**Biofortification of rice to augment iron availability for better health of population**


## Abstract

Aim : Rice is one of the largely consumed cereal and masses have been expressed anemia conditions. Iron augmentation of rice varieties had been carried out by agronomical biofortification as established an easy way to reach the poor rural masses for enhancing the concentration of particular minerals.

Methodology : Six rice varieties were evaluated during kharif in the net houses of the Department of Chemistry and Biochemistry, CCS HAU augmented with 0 mM 0.1m 0.5 mM Ethylene diamine tetra acetic acid (EDTA-Fe(II)). Reactive Oxygen Species (ROS) related metabolites along antioxidative metabolites were estimated in grains, upper shoots & lower shoots.

Results : Roots of HBC19 and Palman579 and lower and upper shoots of PUSA1121 contained higher iron. Highest iron in dehusked grains was recorded in Palman579 followed by HBC19, PUSA1121, HKR120, Super and Govind. Production of toxic super oxide radical ($O_2$- ) and hydrogen peroxide ($H_2O_2$) and lipid peroxidation (MDA), enhanced in all the varieties with increase in Fe concentration. Antioxidative metabolites' contents (ascorbic acid and glutathione) and activities of antioxidative enzymes [super oxide dismutase (SOD), catalase (CAT), peroxidase (POX), ascorbate peroxidase (APX) and glutathione reductase (GR)] invariably increased with increasing iron treatment in both root and shoot.

*Interpretation:* Less accumulation of reactive oxygen species along with the gradual increase in antioxidative metabolites' contents and enzymes' activities at higher iron treatments suggest that a better ROS scavenging ability to restrict the damage to cellular membranes due to lipid peroxidation may be responsible for the adaptation of these varieties at high iron levels.



**Key words :** Agronomic bio fortification, Iron augmentation, Reactive Oxygen Species related metabolites


## 1. Introduction

Rice is one of the largely consumed cereals in world. In developing countries, people often rely on rice as their sole source of nutrition similar to India (Bharadva *et al*., 2019). Iron in plants has an essential role in the development and formation of chlorophyll, maintains chloroplast structure to improve the photo systems along with the formation and activation of enzymes (Kumar *et al*., 2019; Aung and Masuda, 2020). Fe application plays an important role in growth of plant, different cellular functions and in the process of photosynthesis (Beasley *et al*., 2019). Fe supports the process of oxidation. Plant growth positively affects with the application of Fe, if applied at suitable level and time (Bouis & Saltzman, 2017). Fe is essential for the completion of crop's life cycle (Prity *et al*., 2021). Biofortification is the procedure of increasing the concentration of certain micronutrients in the edible part of crop plants by application of mineral

fertilizers or through conventional breeding methods to develop varieties with higher amount of micronutrients (Bouis *et al*., 2011; Garg et al 2018). Biofortification through breeding is a long-term exercise (Ramzan *et al*., 2020). Soil and foliar application of fertilizer is an agronomic tool which helps in higher accumulation of Zn and Fe in edible parts of plants (Jalal *et al*., 2020), which is known as agronomic biofortification. Agronomic biofortification is an easy way to reach the poor rural masses for enhancing the concentration of particular minerals (Zn and Fe) in their diet (Yadav *et al*., 2015). Studies suggest that fertilization is a cost-effective method for increasing the concentration of micronutrient (Zuo *et al*., 2011). Application of iron-based fertilizers and/or improving the solubilization and mobilization of iron in the soil will be helpful to enhance the iron status of plant and bioavailability of iron (Giordano *et al*., 2019). Soil application of iron fertilizer improves the available iron content of soil in iron deficient regions (Hassan *et al*., 2019). With increasing the daily intake of food derived from Fe-rich crops, has proven to be the most economical and sustainable approach for relieving micronutrient deficiency in the last decade worldwide (Haas et al 2016; Niyigaba *et al*., 2019).

**2. 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 standard cultivation practices and the pots were divided in three sets after 20 days of sowing and following treatment were given: One set was given Yoshida nutrient medium without Fe (0 mM EDTA-Fe(II)) (Sikirou et al 2016). 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)). Reactive Oxygen Species (ROS) related metabolites; malondialdehyde (MDA), superoxide radical ($O_2^{\cdot-}$), hydrogen peroxide ($H_2O_2$), antioxidative metabolites viz. ascorbic acid, 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 analyzed at the in root and shoot tissues of the rice varieties. Iron content in grains, upper shoots & lower 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). Hydrogen Peroxide ($H_2O_2$) 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). Peroxidase enzyme activity was estimated by the method of Shannon et al., (1966). The research data generated under present investigation was subjected to two way analysis of variance (ANOVA) to find out differences if any were significant at 5% level of significance.

