2.25 × 2.0 m. The experiment consisting of nine treatments including control (only water sprayed) were arranged in a factorial randomized block design with three replications of each treatment at the In-check Research Farm, Bidhan Chandra Krishi Viswavidyalaya, Kalyani, Nadia, West Bengal, India. The detail treatments are summarized in Table 1.

Since onion is a shallow rooted crop, supplementary irrigations (12 weekly irrigations providing approximately 5 cm of water were applied with the first being before transplanting) were given. There were four light hoeings within 9 weeks of transplanting. The application of fungicide Chlorothalonil at 2 g•L^{-1} with sticker Sandovit at 1 ml•L^{-1} was applied four to five times beginning from 15 days after transplanting and at 15 days intervals to control purple blotch disease [Alternaria porri (Ellis) Cif.].

Onion bulbs from each plot were harvested at 127-128 days after transplanting (when more than 80% plant leaves were dried). Sample (15-20 pieces of bulbs) from each replication was collected after harvesting. Fresh materials were washed, dried with soft tissue, and chopped with a sharp knife into small pieces before analysis of the content of non-enzymatic antioxidants, activity of enzymatic antioxidants and antioxidant activity under different systems of assay [MRAP, DPPH radical scavenging activity and lipid peroxidation] as well as pyruvic acid development (PAD) which is a measure of pungency.

Chemical analysis

Analysis of ascorbic acid content (AAC)

One gram of finely chopped onion tissue was extracted with 20 ml of 4% oxalic acid following maceration in a pestle and mortar and the material centrifuged for 30 min at 10,000 *g*. Ascorbic acid content was determined using the dichlorophenol indophenol titration procedure (AOAC).

Analysis of total phenol content (TPC) and free phenol content (TPC)

Total phenol was extracted in 50% methanolic 1.2 N HCl and free phenol was extracted in 50% aqueous methanol by boiling one gram of finely chopped tissue for 1.5 h at 80-90°C following the method of Vinson et al. (1998) and subsequent analysis was with the Folin-Ciocalteau reagent using gallic acid as standard.

Analysis of super oxide dismutase (SOD) activity

One gram finely chopped bulb tissue extracts to inhibit photochemical reduction of nitroblue tetrazolium (NBT) in riboflavin light NBT system (Beauchamp and Fridovich, 1971) was used to determine super oxide dismutase (SOD) activity. Absorbance at 560 nm was recorded and percentage inhibitory activity was calculated as $[(A_0-A_e)/A_0] \times 100$ where A_0 = absorbance without extract and A_e = absorbance with extract.

Table 1. Detail doses of treatments used as foliar spray for onion.

Treatment	Manganese (Mn) (%)	Iron (Fe) (%)
T_1 (Mn_1Fe_1)	0.0	0.0
T_2 (Mn_1Fe_2)	0.0	0.5
T_3 (Mn_1Fe_3)	0.0	1.0
T_4 (Mn_2Fe_1)	0.5	0.0
T_5 (Mn_2Fe_2)	0.5	0.5
T_6 (Mn_2Fe_3)	0.5	1.0
T_7 (Mn_3Fe_1)	1.0	0.0
T_8 (Mn_3Fe_2)	1.0	0.5
T_9 (Mn_3Fe_3)	1.0	1.0

The source of Mn was $MnSO_4.H_2O$ and that of Fe was $FeSO_4.7H_2O$. Each spraying was done four times with sticker starting from 30 days after transplanting and subsequent ones at an interval of 10 days during vegetative stage.

Analysis of peroxidase (POD) activity

One gram of fresh onion tissue from each treatment was macerated in a pestle and mortar and extracted with 10 ml tris-HCL buffer (pH 7.6) to determine POD activity. Triturated samples were centrifuged at 10,000 g for 30 min at 4°C and supernatants were assessed for enzyme activity. The POD was estimated by mixing 0.1 ml chilled enzyme extract with 2.8 ml reaction mixture (0.5% o-dianisidine dissolved in methanol, 0.28 ml Na-acetate buffer, 2.4 ml water). The reaction was initiated by adding 0.1 ml H_2O_2 (30%). Changes in absorbance at 430 nm were monitored at 1 min intervals for up to 3 min. POD activity was measured using o-dianisidine as the substrate (Bhattacharya et al., 2010) and expressed as mM o-dianisidine oxidized•min^{-1}•g^{-1} of fresh tissue using the extinction coefficient 1.13×10^4•M^{-1}•cm^{-1}.

