



Study of Iron Supplementation on Rice Genotypes

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ABSTRACT

Promising rice genotypes were evaluated during June to October in the Department of Chemistry and Biochemistry, CCS HAU, Hisar, Haryana, for 2015–2016 and 2016–2017 cropping seasons. Biochemical changes in root tissues were estimated in response to augmentation of iron concentrations during vegetative to reproductive stages. Estimated values of metabolites observed variation in ascorbic acid from 343.3–680.2 among treatments for this study. Values of hydrogen peroxide expressed deviation from 506.8–230.8, while malondialdehyde deviated from 10.9–30.8. Very large deviation had expressed by peroxidase values i.e. 38.1–160.8. Larger values expressed by hydrogen per oxide as varied from 187.3–457.5, and least total deviation from 9.9–24.2 for malondialdehyde values. The first principal component accounted for 73.8% of the total variation among estimated values and larger contribution expressed by peroxidase, catalase, superoxide dismutase of vegetative stage and superoxide dismutase, catalase, ascorbate peroxidase of reproductive stage etc. Total of 22.6% to the total variation contributed by second principal component with major contributors were malondialdehyde, hydrogen peroxide, superoxide radicals of vegetative stages along with malondialdehyde, hydrogen peroxide, superoxide radicals, of reproductive stages. Agronomic biofortification with a proper balance of iron augmentation induce desirable effect on the physiological process of the plants.

KEYWORDS: Antioxidative metabolites, ROS related metabolites, synthetic chelated micronutrient

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1. INTRODUCTION

Larger proportion of people in developing countries relies on rice as their staple foods for daily consumption (Masuda et al., 2020). Resources poor populations face hardship to afford other micronutrients-rich non staple food for balance diet to ensure good health (Aung et al., 2013; Bouis et al., 2017). Rice the prime source of nutrition of Indian population would be fortified with Iron to overcome the problem of Anemia in children and lactating women (Samtiya et al., 2020). The problem of micronutrient deficiency among human beings has been tackled by biofortification interventions at worldwide (Boonyaves et al., 2017; Masuda et al., 2020). Recently, the biofortified crops have been advocated to cultivate on larger areas to supplement the daily requirement of micronutrients intake on economical basis (Slamet et al., 2015; Jan et al., 2020). 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 (Bouis & Saltzman, 2017; Gargett et al., 2018). The predominance of cereal-based diet mostly in Africa and South Asia, approximately 2 billion people has been affected due to Micronutrient deficiency (Trijatmiko et al., 2016; Jalal et al., 2020). Recent food protection policies have emphasized more on Micronutrient Safety, i.e. development and availability of nutrition's rich healthy food (Bharadva et al., 2019). This method is beneficial for ensuring the availability of micronutrients that can be directly absorbed by the plant, such as iron (de Valença et al., 2017; Kumar et al., 2019). Among various approaches chosen to increase the Fe concentration in rice grains, fertilization is considered as a rapid and efficient method (Ramzan et al., 2020). 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 (Giordano et al., 2019).

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

Genotype/ iron augmentation	0 mM EDTA- Fe(II)	0.1 mM EDTA- Fe(II)	0.5 mM 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

2. MATERIALS AND METHODS

Promising six rice varieties were evaluated under field trials during June to October in the net houses of the Department of Chemistry and Biochemistry, CCS HAU, Hisar, Haryana, India 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., 2006) and the pots were divided in 3 sets after 20 days of sowing for Iron augmentation as : 1st set was given Yoshida nutrient medium without Fe (0 mM EDTA-Fe(II)). 2nd set was given Yoshida nutrient medium with 0.1 mM EDTA-Fe(II) concentration. 3rd set was given Yoshida nutrient medium with high Fe concentration (0.5 mM EDTA-Fe (II)). ROS related metabolites; malondialdehyde (MDA), superoxide radical (O_2^-), hydrogen peroxide (H_2O_2), antioxidative metabolites viz. ascorbic acid, glutathione (GSH and GSSG), enzymes; superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX), glutathione reductase (GR) and isozymes of SOD, CAT, APX and GR were estimated in the root tissues of the varieties. Malondialdehyde content (MDA) was estimated according to the method of Heath and Packer (1968). Superoxide radical (SOR) 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 and Chaudhari (1983), which was based on the reduction of 2, 4-dinitrophenyl 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.