## 3. Results and discussion

### Differential Pattern of metabolites

ANOVA analysis had observed highly significant variations among the estimated values among genotypes as well as for doses of iron supplementation (Stein *et al*., 2009). Variability among the estimated values had been bifurcated into different treatment combinations comprise of genotypes as well as doses of (EDTA) for meaningful interpretations (Niyigaba *et al*., 2019) (Figure 1). Combination C1G5 had expressed minimum value (96.2) for Superoxide radicals in shoots while C3G1 achieved maximum value of 243.8. Estimation in roots observed minimum value by C1G5 and maximum value of 173.9 by C3G1. Minimum value of Ascorbic acid estimated in shoots for C1G2 (411.9) while maximum value scored by C3G4 (978.2). Treatment combination C1G2 had minimum value 142.2 in roots whereas largest estimation expressed by

C3G5 (421.2) for Ascorbic Acid. Values of Hydrogen Peroxide in roots estimated had lower limits as compared to shoots of rice genotypes. Maximum value of Hydrogen peroxide had exhibited by C3G1 (664) and minimum value by C1G5 (375.3) as per for shoots estimation. Hydrogen peroxide had showed lower range in roots as varied from 187.3(C1G2) to 457.5(C3G4). Similar trends in POX values expressed as lower value in roots estimation as compared to shoots value i.e. 85(C1G2) to 370.2(C3G4) vis-à-vis 38.5(C1G1) to 76.4(C3G5). Wide variation observed among values for Malondialdehyde (MDA) as ranged from 10.4(C1G2) to 28.8(C3G5) in shoots as compared to 14.5(C1G2) to 59.5(C3G4) values estimated in roots (Figure 2). Large values of estimated Catalase observed in shoots than in roots as 7.7(C1G1) to 27.6(C3G6) in comparison to 16.1(C1G1) to 82.9(C3G5). Values of SOD expressed the difference of 12.2(C1G2) to 30.5(C3G5) for shoots in comparison to 7.7(C1G5) to 22.1(C3G1) for roots estimation. APX values were more in roots than shoots values for as evident from 9.9(C1G5) to 24.2(C3G1) in shoots and 15.5(C1G1) to 40.8(C3G4) corresponding to roots. Very contrasting values had observed for GR in shoots as showed deviation of 8.3(C1G2) to 23.9(C3G5).

**Variation Pattern of Superoxide radicals**

Variations in values of Superoxide radicals had observed (89.8 to 243.8) among genotypes vis-à-vis with quantity of EDTA-Fe(II) in shoot samples of the rice varieties (Kabir *et al*., 2016) at their reproductive stage as accompanied with root variation from (52.4 to 173.9) (Table 1). Govind variety has been expressed maximum increase in Superoxide in shoot and roots both. Maximum increase was associated with 0.5mM EDTA-Fe(II) for Govind followed by Super showed increase in shoot and root both with augmented application of EDTA. Highly significant differences had been exhibited by varieties in overall mean values of Superoxide over shoot and roots samples. The same trend of significant variation observed for various doses of EDTA for mean values over the varieties. Values of Critical Difference at 5% level of significance pointed

significant differences between overall mean values for shot and root samples estimation of Superoxide radicals.

**Variability in Ascorbic acid**

Significant Variations in values of Ascorbic acid had been expressed by varieties (Table 2) as differences carried from (411.1 to 978.2) vis-à-vis with quantity of EDTA-Fe(II) in shoot samples whereas root variation seen (142.2 to 387.4) (Kabir *et al*., 2016). PUSA1121 rice variety has been expressed maximum increase in Ascorbic acid in shoot followed by HBC19 while HBC19 and Palman579 for the roots estimation values. Maximum increase was observed in PUSA1121associated with 0.5mM EDTA-Fe(II) for shoot whereas HBC19 showed increase in root values for augmented application of EDTA. Highly significant differences had been exhibited by varieties in overall mean values over shoot and roots samples as variety Palman579 was of choice. The significant variations were observed for various doses of EDTA for mean values over the varieties. Critical Difference values at 5% level of significance pointed significant differences between overall mean values for shot and root samples estimation of Ascorbic acid.

**Differences of Hydrogen Peroxide**

Hydrogen Peroxide values showed significant variations had been expressed by varieties as differences carried from (375.3 to 664) vis-à-vis with quantity of EDTA-Fe(II) in shoot samples as accompanied with root variation from (187.3 to 387.3) (Table 3). Govind variety has been expressed maximum increase in values in shoot followed by Super and same two varieties had expressed larger values for roots estimation also. Maximum increase was observed in Govind associated with 0.5mM EDTA-Fe(II) for shoot and root with augmented application of EDTA. Highly significant differences had been exhibited by varieties in overall mean values over shoot and roots samples as Govind were the rice variety of choice. The significant variations were also observed for various doses of EDTA for mean values over the varieties. Overall mean values for

shot and root samples estimation of Hydrogen Peroxide pointed significant differences between as per Critical Difference values at 5% level of significance.

**Pattern of Malondialdehyde**

Significant variations had been observed among estimated Malondialdehyde (MDA) values as differences carried from (7.7 to 22.1) vis-à-vis with quantity of EDTA-Fe(II) in shoot samples as accompanied with root variation from (9.9 to 24.2) (Table 4) (Aung and Masuda, 2020). Govind variety has been expressed maximum increase in shoot values followed by Super and same two varieties had expressed larger values for roots estimation also. Maximum increase was observed in Govind associated with 0.5mM EDTA-Fe(II) for shoot and root augmented with application of 0.5mM EDTA. Highly significant differences had been exhibited by varieties in overall mean values over shoot and roots samples as Govind were the rice variety of choice. The significant variations were also observed for various doses of EDTA for mean values over the varieties. Critical Difference values at 5% level of significance pointed significant differences between overall mean values for shot and root samples.