Analysis of catalase (CAT) activity

Two grams of fresh tissue was macerated in a pre-chilled pestle and mortar and extracted with 10 ml phosphate buffer (pH 7.0) to determine CAT activity. Triturated samples were centrifuged at 10,000 g for 30 min at 4°C and supernatants were assessed for enzyme activity. An assay mixture of 3 ml of phosphate buffer, 2 ml of H_2O_2, and 1 ml of enzyme source were pipette into a porcelain crucible and incubated for 1 min at 20°C to estimate CAT activity. The reaction was stopped with addition of 10 ml of 0.7N H_2SO_4 and the reaction mixture titrated against 0.01 N $KMnO_4$ to determine residual H_2O_2 until a faint pink color that persisted for at least 15 s was seen. One unit of CAT activity was defined as amount of enzyme that destroyed 1 μmol of H_2O_2•min^{-1}•g^{-1} of fresh tissue. Changes in activity were measured using the method of Kar and Mishra (1976).

Analysis of total antioxidant activity

A 0.5 gram freshly chopped onion tissue was extracted by macerating with 10 ml distilled water for estimation of total antioxidant activity. Tubes containing extract and reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate) were incubated at 95°C for 90 min. After incubation the mixture cooled to room temperature, absorbance of each solution was measured at 695 nm against a blank (Prieto et al., 1999). Antioxidant capacity was expressed as gallic acid equivalent.

Analysis of 2, 2-diphenyl-β-picrylhydrazyl radical scavenging activity (DPPHRAC)

The scavenging effect of onion for stable 2,2-diphenyl-$β$-picrylhydrazyl (DPPH) radical was monitored according to the method of Braca et al. (2001). One gram of fresh, finely chopped onion tissue was extracted with 10 ml distilled water. A 0.2 ml aqueous extract was added to 6 ml of a 0.004% methanolic solution of DPPH. Absorbance at 517 nm was recorded after 30 min and percentage inhibitory activity was calculated as $[(A_0 − A_e)/A_0] \times 100$ where A_0 = absorbance without extract and A_e = absorbance with extract.

Analysis of lipid peroxidation (LP)

Lipid peroxidation was measured as the amount of malondialdehyde (MDA) produced by the thiobarbituric acid (TBA) reaction as described by Dhindsa et al. (1981). A one gram sample of chopped fresh onion tissue was homogenized in 2 ml of 20% trichloroacetic acid (TCA) followed by centrifugation at 5000 g. A 20% TCA solution containing 0.5% TBA and 4% butylated hydroxytoluene was added to a 1 ml aliquot before heating at 95°C for 30 min and then cooled in an ice bath. The contents were centrifuged at 10,000 g and absorbance read at 532 nm. The concentration of MDA was calculated using an extinction coefficient of 155•mM^{-1}•cm^{-1}.

Analysis of pyruvic acid development (PAD)

Pungency of onion bulbs was determined as pyruvic acid (Anthon and Barrett, 2003; Schwimmer and Weston, 1961). Pyruvic acid concentration was determined using the method of Schwimmer and Weston (1961). One gram sample of each treatment was crushed in a pestle and mortar and incubated with 2, 4-dinitrophenylhydrazine and the absorbance read at 420 nm on a spectrophotometer for total pyruvic acid concentration, determined against a sodium pyruvate standard curve.

