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Agronomic biofortification to supplement rice genotypes with iron contents

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Abstract
Iron augmentation of rice varieties had been carried out by agronomical biofortification for enhancing the concentration of iron in the present study. Biochemical changes in shoots tissues of rice varieties were investigated in response to augmented iron concentrations at vegetative and reproductive stages. Large values of Ascorbic Acid varied from 701.1 to 1358.9 among treatment combinations at the vegetative stage of genotypes. Values expressed by hydrogen peroxide varied from 413.7 to 764.4, a total deviation from 9.4 to 30.1 for malondialdehyde values. Short range that varied from 5.3 to 12.1 was seen for the values of total glutathione while catalase expressed variation from 19.1 to 55.8 among treatment combinations. Estimation of shoots tissues at reproductive stage seen short range from 4.4 to 9.6 was seen for of the total glutathione values of while catalase expressed variation from 15.5 to 40.8 among treatment combinations. Superoxide dismutase ranged from 38.5 to 76.4 while as corbate peroxidase values deviated from 14.5 to 59.5 as observed in present study. Very large deviation had expressed by peroxidase values i.e. 85.1 to 370.2. Least deviation had observed among values of glutathione reductase among the treatment combinations *viz* 12.2 to 30.5.Biplot analysis observed the total variation of 97.2% had been explained by first two principal components. Major contributors were catalase peroxidise of vegetative stage and ascorbic acid, catalase, ascorbate peroxidise, peroxidase of reproductive stage of genotypes. Agro Biofortification with a proper balance of iron augmentation induce desirable effect on the physiological process of the plants.

Keywords: Synthetic chelated micronutrient, ROS related metabolites, antioxidative metabolites

Introduction
Green revolution significantly enhanced the crop production primarily rice, wheat and maize production was boosted to meet the energy needs of growing population. Nearly 2 billion people consequence of the predominance of cereal-based, Africa and South Asia, have been affected more severely due to Micronutrient deficiency (Masuda *et al*. 2020) [10]. The problem of malnutrition reported in major portions of the globe predominantly in developing countries where common people consume mostly cereal based staple foods that are really not a good source of micronutrient (Jalal *et al*. 2020) [7]. Recent food protection policies have emphasized more on Micronutrient Safety, i.e. development and availability of nutrition's rich healthy food (Jan *et al*. 2020) [2].

The biofortification technique has been developed as one of the world's leading methods for tackling micronutrient deficiency (Bouis & Saltzman 2017) [3]. Moreover, biofortified crops help to improve the daily availability of micronutrient intakes on economical basis (deValença *et al*. 2017) [4]. Agronomic bio fortification is the application of a micronutrient containing mineral fertilizer to soil and/or plant leaves (foliar) to improve the micronutrient quality of the edible portion of food crops (Bharadva *et al*. 2019) [1]. Though temporarily increased the nutritional value of the crop, thereby fulfilling the human nutritional requirement (Garg *et al*. 2018) [5]. This method is beneficial for augmenting the micronutrients that can be directly absorbed by the plant. Among various approaches chosen to increase the Fe concentration in rice grains, fertilization is considered as a rapid and efficient method (Giordano *et al*. 2019) [6]. Generally, the solubility of iron compounds is relatively low but reduced conditions and/or lowering of pH favours conversion of insoluble ferric oxides (Fe3+) to plant available ferrous oxides (Fe^{2+}). The effectiveness of agronomic biofortification can be enhanced by application of synthetic chelated micronutrient fertilizers and/or organic fertilizers fortified with micronutrients in combination with NPK ensuring proper nourishment of crops with adequate nutrient supply by slow release of nutrients in soil solution (Kumar *et al*. 2019; Ramzan *et al*. 2020) [9, 12].