3. RESULTS AND DISCUSSION

3.1. Analysis of yield and contributing traits

ANOVA analysis had observed highly significant variations among the estimated values among treatment comprises of genotypes as well as doses of iron supplementation at vegetative and reproductive stages (Shi et al., 2016).

3.2. Vegetative stage of genotypes

Variations among the mean of ROS related ten metabolites



at the vegetative stage of genotypes were displayed in table 2. Range of 63.8–233.5 observed for superoxide radicals (SOR) values among genotypes augmented with three doses of iron augmentation. Values of ascorbic acid (AA) varied from 343.3–680.2 among treatment combinations for this study (Yang et al., 2016). Larger values expressed by hydrogen peroxide (HPO) as varied from 506.8–230.8, total deviation from 10.9–30.8 for malondialdehyde (MDA) values. Short range that varied from 4.1–9.9 was seen for the values of

total glutathione (TOG) while catalase (CAT) expressed variation from 14.2–33.9 among treatment combinations. Estimated values of superoxide dismutase (SOD) ranged from 28.2–61.5 while deviation from 10.3–42.4 observed for ascorbate peroxidase (APX) values. Very large deviation had expressed by peroxidase (POX) values i.e. 38.1–160.8. Least deviation had observed among values of glutathione reductase (GR) among the treatment combinations (Kabir et al., 2016).

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

Treat-ments	Super-oxide radicals	Ascor-bic acid	Hydrogen peroxide	Malondi-aldehyde	Total Gluta-thione	Cat-alase	Superoxide dismutase	Ascorbate peroxidase	Perox-idase	Glutathione reductase
C1G1	139.29	343.33	331.80	17.17	4.43	14.17	28.25	10.30	44.83	3.83
C1G2	132.54	355.56	350.86	18.05	4.08	15.61	31.66	11.16	38.17	4.01
C1G3	107.30	375.76	305.81	13.47	5.31	19.43	36.04	14.59	62.67	4.62
C1G4	86.30	416.16	296.64	14.98	4.78	17.43	33.11	16.79	67.50	4.27
C1G5	69.97	458.89	230.89	10.98	5.93	22.08	42.22	18.04	93.63	5.35
C1G6	63.81	430.30	253.06	11.75	5.53	21.43	39.23	22.64	83.00	5.01
C2G1	205.73	377.98	425.20	23.15	4.99	16.39	31.73	13.35	54.67	4.26
C2G2	198.21	386.32	465.84	24.19	4.63	17.89	36.53	14.10	43.17	4.64
C2G3	148.79	442.61	376.38	15.56	6.37	23.38	43.65	21.13	85.43	5.83
C2G4	116.05	503.95	356.15	17.95	6.09	21.67	40.60	23.43	94.17	5.43
C2G5	89.89	618.18	259.50	12.05	8.18	29.29	55.30	29.74	133.50	7.42
C2G6	84.16	581.82	280.86	13.01	7.45	27.96	50.02	35.72	123.50	6.75
C3G1	233.49	413.33	496.16	28.35	5.60	17.95	34.50	15.81	62.50	5.36
C3G2	226.78	421.03	506.88	30.85	5.08	20.21	38.29	16.15	55.83	5.65
C3G3	159.33	466.38	413.95	21.70	7.50	26.74	48.77	24.67	94.33	7.31
C3G4	125.08	540.10	388.53	23.74	7.22	23.73	44.18	28.06	104.83	6.90
C3G5	96.03	680.23	284.02	15.60	9.92	33.99	61.50	34.69	160.83	9.12
C3G6	92.84	631.43	304.84	17.09	9.05	32.01	55.29	42.41	135.04	8.80
CD	3.99	10.34	10.68	1.14	0.80	4.31	2.48	3.04	7.13	0.48