**Total Glutathione Pattern among genotypes**

Variations of significant nature had been observed among Total Glutathione content values as deviated from (4.6 to 9.6) vis-à-vis with quantity of EDTA-Fe(II) in shoot samples as accompanied with root variation from (2.8 to 7.8) (Table 5) (Shi *et al*., 2016). PUSA1121 variety followed by HBC19 has expressed maximum increase in shoot values and HBC19 along with Palman579 had expressed larger values for roots estimation. Large change was observed in PUSA1121 associated with 0.5mM EDTA-Fe(II) for shoot and HBC19 expressed maximum root values estimation augmented with application of 0.5mM EDTA. Highly significant differences had been exhibited by varieties in overall mean values over shoot and roots samples as HBC19 were the rice variety of choice. The significant variations were also observed for various doses of EDTA for mean values over the varieties. Mean values for shot and root samples estimation

of Total Glutathione content expressed significant differences as expressed the Critical Difference values at 5% level of significance.

**Differential expression of Catalase**

Catalase values showed significant amount of variations (Table 6) with increased quantity of EDTA-Fe(II) as differences showed from (16.5 to 40.8) in shoot samples and accompanied root variations from (8.3 to 23.9). PUSA1121 variety has been expressed maximum increase in values in shoot followed by HBC19 and HBC19 and Palman579 for the roots estimation values. Maximum increase was observed in PUSA1121 associated with 0.5mM EDTA-Fe(II) for shoot whereas HBC19 showed increase in root augmented application of EDTA. Highly significant differences had been exhibited by varieties in overall mean values over shoot and roots samples as HBC19 were the rice variety of choice. The significant variations were also observed for various doses of EDTA for mean values over the varieties. Values of Critical Difference at 5% level of significance observed significant differences between overall mean values for shot and root samples estimated values.

**Pattern of Superoxide dismutase**

Significant variations had been expressed by Superoxide dismutase (SOD) values as differences varied from (38.5 to 76.4) vis-à-vis with quantity of EDTA-Fe(II) in shoot samples and accompanied with root variations from (10.4 to 28.8) (Table 7). Rice variety has been expressed maximum increase in shoot values followed by PUSA1121 and HBC19 for the roots estimated values followed by Palman579. Maximum increase was observed in HBC19 associated with 0.5mM EDTA-Fe(II) for shoot and root augmented application of EDTA. Highly significant differences had been exhibited by varieties in overall mean values over shoot and roots samples as Palman579 were the rice variety of choice. The significant variations were also observed for various doses of EDTA for mean values over the varieties. Critical Difference values at 5% level of significance pointed significant differences between overall mean values for shot and root samples estimation of Superoxide dismutase.

**Ascorbate peroxidase variation among genotypes**

Significant variations in amounts of estimated values Ascorbate peroxidase (APX) had been expressed by varieties with increased applications of EDTA-Fe(II) as differences observed from (14.5 to 59.5) in shoot samples as accompanied with root variation from (7.7 to 27.6) (Table 8). PUSA1121 variety has been expressed maximum increase in APX in shoot followed by HBC19 and Palman579 & HBC19 for the roots estimation. Maximum increase was observed in PUSA1121 associated with 0.5mM EDTA-Fe(II) for shoot whereas Palman579 showed increase in roots with augmented application of EDTA. Highly significant differences had been exhibited by varieties in overall mean values over shoot and roots samples as HBC19 were the rice variety of choice. The significant variations were observed for various doses of EDTA for mean values over the varieties. Overall mean values for shot and root samples estimation of Ascorbate peroxidase at Critical Difference values at 5% level of significance pointed significant differences.

**Differential Pattern of Peroxidase**

Variations in values of Peroxidase (POX) (Table 9) had observed (85.0 to 370.2) among genotypes vis-à-vis with augmentation of EDTA-Fe(II) in shoot samples as accompanied with drastic low values for root estimation as values ranged from (16.1 to 82.9). PUSA1121 variety has been expressed maximum increase in POX in shoot as followed by HBC19 and Palman579 & HBC19 for roots estimated values. Maximum increase was associated with 0.5mM EDTA-Fe(II) for shoot and root stages whereas PUSA1121 showed increase in shoot and Palman579 for root with augmented application of EDTA. Highly significant differences had been exhibited by varieties in overall mean values of POX (73.7 to 166.4) over shoot and roots samples. The same trend of significant variation observed for various doses of EDTA for mean values over the varieties (89.3 to 150.2). CD values at 5% level of significance pointed significant differences between overall mean values for shot and root samples estimation of Peroxidase.

**Pattern of Glutathione reductase**

Significant variations in estimated values Glutathione reductase (GR) had been expressed by varieties with increased applications of EDTA-Fe(II) as differences observed from (12.2 to 30.5) in shoot samples whereas associated root variations from (2.7 to 6.8) (Table 10). HBC19 has shown maximum increase in shoot values followed by Palman579 while large values achieved by HBC19 & Palman579 for the roots estimation. Maximum increase was observed in HBC19 associated with 0.5mM EDTA-Fe(II) for shoot whereas HBC19 also showed increase in roots with augmented application of EDTA. Highly significant differences had been exhibited by varieties in overall mean values over shoot and roots samples as HBC19 were the rice variety of choice. The significant variations were observed for various doses of EDTA for mean values over the varieties. Critical Difference values at 5% level of significance pointed significant differences between overall mean values for shot and root samples estimation of Glutathione reductase.