Materials and Methods

Six rice varieties were evaluated under field trials during kharif in the net houses of the Department of Chemistry and Biochemistry, CCS HAU, Hisar during cropping seasons 2015-2016 and 2016-2017. Seeds of all rice varieties were sown directly in pots at 2-3 cm depth in light textured (loamy) soil with recommended agronomical practices (Sikirou *et al*. 2016) [14] and the pots were divided in three sets after 20 days of sowing for Iron augmentation as: One set was given Yoshida nutrient medium without Fe (0 mM EDTA-Fe(II)). Second set was given Yoshida nutrient medium with 0.1mM EDTA-Fe(II)) concentration. Third set was given Yoshida nutrient medium with high Fe concentration (0.5 mM EDTA-Fe (II)). ROS related metabolites; malondialdehyde (MDA), superoxide radical (SOR), hydrogen peroxide (H_2O_2), antioxidative metabolites *viz.* ascorbic acid (AA), glutathione (GSH & GSSG), enzymes; superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and isozymes of SOD, CAT, APX & GR were estimated in the shoot tissues of the varieties. Iron content in shoots of rice varieties was analyzed by method of Lindsey and Norwell (1978). Malondialdehyde content (MDA) was estimated according to the method of Heath and Packer (1968). Superoxide ($O_2^{\cdot-}$) radical was measured by monitoring the nitrite formation from hydroxylamine following the method of Elstner and Heupel (1976). H_2O_2 was estimated by the method of Sinha (1972). Ascorbic acid content was estimated by the method of Mukherjee & Chaudhari, (1983), which was based on the reduction of 2, 4 – dintrophynel hydrazine. Glutathione was estimated by the method of Griffith (1980). Superoxide dismutase was assayed by measuring its ability to inhibit the photochemical reduction of nitroblue tetrazolium by the method of Giannopolities and Ries (1977). Catalase activity was determined by the procedure of Sinha (1972). The Peroxidase enzyme activity was estimated by the method of Shannon *et al*., (1966). The data obtained in the present investigation was subjected to analysis of variance (ANOVA) technique and critical differences in values at 5% level of significance was used for making comparisons among the rice genotypes augmented with additional iron levels and changes were studied in metabolites estimation from vegetative to reproductive stages of the genotypes.

Results and Discussion

Analysis of yield and contributing traits

ANOVA analysis had observed highly significant variations among the estimated values in shoots at vegetative and reproductive stages of genotypes (Shi *et al*. 2016) [13].

Vegetative stage of genotypes

Range of (108.4 to 282.4) observed for Superoxide radicals (SOR) values at vegetative stage of genotypes. Large values of Ascorbic acid (AA) varied from 701.1 to 1358.9 among treatment combinations for this study (Yang *et al*. 2016) [16]. Values expressed by HPO varied from 413.7 to 764.4, a total deviation from 9.4 to 30.1 for Malondialdehyde (MDA) values. Short range that varied from 5.3 to 12.1 was seen for the values of Total Glutathione (TOG) while Catalase (CAT) expressed variation from 19.1 to 55.8 among treatment combinations. Estimated values of Superoxide dismutase (SOD) ranged from 39.3 to 96.6 while deviation from 18.3 to 84.1 observed for Ascorbate peroxidase (APX) values. Very large deviation had expressed by Peroxidase (POX) values i.e. 137.6 to 476.3. Least deviation 12.6 to 34.3 had observed among values of Glutathione reductase (GR) among the treatment combinations (Kabir *et al*. 2016) [8].

Reproductive stage of genotypes

SOR values Ranged from (89.8 to 243.9) among genotypes augmented with three doses of iron augmentation at reproductive stage. Values of AA varied from 411.9 to 978.2 among treatment combinations for this study. Larger values expressed by HPO as varied from 375.3 to 664.1, and least total deviation from 7.7 to 22.1 for MDA values. Similarly, short range that varied from 4.4 to 9.6 was seen for the values of TOG while CAT expressed variation from 15.5 to 40.8 among treatment combinations (Yadav *et al*. 2015) [15]. Estimated values of SOD ranged from 38.5 to 76.4 while APX values deviated from 14.5 to 59.5 as observed in present study. Very large deviation had expressed by POX values i.e. 85.1 to 370.2. Least deviation had observed among values of GR among the treatment combinations *viz* 12.2 to 30.5.