(p=0.05)

3.3. Reproductive stage of genotypes

Variations in ROS related 10metabolites among the genotypes were displayed at the reproductive stage in the terms of average values of metabolites in table 3. Range of (52.4–173.9) observed for SOR values among genotypes augmented with three doses of iron augmentation at reproductive stage. Values of AA varied from 142.2–421.2 among treatment combinations for this study. Larger values expressed by HPO as varied from 187.3–457.5, and least total deviation from 9.9–24.2 for MDA values. Similarly, short range that varied from 2.8–7.8 was seen for the values of TOG while CAT expressed variation from 8.3–23.9

among treatment combinations (Yadav et al., 2015). Estimated values of SOD ranged from 10.4–28.8 while APX values deviated from 7.7–27.6 as observed in present study. Very large deviation had expressed by POX values i.e. 16.1–82.9. Least deviation had observed among values of GR among the treatment combinations.

3.4. Relative change in estimated values

Changes in ROS related 10 metabolites among the rice genotypes from vegetative to reproductive stages were displayed in the terms of mean values of metabolites in Figure 1. Maximum change on average basis expressed by SOD, POX, AA whereas least change observed in HPO

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

Treat-ments	Superox-ide radi-cals	Ascor-bic acid	Hydro-gen per-oxide	Malondi-aldehyde	Total glutathi-one	Cata-lase	Superox-ide dismutase	Ascorbate peroxidase	Per-oxi-dase	Glutathione reductase
C1G1	106.98	151.58	307.28	13.79	2.89	9.87	11.09	7.70	16.10	3.08
C1G2	101.11	142.27	288.23	13.16	3.19	8.38	10.45	8.26	21.40	2.72
C1G3	74.29	177.78	262.23	11.94	4.00	11.98	16.23	12.79	37.57	3.55
C1G4	63.02	210.10	253.06	12.39	3.58	12.06	15.72	10.85	33.63	3.42
C1G5	52.49	258.93	187.31	9.91	4.79	16.10	19.56	15.33	46.63	4.01
C1G6	57.78	242.42	209.48	10.47	4.22	14.63	18.77	16.99	47.30	3.85
C2G1	153.62	184.34	397.03	18.37	3.19	11.14	13.19	9.03	20.60	3.65
C2G2	144.06	169.00	384.10	17.88	3.65	9.71	12.73	10.18	28.61	3.16
C2G3	98.02	236.67	320.81	14.72	5.01	14.58	21.06	17.49	50.80	4.51
C2G4	81.70	288.99	295.23	15.67	4.57	15.02	20.45	15.16	47.07	4.30
C2G5	64.78	392.22	206.93	11.27	6.91	21.53	27.13	23.26	66.92	5.55
C2G6	72.73	362.88	235.47	12.18	6.01	18.97	25.21	24.81	70.37	5.22
C3G1	173.95	192.41	457.58	24.25	3.41	12.22	14.09	11.04	23.05	4.10
C3G2	166.03	182.07	436.30	22.48	3.80	10.17	13.43	11.32	31.73	3.78
C3G3	114.43	248.48	346.37	17.81	5.59	16.32	21.77	19.59	58.23	5.42
C3G4	94.19	300.12	324.95	19.41	5.11	16.40	20.60	16.10	53.52	5.03
C3G5	73.32	421.21	225.44	13.07	7.89	23.95	28.84	26.19	82.98	6.87
C3G6	81.57	387.47	255.26	14.09	6.68	21.39	26.74	27.66	79.53	6.33
CD	9.89	11.52	8.51	0.974	0.40	1.12	2.60	1.16	2.85	0.39

($p=0.05$)