**Conclusions**

HBC19 and Palman579 contain higher iron in root tissues as compared to Govind and Super rice varieties. The iron content in roots increased with increasing iron concentration in all the six varieties however, the maximum increase was observed in Govind at 0.5 mM Fe dose. Superoxide radical, $H_2O_2$ and MDA contents were significantly higher in (Govind and Super) as compared to other varieties in both shoot and root tissues. Higher increase in superoxide ions , $H_2O_2$ and MDA content was observed in shoots of Govind and Super at at 0.5 mM Fe treatment as compared to the varieties. Oxidized glutathione content on the other hand was significantly higher in Govind and Super. Ascorbate, reduced glutathione and oxidized glutathione exhibited progressive increase at 0.1 and 0.5 mM Fe treatments in the two tissues of six rice varieties at all the stages. Antioxidative enzymes viz. super oxide dismutase (SOD), catalase (CAT), peroxidase(POX), ascorbate peroxidase (APX) and glutathione reductase (GR) exhibited similar pattern as observed for ascorbate and reduced glutathione. Iron treatment resulted in enhanced activities of SOD, CAT, POX, APX and GR in both roots and shoots of the six rice varieties at

reproductive stage. Photo-oxidative damage is often observed in Fe-deficient plants. Plants starving with Fe are more prone to oxidative stress as Fe is a co-factor of many antioxidant enzymes. In this study, less accumulation of reactive oxygen species along with the gradual increase in antioxidative metabolites' contents and enzymes' activities at higher iron treatments suggest that a better ROS scavenging ability to restrict the damage to cellular membranes due to lipid peroxidation may be responsible for the adaptation of these varieties at high iron levels.

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Table 1: Superoxide radicals of rice genotypes at the shoot and root stage

| Genotype | Shoot | | | | Root | | | | Overall mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | |
| Govind | 145.40 | 203.94 | 243.86 | 197.73 | 106.98 | 153.62 | 173.95 | 144.85 | 171.29 [a] |
| Super | 136.19 | 196.11 | 235.57 | 189.29 | 101.11 | 144.06 | 166.03 | 137.07 | 163.17 [b] |
| HKR120 | 126.67 | 169.94 | 196.97 | 164.52 | 74.29 | 98.02 | 114.43 | 95.58 | 130.05 [c] |
| PUSA1121 | 89.83 | 112.29 | 136.29 | 112.80 | 63.02 | 81.70 | 94.19 | 79.63 | 96.21 [e] |
| HBC19 | 96.22 | 121.22 | 132.41 | 116.62 | 52.49 | 64.78 | 73.32 | 63.53 | 90.07 [f] |
| Palman579 | 107.94 | 138.57 | 154.03 | 133.51 | 57.78 | 72.73 | 81.57 | 70.69 | 102.10 [d] |
| Mean | 117.04 | 157.01 | 183.18 | 152.41 | 75.94 | 102.48 | 117.24 | 98.55 | |
| Overall mean | 96.49 [c] | 129.74 [b] | 150.21 [a] | | | | | | |
| CD at 5% for genotypes | 2.86 | | | | | | | | |
| CD at 5% for chemical | 2.02 | | | | | | | | |
| CD at 5% for stages | 1.65 | | | | | | | | |

Table 2: Ascorbic acid (µg/g FW) of rice genotypes at the shoot and root stage

| Genotype | Shoot | | | | Root | | | | Overall mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | |
| Govind | 420.20 | 468.99 | 511.21 | 466.80 | 151.58 | 184.34 | 192.41 | 176.11 | 321.45 [e] |
| Super | 411.96 | 452.53 | 508.69 | 457.72 | 142.27 | 169.00 | 182.07 | 164.45 | 311.08 [f] |
| HKR120 | 472.73 | 609.74 | 645.86 | 576.11 | 177.78 | 236.67 | 248.48 | 220.98 | 398.54 [d] |
| PUSA1121 | 634.34 | 896.16 | 978.28 | 836.26 | 210.10 | 288.99 | 300.12 | 266.40 | 551.33 [b] |
| HBC19 | 608.08 | 874.58 | 934.95 | 805.87 | 258.93 | 392.22 | 421.21 | 357.45 | 581.66 [a] |
| Palman579 | 565.66 | 789.79 | 857.32 | 737.59 | 242.42 | 362.88 | 387.47 | 330.93 | 534.25 [c] |
| Mean | 518.83 | 681.96 | 739.39 | 646.73 | 197.18 | 272.35 | 288.63 | 252.72 | |
| Overall mean | 358.00 [c] | 477.15 [b] | 514.00 [a] | | | | | | |
| CD at 5% for genotypes | 4.79 | | | | | | | | |
| CD at 5% for chemical | 3.38 | | | | | | | | |
| CD at 5% for stages | 2.76 | | | | | | | | |