Relative change in estimated values

Combination C2G1 had expressed maximum change in values for Superoxide radicals (SOR) followed by C1G6 &C1G2 in shoots while C3G4 achieved minimum value of 3.9. Estimation of Ascorbic acid (AA) in shoots observed minimum change in value for C2G6 while maximum change had expressed by C1G1, C2G1 & C3G1. Treatment combination C1G3 had minimum value 4.9 whereas larger estimation exhibited by C3G1, C3G6, C3G2 for Hydrogen peroxide (Zhang *et al*. 2018) [17]. MDA observed maximum change for C2G2 followed by C1G2& C2G1 whereas minimum value by C3G4(13.1) as per for estimation. Wide variation observed among values for TOG () as ranged from 59.4(C3G3) to 6.8(C2G5) as compared to catalase (CAT) values from 47.1(C3G6) to 23.1(C1G1) values estimated in shoots. Larger values of Superoxide dismutase (SOD) expressed by the C2G6 (32.6) followed by C3G4 &C3G6 as compared to least value mentioned by 2.1(C1G1) for shoots estimation. Ascorbate peroxidise (APX) values were more in C3G2, C3G5, C3G6 as compared to least value by C2G3(4.3). Peroxidase (POX) values expressed as lower value in estimation i.e. 19.4(C3G3) while maximum observed for C2G6 followed by C3G6 & C1G6. Lower values had observed for Glutathione reductase (GR) for C3G1 as compared to corresponding larger C3G4, C1G4, C2G4 (Bouis *et al*. 2011).

Biplot analysis of metabolites versus treatments

Total variation of 97.2% among the treatment combinations had been explained by first two principal components as evident from (Table 4). The first principal component (PC) accounted for 70.6% of the total variation among estimated values and larger contribution expressed by CAT, POX at vegetative stage andAA, CAT, APX,POX of reproductive stages etc. Principal component two contributed 26.6% to the total variation. Six contributors were SOR, MDA, HPO of vegetative stages and HPO, MDA, SOR of reproductive stages. Out of the 20 traits evaluated, 06 contributed most to the first two principal components (Table 4) and these considered most desirable to summarize variation among the accessions through hierarchical cluster analysis. Metabolites had formed two clusters among themselves and estimated

values at vegetative and reproductive stages did not show clear cut distinguishes (Fig. 3). The biplot analysis is an appropriate method to analyse the association among estimated metabolites in turn assist to narrowing down the number of metabolites to the ones contributing a major portion to the variability Prity *et al.* (2021) [11]. The vector length of the metabolites from the biplot origin is a measure of the distinctiveness of the metabolite from other metabolites as reviewed by Kumar *et al.,* 2020. In the biplot vectors of metabolites showing acute angles are positively correlated whereas those showing obtuse or straight angles are negatively correlated and those with right angles have no correlation. HPO, MDS and SOR expressed strong association ship as observed together in one cluster. This showed the estimation of these metabolites at vegetative stage would be appropriate and estimation at reproductive stages may be avoided to reduce the work load (Fig. 4). Similar type of behaviour exhibited by AA, APX, CAT, POX, TOG, and GR metabolites. Group of these metabolites had maintained distance from earlier group as observed in different cluster. More over these would be not correlated with HPO, MDS and SOR metabolites as nearly right angles observed among the rays connecting to the estimated metabolites values in the biplot analysis.