Statistical analysis

Data were subjected to ANOVA of a factorial randomized block design and by Duncan's multiple range tests, to determine differences among means. Principal component analysis (PCA), as the method of identifying the factor dimension of the data, was used to summarize the treatment informing in a reduced number of factors for selection of the best performing treatment. Statistical analyses were done using SPSS Professional Statistics ver. 7.5 (SPSS Inc., Irvine, California).

RESULTS AND DISCUSSION

Non-enzymatic antioxidants

Ascorbic acid and phenol

Ascorbic acid is one of the most important hydrophilic antioxidants that scavenge harmful free radicals and other ROS and also regenerate other antioxidants like tocopherol to its functional state. In the present

Table 2. Effect of Mn and Fe on antioxidants content and antioxidant activity together with pyruvic acid development in onion.

Treatment	AAC (mg/100 g)	TPC (mg/100 g GAE)	FPC (mg/100 g GAE)	SOD (IC_{50}:mg/ml)	POD (mM *o*-dianisidine oxidized/g/min)	CAT (units)	MRAP (mg/g GAE)	DPPHRAC (IC_{50}: mg/ml)	LP (nmol/ 100 g)	PAD (µmol/g)
Mn										
1	11.11	115.28	73.30a[a]	5.02	0.26b	0.98b	64.22a	15.19a	64.11a	8.13b
2	11.32	121.02	50.31c	5.01	0.24c	0.94b	42.46b	13.13b	66.33a	8.44b
3	12.63	115.87	54.12b	5.49	0.41a	1.04a	41.98b	13.10b	60.78b	10.75a
Fe										
1	11.76	107.55b	52.25c	5.93a	0.26c	0.97	53.60a	16.93a	63.67	8.35b
2	11.76	121.52a	56.87b	4.94b	0.33a	1.00	38.53b	12.92b	63.89	9.25a
3	11.54	123.10a	68.61a	4.65b	0.31b	0.99	56.53a	11.57c	63.67	9.72a
Mn_1										
Fe_1	10.45	117.81	62.26	5.23	0.11	0.91	56.49	14.67	58.33	7.39
Fe_2	11.11	112.97	73.06	5.17	0.35	1.03	65.27	16.93	68.67	9.29
Fe_3	11.76	115.06	84.59	4.64	0.33	0.99	70.88	13.98	65.33	7.70
Mn_2										
Fe_1	11.11	99.22	35.82	5.95	0.27	0.96	37.67	18.52	66.67	8.10
Fe_2	12.41	135.05	59.41	4.39	0.28	0.95	26.90	8.52	63.67	9.41
Fe_3	10.45	128.81	55.71	4.70	0.17	0.92	62.82	12.35	68.67	7.81
Mn_3										
Fe_1	13.72	105.64	58.67	6.61	0.41	1.05	66.63	17.60	66.00	9.56
Fe_2	11.76	116.53	38.14	5.24	0.37	1.04	23.41	13.32	59.33	9.04
Fe_3	12.41	125.42	65.55	4.62	0.44	1.05	35.89	8.38	57.00	13.66
Analysis of variance (F values)										
Mn	NS	NS	102.661**	NS	652.155**	18.220**	17.907**	27.500**	11.792**	41.944**
Fe	NS	10.240**	48.117**	14.065**	109.367**	NS	10.357**	147.843**	NS	9.905**
Mn x Fe	NS	5.854**	31.031**	NS	238.373**	3.659*	11.233**	82.056**	14.054**	22.838**

*Significant at 5%, **significant at 1%, NS = non significant; [a]Values followed by the same letter in a column are not significant at $P < 0.05$, Duncan's multiple range test (DMRT).

experiment, application of Mn and Fe through different treatments could produce no significant differences in the content of ascorbic acid (Table 2). Therefore, no significant effects could be observed by application of Mn and Fe on the content of ascorbic acid. Phenols are ubiquitous

phytochemicals that contribute largely to antioxidant potential of any plant. In the present experiment, total (TPC) and free (FPC) phenols were estimated to assess the contents of conjugated and unconjugated (free) phenols in the genotype Sukhsagar of onion. Manganese application alone failed to produce any significant effect in TPC.