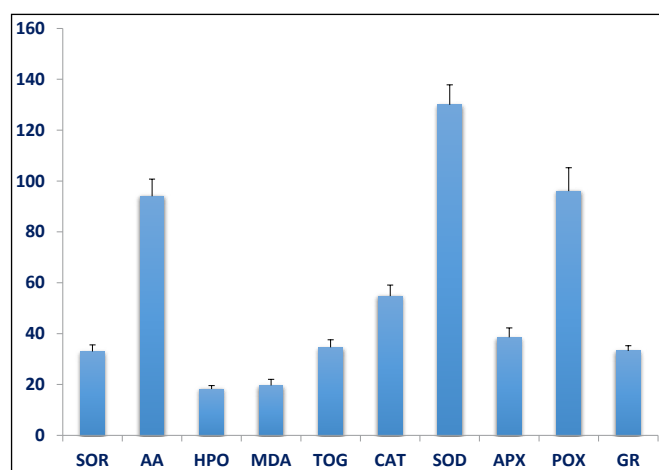


Figure 1: Standard errors in the estimated values

along with MDA estimated values.

Combination C2G3 had expressed maximum change in values for super oxide radicals (SOR) followed by C2G4 and C1G3 in roots while C1G6 achieved minimum value of 10.4 (Figure 2). Estimation in roots observed minimum change in value for C2G5 and maximum value by C1G2, C3G2

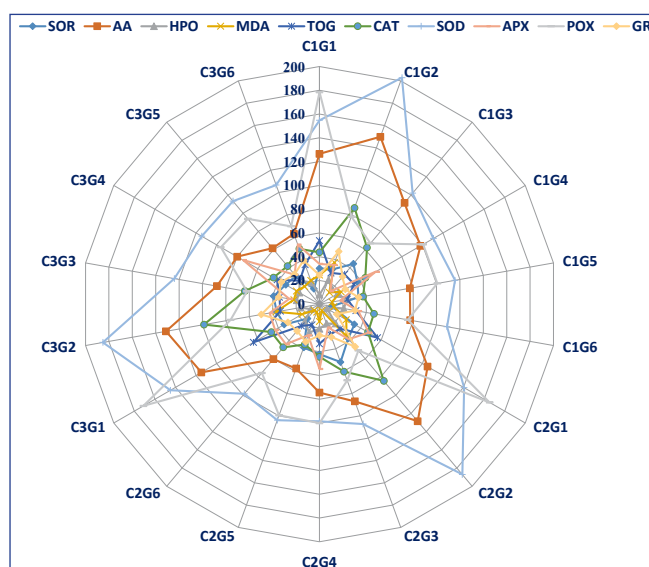


Figure 2: Relative change in values of metabolites with advancement of stages of genotypes

and C2G2 of estimated ascorbic acid (AA). Treatment combination C2G1 had minimum value 7.1 in roots whereas larger estimation exhibited by C3G5, C2G5, C1G5

for hydrogen peroxide (Zhang et al., 2018). MDA observed maximum change for C3G2 followed by C1G2 and C2G2 and minimum value by C2G3 (5.7) as per for estimation.

Larger values of superoxide dismutase (SOD) expressed by the C1G2 (202.9) followed by C2G2 and C3G2 as compared to least value mentioned by 98.4 (C2G6) for roots estimation. Ascorbate peroxidase (APX) values were more in C3G4, C1G4, C2G4 as compared to least value by C1G3(14.1). Lower values had observed for glutathione reductase (GR) in roots estimation for C2G1 as compared to corresponding larger C3G2, C1G2, C2G2. Wide variation observed among values for TOG as ranged from 64.1(C3G1)-18.5(C2G5) as compared to catalase (CAT) values from 98.7(C3G2)-36.1(C2G5) values estimated in roots. peroxidase (POX) values expressed as lower value in roots estimation value i.e. 50.8(C2G2) while maximum observed for C1G1 followed by C3G1 and C2G1 (Bouis et al., 2011).

3.5. Biplot analysis of metabolites versus treatments

First two principal components explained 96.4% of the total variation among the treatments of the study as evident from (Table 4). The first principal component (PC) accounted

for 73.8% of the total variation among estimated values and larger contribution expressed by POX, CAT, SOD of vegetative and SOD, CAT, APX of reproductive stage etc. Principal component two contributed 22.6% to the total variation. Major six contributors were MDA, HPO, SOR, of vegetative and 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 (Figure 3).