Table 1: Details of treatments consisted of genotypes and augmented level of Iron

Genotype/Iron augmentation	0mM EDTA-Fe(II)	0.1mM EDTA-Fe(II)	0.5mM EDTA-Fe(II)
Govind	C1G1	C2G1	C3G1
Super	C1G2	C2G2	C3G2
HKR120	C1G3	C2G3	C3G3
PUSA1121	C1G4	C2G4	C3G4
HBC19	C1G5	C2G5	C3G5
Palman	C1G6	C2G6	C3G6

Table 2: Differential pattern in metabolites estimation in shoots for treatments at vegetative stages

Treatments	Superoxide radicals	Ascorbic acid	Hydrogen Peroxide	Malondi aldehyde	Total Glutathione	Catalase	Superoxide dismutase	Ascorbate peroxidase	Peroxidase	Glutathione reductase
C1G1	173.22	701.01	487.40	16.61	5.35	19.10	39.39	18.38	151.00	13.61
C1G2	168.33	656.57	482.46	17.57	5.57	22.26	42.10	20.24	137.67	12.68
C1G3	145.71	735.18	454.18	13.16	6.60	27.52	48.49	26.42	190.83	15.85
C1G4	108.41	909.49	413.72	9.47	7.04	36.69	56.61	42.49	285.83	19.37
C1G5	114.10	888.89	399.01	10.21	6.77	34.82	60.73	39.91	266.59	20.44
C1G6	133.33	818.35	441.83	12.32	6.10	29.69	53.28	37.33	229.33	17.20
C2G1	252.33	771.82	646.27	26.08	5.84	21.64	44.86	23.12	188.83	15.23
C2G2	239.19	727.27	631.41	26.46	6.10	24.82	48.46	26.05	165.50	14.35
C2G3	190.38	925.05	567.52	19.12	7.83	32.12	61.23	36.06	257.83	19.10
C2G4	125.05	1194.34	480.10	12.48	9.52	45.77	77.66	60.16	425.83	25.99
C2G5	133.57	1177.98	467.22	13.10	8.61	44.09	83.20	58.40	389.50	26.63
C2G6	163.11	1052.32	528.18	17.21	7.70	36.03	71.36	52.61	326.83	21.85
C3G1	282.49	840.10	764.41	31.51	6.76	23.78	49.88	28.83	217.50	18.36
C3G2	270.35	795.45	711.36	30.18	7.22	27.04	54.86	32.61	194.67	17.63
C3G3	211.14	1032.63	622.17	24.40	9.62	37.08	68.92	45.88	308.33	24.11
C3G4	141.67	1358.99	517.87	14.09	12.14	55.86	92.12	84.11	475.33	33.41
C3G5	147.44	1338.54	493.15	15.34	10.97	54.38	96.62	76.14	476.33	34.38
C3G6	176.44	1207.68	583.13	20.45	9.66	42.47	83.03	68.23	393.67	27.77
CD at 5%	5.16	12.97	9.78	1.19	0.84	4.54	4.61	3.25	8.91	3.65

Table 3: Differential pattern in metabolites estimation in shoots for treatments at reproductive stages

Treatments	Superoxide radicals	Ascorbic acid	Hydrogen Peroxide	Malondi aldehyde	Total Glutathione	Catalase	Superoxide dismutase	Ascorbate peroxidase	Peroxidase	Glutathione reductase
C1G1	145.40	420.20	459.65	12.75	4.84	15.52	38.57	16.21	103.92	13.32
C1G2	136.19	411.96	453.24	11.98	4.68	16.53	39.72	14.51	85.07	12.23
C1G3	126.67	472.73	432.72	10.52	4.43	19.78	46.29	24.93	155.07	14.71
C1G4	89.83	634.34	392.49	8.37	5.86	26.68	48.25	31.90	208.95	16.29
C1G5	96.22	608.08	375.38	7.75	5.62	24.78	50.19	29.16	178.18	18.38
C1G6	107.94	565.66	415.90	9.10	5.51	20.02	44.13	27.97	138.60	17.95
C2G1	203.94	468.99	598.54	18.12	5.29	17.28	43.19	20.33	125.93	16.59
C2G2	196.11	452.53	579.46	17.52	4.99	18.52	45.25	19.09	107.30	14.80
C2G3	169.94	609.74	526.47	14.21	5.16	23.54	55.96	34.55	200.63	18.77
C2G4	112.29	896.16	444.12	10.10	8.15	35.06	60.24	47.99	285.30	22.96
C2G5	121.22	874.58	442.76	9.12	8.07	31.65	64.05	45.79	252.80	25.73
C2G6	138.57	789.79	482.19	12.02	6.75	25.25	53.80	39.84	192.32	24.19
C3G1	243.86	511.21	664.07	22.11	5.87	18.79	51.43	24.05	152.72	18.17
C3G2	235.57	508.69	635.02	21.50	5.47	19.51	50.69	22.13	131.28	17.27
C3G3	196.97	645.86	567.50	17.84	6.04	27.29	63.39	40.81	258.03	22.74
C3G4	136.29	978.28	476.94	12.46	9.69	40.85	70.28	59.52	370.22	27.84
C3G5	132.41	934.95	465.40	11.21	9.42	37.38	76.44	52.35	324.48	30.59