On the contrary, the Mn treatment alone produced significant differences among the values of FPC, which might be due to activation of enzymes of the Shikimic acid pathway and subsequent pathways and activates the biosynthesis of flavonoids that are an important class of phenolics (Marschner, 1995). However, application of Fe produced values of both TPC and FPC with significant differences while the FPC in Mn treatment decreased with increasing doses and also with respect to control (Mn_1), both TPC and FPC increased significantly following Fe application. The highest values of TPC and FPC were exhibited by the treatments Mn_2Fe_2 and Mn_1Fe_3 (Table 2) respectively while the treatment Mn_2Fe_1 exhibited the lowest values of both TPC and FPC.

Enzymatic antioxidants

SOD, POD and CAT

SOD are present in all aerobic organisms and convert $O_2^{\bullet-}$ to H_2O_2, which is ultimately neutralized to water, a safe product, by CAT. Despite SODs have several cofactors like Cu and Zn, Fe and Mn; Mn-SOD is not widely distributed in various families of higher plants (Sandmann and Boger, 1983). In the present experiment, Mn alone showed no significant effect on SOD whereas Fe application resulted in a significantly decreasing IC_{50} values indicative of increasing SOD activity with increasing dose of Fe, which seems to be quite likely because SOD has a Fe cofactor.

Regarding POD activity, significant effects were observed in application of both the micronutrients. The highest dose of Mn (Mn_3) resulted in a pretty higher value of POD activity as compared to control (Mn_1). However, application of Fe increased the activity at single dose (Fe_2), and decreased with response to the highest dose (Fe_3).

The fact that Mn activates POD leading to biosyn-thesis of lignin is already established (Marschner, 1995). The initial increase of POD activity with application of Fe is also expected because POD has a heme prosthetic group (Marschner, 1995). The maximum POD activity was observed in the case of the treatment Mn_3Fe_3.

CAT is another antioxidative enzyme, which has heme prosthetic group. CAT detoxifies the H_2O_2 produced from the action of SOD on $O_2^{\bullet-}$ and also from different other sources. In the present experiment, Mn alone only produced a significant increase of CAT activity at the highest dose. The lowest dose, however, could produce no signifi-

cant differences with the value obtained in the control. Presence of no significant differences between the CAT activity value as effectuated by Fe application, was contrary to the observation expected. The interaction effect also shows that the highest set of values was presented by the treatments Mn_3Fe_1, Mn_3Fe_2 and Mn_3Fe_3.

Antioxidant activities

MRAP, DPPHRAC and LP

Antioxidant activity was analyzed under different systems of assay. In our experiment, MRAP, DPPHRAC and LP are the three different systems. The MRAP and DPPHRAC yielded significant differences between the treatments, whereas in the case of LP there were significant differences only in Mn treatments alone. Mn doses alone resulted in significantly decreasing values of MRAP with respect to Mn_1, whereas the Fe_1 and Fe_3 had produced no significant differences in MRAP values, but the single dose of Fe (Fe_2) resulted in a value lesser than the control (Fe_1). The highest value of MRAP assay was offered by the combination of Mn_3Fe_1 and the lowest by Mn_3Fe_2.

The DPPHRAC assay produced IC_{50} values with no significant difference between the Mn treatments alone but there was significant difference with the control or Mn_1. In the case of Fe treatments alone the values were gradually decreased significantly indicating increase in radical scavenging activity with increasing doses of Fe. The most efficient treatment combination with respect to DPPHRAC was Mn_3Fe_3 followed by Mn_2Fe_2.

Regarding LP, the treatment Mn_3 only resulted in significantly higher value of MDA concentration with respect to Mn_1. The Fe treatments could produce no significant effects on LP. With regards to LP the most efficient combination was Mn_3Fe_3 and the combination with the highest MDA concentration was Mn_2Fe_3.