Table 3: Hydrogen Peroxide (µg/g FW) of rice genotypes at the shoot and root stage

| Genotype | Shoot | | | | Root | | | | Overall mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | |
| Govind | 459.65 | 598.54 | 664.07 | 574.08 | 307.28 | 397.03 | 457.58 | 387.30 | 480.69 [a] |
| Super | 453.24 | 579.46 | 635.02 | 555.90 | 288.23 | 384.10 | 436.30 | 369.54 | 462.72 [b] |
| HKR120 | 432.72 | 526.47 | 567.50 | 508.90 | 262.23 | 320.81 | 346.37 | 309.80 | 409.34 [c] |
| PUSA1121 | 392.49 | 444.12 | 476.94 | 437.85 | 253.06 | 295.23 | 324.95 | 291.08 | 364.46 [d] |
| HBC19 | 375.38 | 442.76 | 465.40 | 427.85 | 187.31 | 206.93 | 225.44 | 206.56 | 317.20 [f] |
| Palman579 | 415.90 | 482.19 | 518.51 | 472.20 | 209.48 | 235.47 | 255.26 | 233.40 | 352.80 [e] |
| Mean | 421.56 | 512.25 | 554.57 | 496.13 | 251.26 | 306.59 | 340.98 | 299.61 | |
| Overall mean | 336.41 [c] | 409.42 [b] | 447.77 [a] | | | | | | |
| CD at 5% for genotypes | 3.67 | | | | | | | | |
| CD at 5% for chemical | 2.58 | | | | | | | | |
| CD at 5% for stages | 2.10 | | | | | | | | |

Table 4: Malondialdehyde (MDA) (µg/g FW) of rice genotypes at the shoot and root stage

| Genotype | Shoot | | | | Root | | | | Overall mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | |
| Govind | 12.75 | 18.12 | 22.11 | 17.66 | 13.79 | 18.37 | 24.25 | 18.80 | 18.23 [a] |
| Super | 11.98 | 17.52 | 21.50 | 17.00 | 13.16 | 17.88 | 22.48 | 17.84 | 17.42 [b] |
| HKR120 | 10.52 | 14.21 | 17.84 | 14.19 | 11.94 | 14.72 | 17.81 | 14.82 | 14.50 [c] |
| PUSA1121 | 8.37 | 10.10 | 12.46 | 10.31 | 12.39 | 15.67 | 19.41 | 15.82 | 13.06 [d] |
| HBC19 | 7.75 | 9.12 | 11.21 | 9.36 | 9.91 | 11.27 | 13.07 | 11.41 | 10.38 [f] |
| Palman579 | 9.10 | 12.02 | 14.37 | 11.83 | 10.47 | 12.18 | 14.09 | 12.25 | 12.03 [e] |
| Mean | 10.08 | 13.51 | 16.58 | 13.39 | 11.94 | 15.01 | 18.52 | 15.16 | |
| Overall mean | 11.01 [c] | 14.26 [b] | 17.55 [a] | | | | | | |
| CD at 5% for genotypes | 0.43 | | | | | | | | |
| CD at 5% for chemical | 0.30 | | | | | | | | |
| CD at 5% for stages | 0.25 | | | | | | | | |

Table 5: Total glutathione content (nmoles/ g FW) of rice genotypes at the shoot and root stage

| Genotype | Shoot | | | | Root | | | | Overall mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | |
| Govind | 4.84 | 5.29 | 5.87 | 5.33 | 2.89 | 3.19 | 3.41 | 3.16 | 4.24 [f] |
| Super | 4.68 | 4.99 | 5.47 | 5.05 | 3.19 | 3.65 | 3.80 | 3.55 | 4.29 [e] |
| HKR120 | 4.43 | 5.16 | 6.04 | 5.21 | 4.00 | 5.01 | 5.59 | 4.87 | 5.03 [d] |
| PUSA1121 | 5.86 | 8.15 | 9.69 | 7.90 | 3.58 | 4.57 | 5.11 | 4.42 | 6.16 [c] |
| HBC19 | 5.62 | 8.07 | 9.42 | 7.70 | 4.79 | 6.91 | 7.89 | 6.53 | 7.11 [a] |
| Palman579 | 5.51 | 6.75 | 7.83 | 6.69 | 4.22 | 6.01 | 6.68 | 5.64 | 6.16 [b] |
| Mean | 5.16 | 6.40 | 7.39 | 6.32 | 3.78 | 4.89 | 5.41 | 4.69 | |
| Overall mean | 4.46 [c] | 5.64 [b] | 6.40 [a] | | | | | | |
| CD at 5% for genotypes | 0.18 | | | | | | | | |
| CD at 5% for chemical | 0.13 | | | | | | | | |
| CD at 5% for stages | 0.10 | | | | | | | | |

Table 6: Catalase (µmole/g FW) of rice genotypes at the shoot and root stage

| Genotype | Shoot | | | | Root | | | | Overall mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | |
| Govind | 15.51 | 17.27 | 18.78 | 17.19 | 9.87 | 11.14 | 12.22 | 11.07 | 14.13 [e] |
| Super | 16.52 | 18.52 | 19.51 | 18.18 | 8.38 | 9.71 | 10.17 | 9.42 | 13.80 [f] |
| HKR120 | 19.77 | 23.54 | 27.29 | 23.53 | 11.98 | 14.58 | 16.32 | 14.29 | 18.91 [d] |
| PUSA1121 | 26.67 | 35.05 | 40.85 | 34.19 | 12.06 | 15.02 | 16.40 | 14.49 | 24.34 [b] |
| HBC19 | 24.77 | 31.65 | 37.37 | 31.26 | 16.10 | 21.53 | 23.95 | 20.52 | 25.89 [a] |
| Palman579 | 20.01 | 25.25 | 28.87 | 24.71 | 14.63 | 18.97 | 21.39 | 18.33 | 21.52 [c] |
| Mean | 20.54 | 25.21 | 28.78 | 24.84 | 12.17 | 15.15 | 16.74 | 14.69 | |
| Overall mean | 16.35 [c] | 20.18 [b] | 22.76 [a] | | | | | | |
| CD at 5% for genotypes | 0.57 | | | | | | | | |
| CD at 5% for chemical | 0.42 | | | | | | | | |
| CD at 5% for stages | 0.34 | | | | | | | | |