C3G6	154.03	857.32	518.51	14.37	7.83	28.87	63.70	47.11	236.63	29.21
CD at 5%	6.93	12.65	9.82	1.18	0.49	1.74	2.47	3.82	10.64	1.58

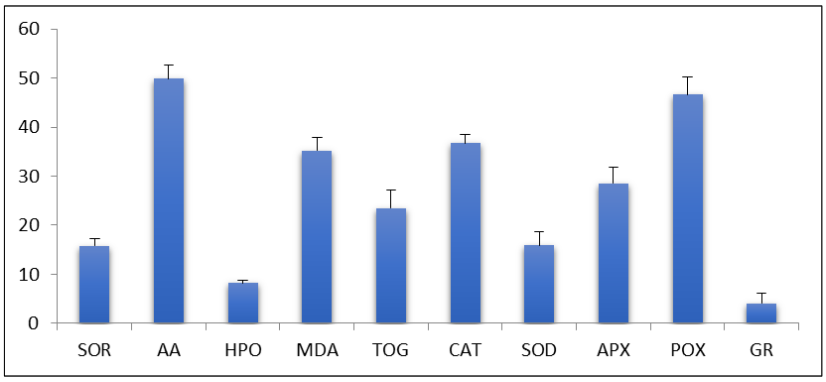


Fig 1: Standard errors in metabolites for change over the stages of genotypes

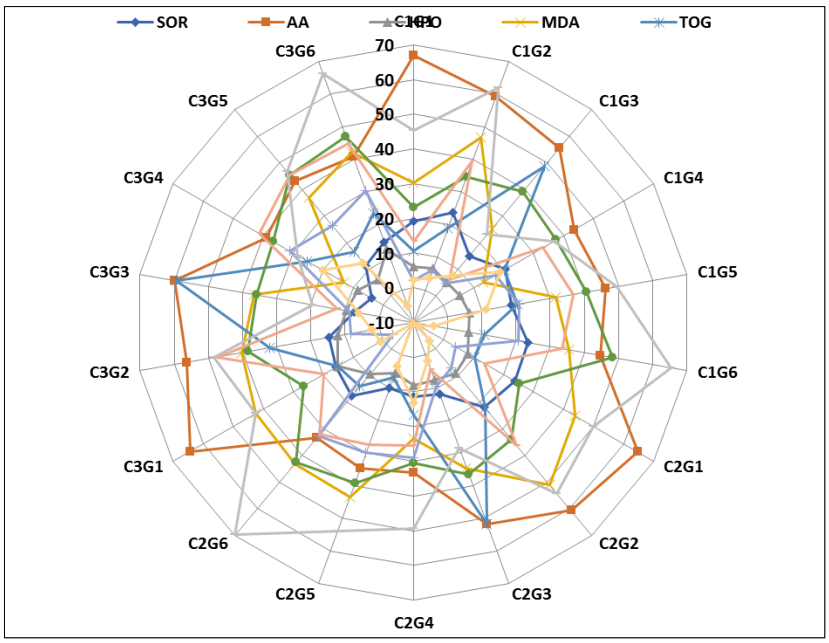
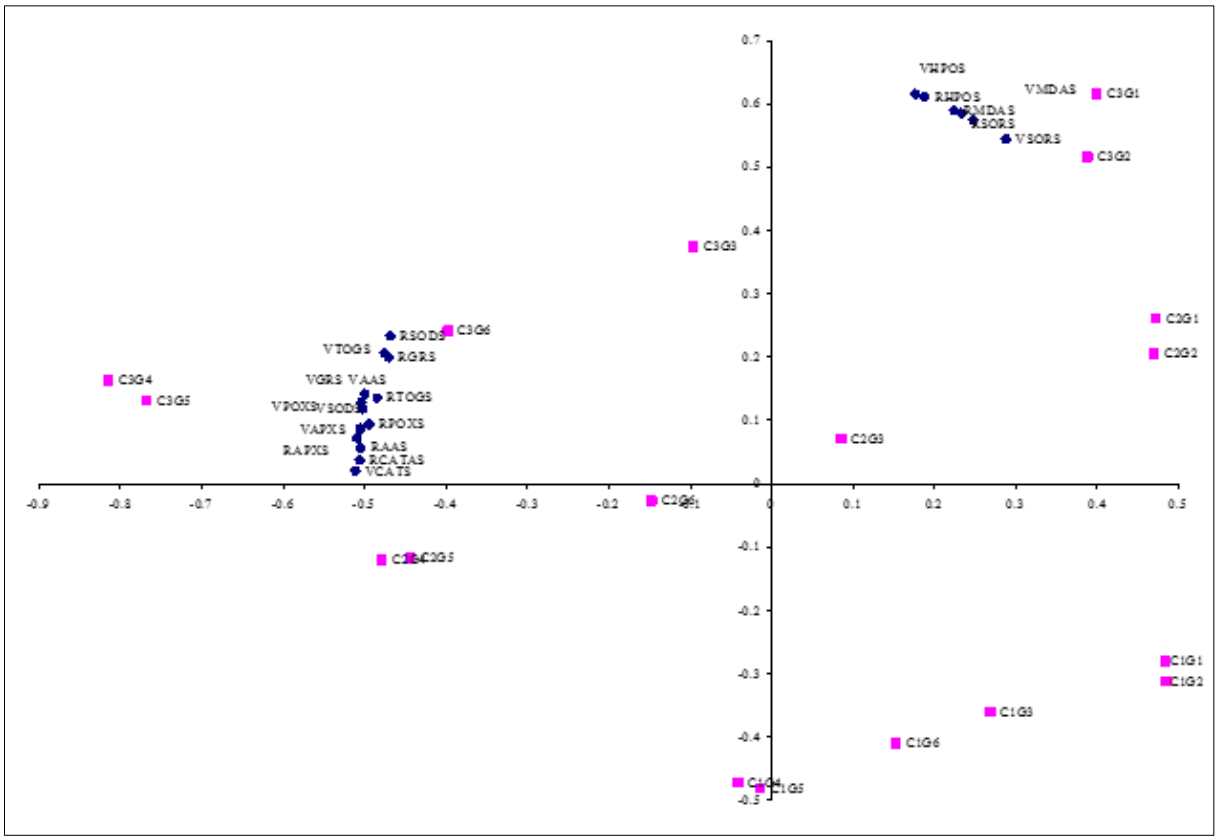


Fig 2: Relative change in values of metabolites with advancement of stages of genotypes

Table 4: Contribution of metabolites in Principal components

Traits	PC1	PC2
VSORS	0.1485	0.3583
VAAS	-0.2597	0.0846
VHPOS	0.0910	0.4050
VMDAS	0.1276	0.3781
VTOGS	-0.2452	0.1358
VCATS	-0.2637	0.0132
VSODS	-0.2589	0.0778
VAPXS	-0.2605	0.0569
VPOXS	-0.2629	0.0475
VGRS	-0.2577	0.0932
RSORS	0.1200	0.3852
RAAS	-0.2605	0.0373
RHPOS	0.0970	0.4023
RMDAS	0.1156	0.3879
RTOGS	-0.2500	0.0892
RCATAS	-0.2610	0.0243
RSODS	-0.2416	0.1537
RAPXS	-0.2605	0.0578
RPOXS	-0.2551	0.0619
RGRS	-0.2427	0.1314
% variance explained	70.68	26.61