Pungency

PAD

The pungency of onion is one of its culinary properties. It is measured by the development of pyruvic acid produced upon rupturing the cells. The single dose of Mn (Mn_2) and the absence of Mn (Mn_1) resulted in no significant differences whereas Mn_3 exhibited a significantly high value of PAD. The Fe treatments alone (Fe_2 and Fe_3) produced no significant differences between them but the values these treatments produced were significantly higher than that of Fe_1. The highest value of PAD was exhibited by Mn_3Fe_3 followed by Mn_3 alone.

Table 3. Results of principal component analysis (PCA) for effects of Mn and Fe on antioxidants content and antioxidant activity together with pyruvic acid development in onion.

Principal component	Eigen value	Variance (%)	Cumulative variance (%)
Eigen values and variance accounted for (%) by PCA based on correlation matrix			
1	3.50	35.01	35.01
2	3.19	31.91	66.92
3	1.79	17.89	84.81
Variables	**PC1**	**PC2**	**PC3**
Factor loadings due to PCs with Eigen values greater than 1			
Ascorbic acid	0.60	0.59	0.13
Total phenol (GAE)	0.47	-0.80	0.17
Free phenol (GAE)	0.09	-0.15	0.94
SOD	-0.36	0.83	-0.28
POD	0.69	0.66	0.17
CAT	0.60	0.72	0.17
MRAP (GAE)	-0.54	0.18	0.77
DPPHRAC	-0.73	0.66	-0.05
LP	-0.59	0.22	0.38
PAD	0.88	0.18	0.04
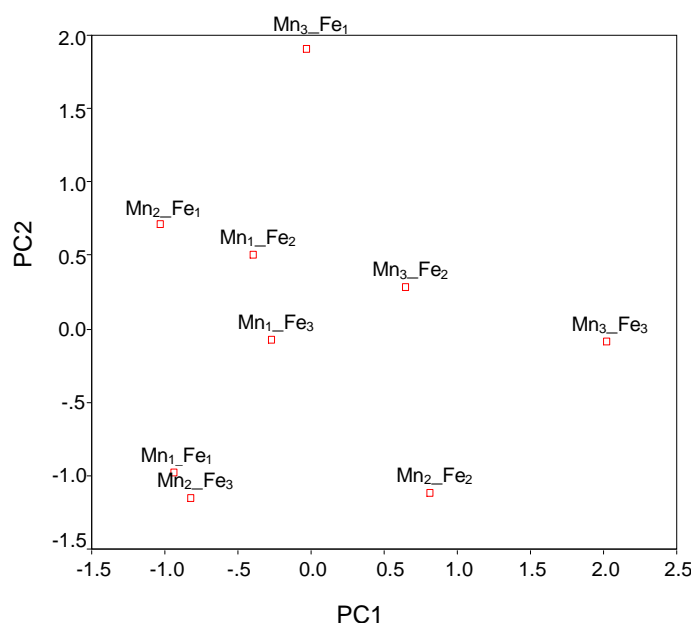
Figure 1. Scatter diagram of the regression factor scores for the first and second components produced by PCA.

## Principal component analysis

Principal component analysis was used to obtain a simplified view of the relationship between the variables and the PCA component loadings for the first three factors are presented in Table 3. These factors were

chosen because the Eigen values were greater than 1.0, which explained 84.81% of total variance.

The first component (PC1) alone explained 35% of total variance in which AAC, TPC, FPC, POD, CAT and PAD restrict SOD, MRAP, DPPHRAC and LP. The negative values of SOD, DPPHRAC and LP are desirable. So, on the basis of PC1 the treatment Mn_3Fe_3, followed by Mn_2Fe_2, Mn_3Fe_2, and Mn_3Fe_1 can be selected as having all the desirable characters apart from MRAP.

The second component (PC2) explained an additional 32% of total variance in which all the variables except phenols assumed positive values. Therefore, AAC, POD, CAT, MRAP, SOD, DPPHRAC and LP and PAD are increased along the Y axis in the scatterplot (Figure 1) while TPC, FPC are decreased. Here also, the increase in IC_{50} values of SOD and DPPHRAC and MDA concentration of LP are not desirable. Therefore, on the basis of PC2 the treatments Mn_1Fe_2, Mn_3Fe_2, Mn_3Fe_3, Mn_1Fe_3 and Mn_2Fe_2 can be selected as having the better combinations of all the antioxidants. Considering PC1 and PC2, the treatment Mn_3Fe_3 followed by Mn_3Fe_2 and Mn_2Fe_2 can be selected as performers.