Table 7: Superoxide dismutase (SOD) (µmole/g FW) of rice genotypes at the shoot and root stage

| Genotype | Shoot | | | | Root | | | | Overall mean |
|---|---|---|---|---|---|---|---|---|---|
| | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | |
| Govind | 38.57 | 43.19 | 51.43 | 44.40 | 11.09 | 13.19 | 14.09 | 12.79 | 28.59 [f] |
| Super | 39.72 | 45.25 | 50.69 | 45.22 | 10.45 | 12.73 | 13.43 | 12.20 | 28.71 [e] |
| HKR120 | 46.29 | 55.96 | 63.39 | 55.21 | 16.23 | 21.06 | 21.77 | 19.69 | 37.44 [d] |
| PUSA1121 | 48.25 | 60.24 | 70.28 | 59.59 | 15.72 | 20.45 | 20.60 | 18.92 | 39.25 [b] |
| HBC19 | 50.19 | 64.05 | 76.44 | 63.56 | 19.56 | 27.13 | 28.84 | 25.18 | 44.36 [a] |
| Palman579 | 44.13 | 53.80 | 63.70 | 53.88 | 18.77 | 25.21 | 26.74 | 23.57 | 38.72 [c] |
| Mean | 44.53 | 53.75 | 62.66 | 53.64 | 15.30 | 19.96 | 20.91 | 18.73 | |
| Overall mean | 29.91 [c] | 36.85 [b] | 41.78 [a] | | | | | | |
| CD at 5% for genotypes | 1.00 | | | | | | | | |
| CD at 5% for chemical | 0.71 | | | | | | | | |
| CD at 5% for stages | 0.58 | | | | | | | | |

Table 8: Ascorbate peroxidase (APX) (µmole/g FW) of rice genotypes at the shoot and root stage

| | Shoot | | | | Root | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | mean |
| Govind | 16.21 | 20.33 | 24.05 | 20.19 | 7.70 | 9.03 | 11.04 | 9.26 | 14.72 [e] |
| Super | 14.51 | 19.09 | 22.13 | 18.57 | 8.26 | 10.18 | 11.32 | 9.92 | 14.24 [f] |
| HKR120 | 24.93 | 34.55 | 40.81 | 33.43 | 12.79 | 17.49 | 19.59 | 16.62 | 25.02 [d] |
| PUSA1121 | 31.90 | 47.99 | 59.52 | 46.47 | 10.85 | 15.16 | 16.10 | 14.04 | 30.25 [c] |
| HBC19 | 29.16 | 45.79 | 52.35 | 42.43 | 15.33 | 23.26 | 26.19 | 21.59 | 32.01 [a] |
| Palman579 | 27.97 | 39.84 | 47.11 | 38.31 | 16.99 | 24.81 | 27.66 | 23.16 | 30.73 [b] |
| Mean | 24.11 | 34.60 | 40.99 | 33.23 | 11.99 | 16.66 | 18.65 | 15.76 | |
| Overall mean | 18.04 [c] | 25.62 [b] | 29.82 [a] | | | | | | |
| CD at 5% for genotypes | 1.11 | | | | | | | | |
| CD at 5% for chemical | 0.79 | | | | | | | | |
| CD at 5% for stages | 0.64 | | | | | | | | |

Table 9: Peroxidase (POX)(µmole/g FW) of rice genotypes at the shoot and root stage

| | Shoot | | | | Root | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | mean |
| Govind | 103.92 | 125.93 | 152.72 | 127.52 | 16.10 | 20.60 | 23.05 | 19.92 | 73.71 [e] |
| Super | 85.07 | 107.30 | 131.28 | 107.88 | 21.40 | 28.61 | 31.73 | 27.25 | 67.56 [f] |
| HKR120 | 155.07 | 200.63 | 258.03 | 204.58 | 37.57 | 50.80 | 58.23 | 48.87 | 126.72 [d] |
| PUSA1121 | 208.95 | 285.30 | 370.22 | 288.16 | 33.63 | 47.07 | 53.52 | 44.74 | 166.44 [a] |
| HBC19 | 178.18 | 252.80 | 324.48 | 251.82 | 46.63 | 66.92 | 82.98 | 65.51 | 158.66 [b] |
| Palman579 | 138.60 | 192.32 | 236.63 | 189.18 | 47.30 | 70.37 | 79.53 | 65.73 | 127.45 [c] |
| Mean | 144.96 | 194.05 | 245.56 | 194.86 | 33.77 | 47.39 | 54.84 | 45.34 | |
| Overall mean | 89.36 [c] | 120.72 [b] | 150.20 [a] | | | | | | |
| CD at 5% for genotypes | 3.08 | | | | | | | | |
| CD at 5% for chemical | 2.18 | | | | | | | | |
| CD at 5% for stages | 1.78 | | | | | | | | |