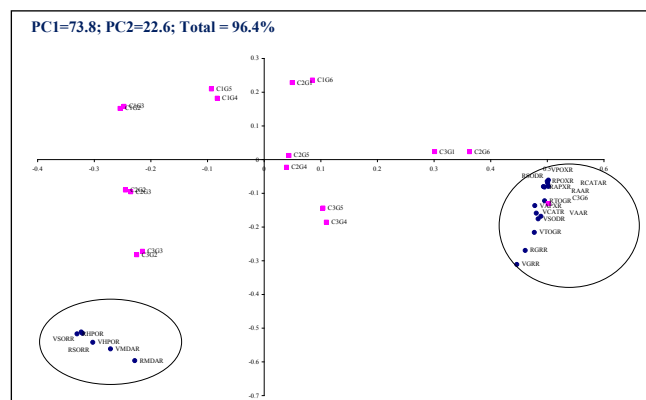


Figure 3: Clustering pattern of metabolites

The biplot analysis is an appropriate method to analyse the association analysis among treatment combinations and estimated values of metabolites and narrowing down the number of metabolites to the ones contributing a major portion to the variability Prity et al. (2021). In the biplot vectors of metabolites showing acute angles are positively correlated whereas those showing obtuse or straight-line angles are negatively correlated and those with right angles have no correlation (Kumar et al., 2020).

POX, HPO, MDA 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 (Figure 4). Similar

Table 4: Contribution of metabolites in principal components

Traits	PC1	PC2
VSORR	-0.1637	-0.3535
VAAR	0.2452	-0.1091
VHPOR	-0.1543	-0.3718
VMDAR	-0.1384	-0.3851
VTOGR	0.2434	-0.1482
VCATR	0.2492	-0.1154
VSODR	0.2468	-0.1207
VAPXR	0.2438	-0.0938
VPOXR	0.2560	-0.0545
VGRR	0.2276	-0.2134
RSORR	-0.1647	-0.3508
RAAR	0.2516	-0.0551
RHPOR	-0.1685	-0.3546
RMDAR	-0.1165	-0.4090
RTOGR	0.2527	-0.0840
RCATAR	0.2554	-0.0481
RSODR	0.2560	-0.0417
RAPXR	0.2525	-0.0560
RPOXR	0.2551	-0.0443
RGRR	0.2352	-0.1849
% variance explained	73.87	22.60

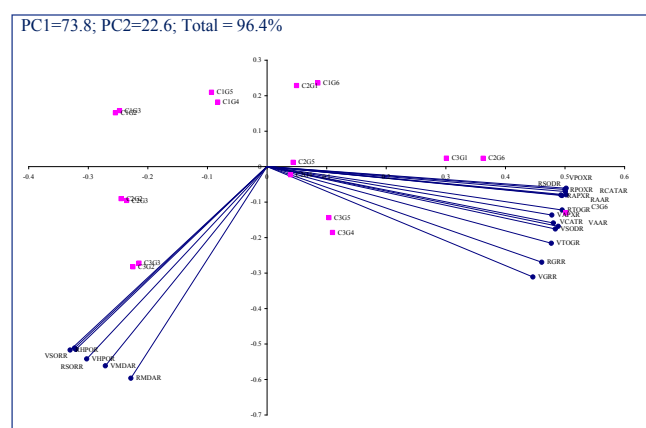


Figure 4: Association analysis among metabolites

type of behaviour exhibited by AA, APX, CAT, POX, SOD, TOG, and GR metabolites as formed large group. 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.

4. CONCLUSION

The less accumulation of reactive oxygen species along with the gradual increase in antioxidative metabolites' contents and enzymes' activities at higher iron augmentation treatments observed a superior ROS scavenging system. Increased the mineral availability in the edible part of the staple crops had proved an effective strategy in alleviating malnutrition economically. This complementary food-based approach would be the safest and cost effective way to ensure bio fortified nutrients on a larger scale to the poor populations.

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