The third component (PC3) explained another 18% of total variance, where all the variables except SOD and DPPHRAC are positively loaded indicating the higher values of all other variables are associated with lower values of SOD and DPPHRAC. This situation is desirable except for LP in which lower values are expected to increase the shelf-life of the crop. Considering PC1 and PC3 (Figure 2), the treatment combination Mn_3Fe_3 followed by Mn_3Fe_1 and Mn_2Fe_2 can be selected to
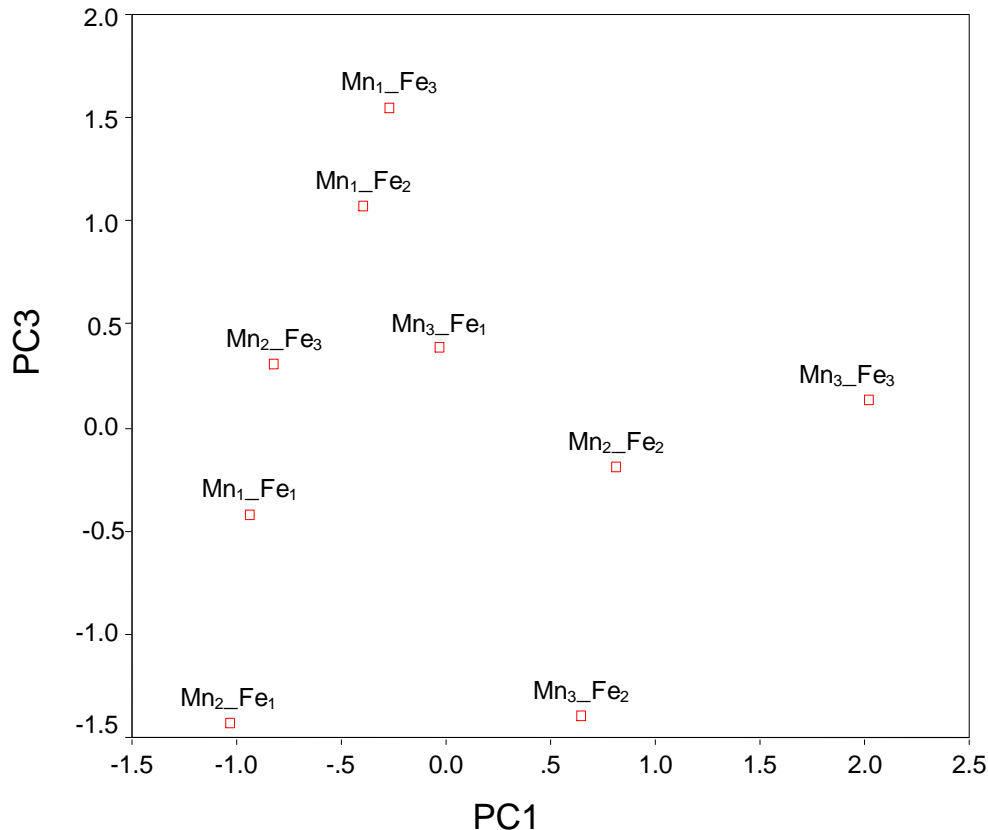
Figure 2. Scatter diagram of the regression factor scores for the first and third components produced by PCA.

ensure all the desirable qualities with respect to anti-oxidant parameters are present in the genotype Sukhsagar following application of manganese and iron.

Conclusions

From the average values of all the parameters and the results of three principal component analyses, it is suggested that the most efficient treatment was the combination of the double doses of both Mn (1.0%) and Fe (1.0%) to ensure all the desirable antioxidant qualities together with pungency development of onion.

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