Table 10: Glutathione reductase (GR)(µmole/g FW) of rice genotypes at the shoot and root stage

| | Shoot | | | | Root | | | | Overall |
|---|---|---|---|---|---|---|---|---|---|
| Genotype | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | 0mM EDTA-Fe(II) | 0.1mM EDTA-Fe(II) | 0.5mM EDTA-Fe(II) | Mean | mean |
| Govind | 13.32 | 16.59 | 18.17 | 16.02 | 3.08 | 3.65 | 4.10 | 3.61 | 9.81 [e] |
| Super | 12.23 | 14.80 | 17.27 | 14.76 | 2.72 | 3.16 | 3.78 | 3.22 | 8.99 [f] |
| HKR120 | 14.71 | 18.77 | 22.74 | 18.74 | 3.55 | 4.51 | 5.42 | 4.49 | 11.61 [d] |
| PUSA1121 | 16.29 | 22.96 | 27.84 | 22.36 | 3.42 | 4.30 | 5.03 | 4.25 | 13.30 [c] |
| HBC19 | 18.38 | 25.73 | 30.59 | 24.90 | 4.01 | 5.55 | 6.87 | 5.47 | 15.18 [a] |
| Palman579 | 17.95 | 24.19 | 29.21 | 23.78 | 3.85 | 5.22 | 6.33 | 5.13 | 14.45 [b] |
| Mean | 15.48 | 20.51 | 24.30 | 20.10 | 3.44 | 4.40 | 5.25 | 4.36 | |
| Overall mean | 9.45 [c] | 12.45 [b] | 14.77 [a] | | | | | | |
| CD at 5% for genotypes | 0.46 | | | | | | | | |
| CD at 5% for chemical | 0.32 | | | | | | | | |
| CD at 5% for stages | 0.26 | | | | | | | | |