PC1=70.6; PC2=26.6; Total = 97.2%

Fig 3: Clustering pattern of metabolites

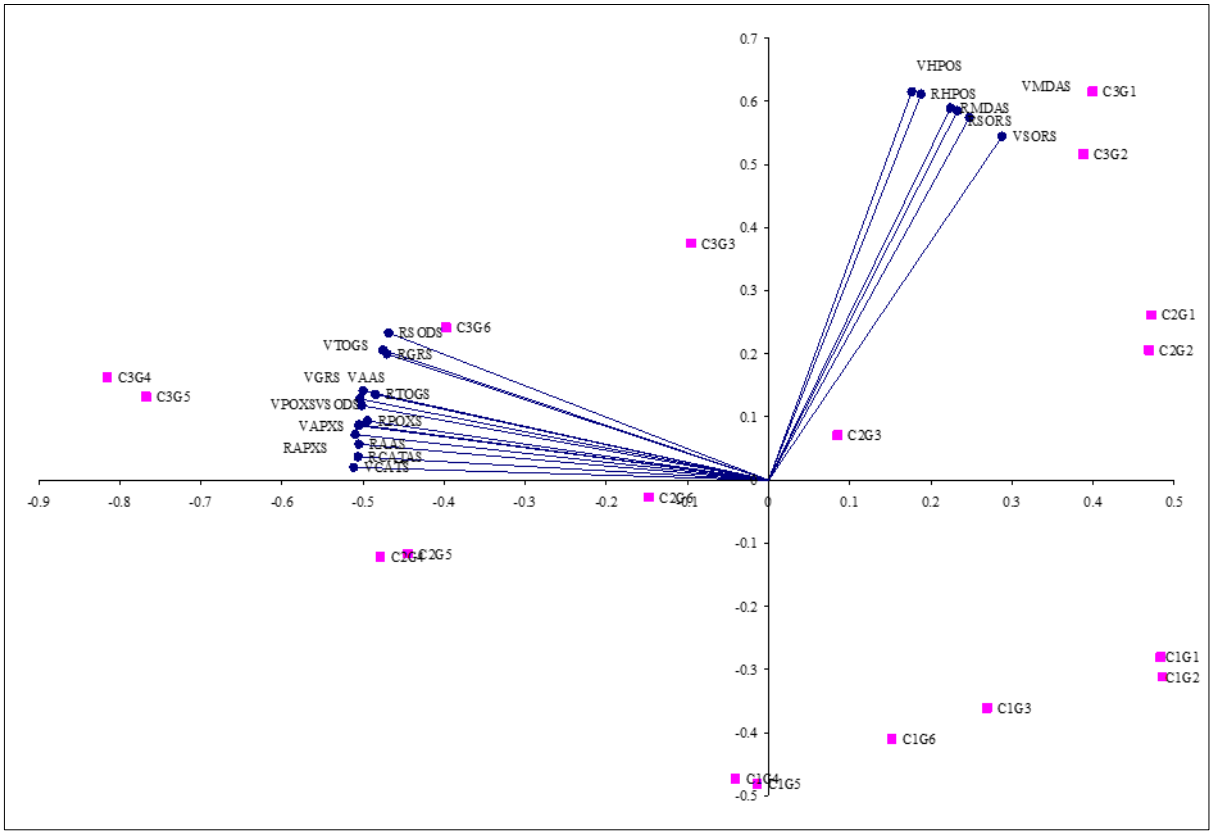


Fig 4: Association analysis among metabolites

Conclusions

The deficiency of micronutrient iron in human nutrition is a grave health concern at worldwide. Agronomic interventions are advocated as an effective approach to enrichment the staple food crops with number of micronutrients. Recently the combination of strategies - seed priming, soil application,

and/or foliar spray of iron have been forwarded to uplift their accumulation in plants.

Competing interests

Authors have declared that no competing interests exist.

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