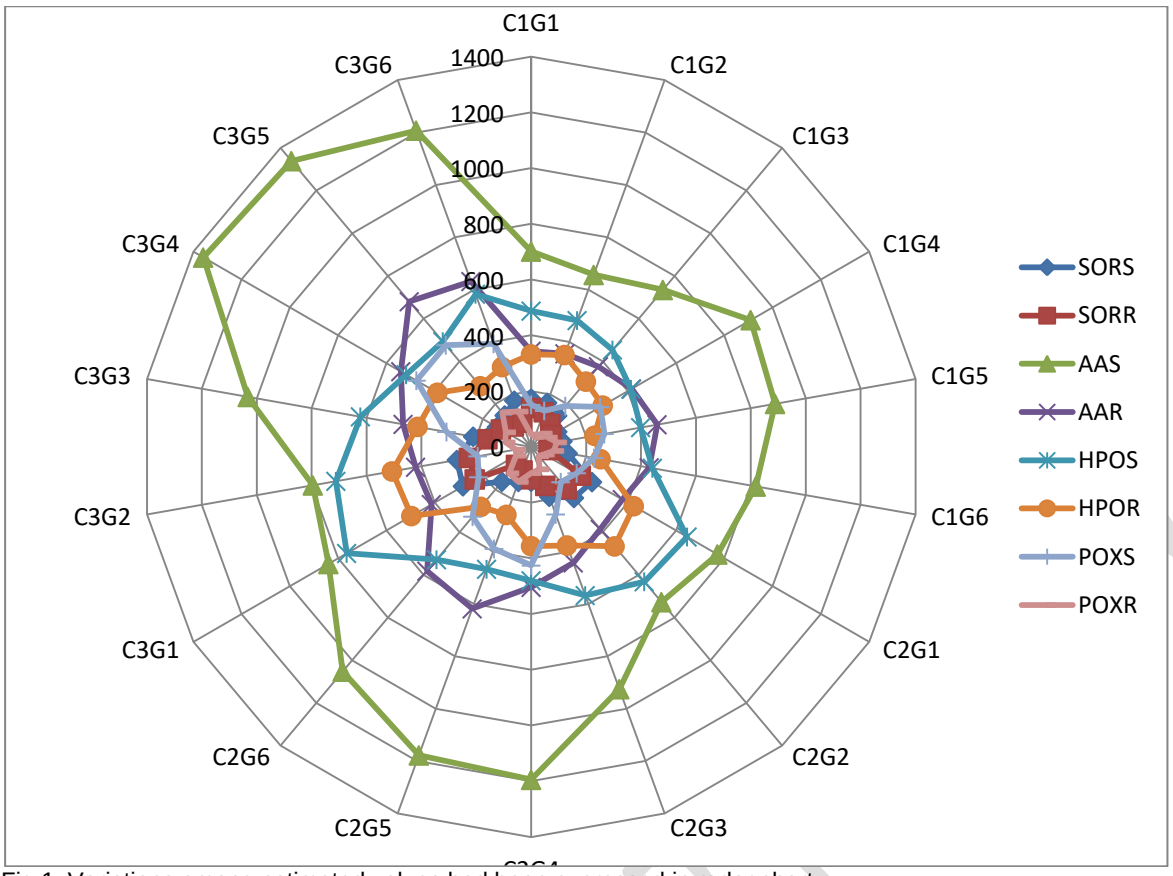


Fig 1. Variations among estimated values had been expressed in radar chart

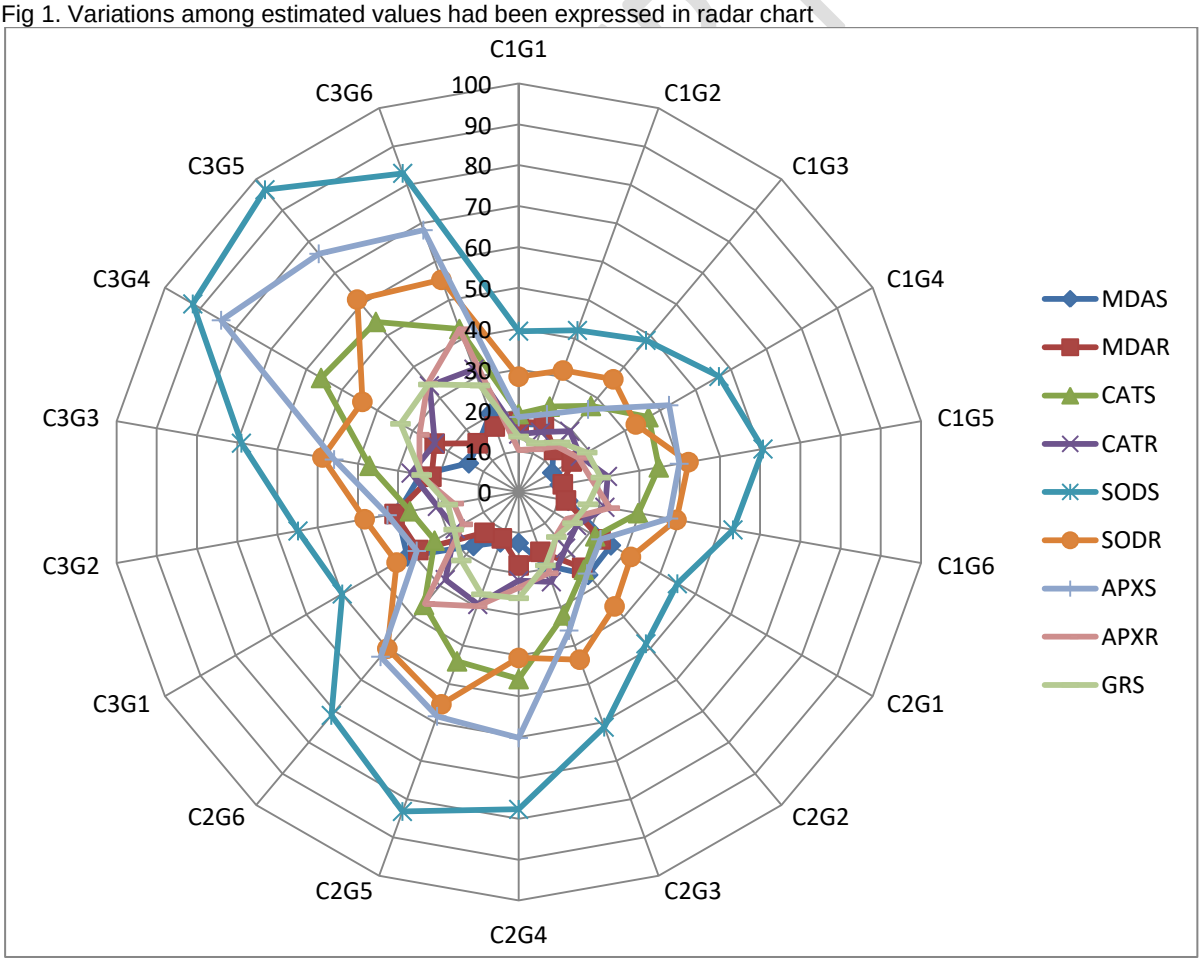


Fig 2. Radar chart expressed the variations among estimated values

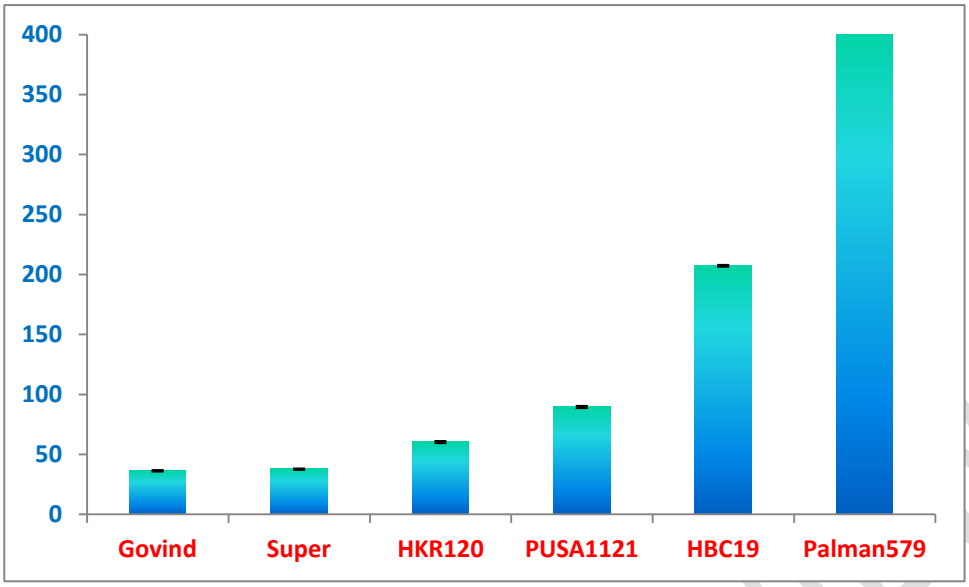


Fig 3: Iron contents in grains (µg/g)