Evaluation of Recombinant Inbred Lines for Higher Iron and Zinc Content Along with Yield and Quality Parameters in Rice (*Oryza sativa* L.)

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**ABSTRACT**

Rice is one of the most important staple food crops for billions of people throughout the world. It is the cheapest source of dietary energy, protein and minerals for people but it is a poor source of micronutrients such as iron (Fe) and zinc (Zn) (Bouis and Welch, 2010). Micronutrients deficiency is a global problem and particularly in developing countries Fe and Zn deficiency affects over three billion people (Welch and Graham, 2004) as well as Fe and Zn ranked fifth and sixth, respectively, among the top ten risk factors contributing to trouble of disease (Rani et al., 2019). Global studies identify that approximately half of this anemic situation is due to iron-deficiency anemia (IDA) (Brotanek et al., 2005). Iron deficiency anemia (IDA) is a common type of anemia in which blood lacks adequate healthy red blood cells. Milled grains of popular rice varieties have approximately 2 μg/g iron and 16 μg/g zinc concentration (Trijatmiko et al. 2016). Iron is predominantly existed in the germ and bran layer which on course of milling got removed (Dexter et al., 1998).

Millions of rice eaters who cannot afford different types of foods are affected by ‘hidden hunger’ due to micronutrient deficiency induced malnutrition which is a serious global challenges faced by mankind (White and Broadley, 2009). Apart from higher yield, genetic up gradation for improving human vitamin and mineral nutrition, especially iron, zinc, selenium and iodine has received considerable attention in recent years. (Grusak et al., 2002). In developing countries deficiencies of zinc, iron and vitamin A in human population is become the major causes of several diseases. The World Health Organization (WHO 2013) states that 2 billion people are suffered by anemia mainly due to iron deficiency. Zinc deficiency is the fifth major cause of diseases and deaths in developing countries (Ravindra babu, 2013). Health problems caused by zinc deficiency include anorexia, dwarfism, weak immune system, skinlesions, hypogonadism and diarrhea (Prasad et al., 2012). Therefore, increasing the zinc and iron content and bioavailability of micronutrients in such as rice grain is an important target for breeders and it offers the potential benefits to a large proportion of human population (Cakmak, 2008; Palmgren et al., 2008). Genetic

**INTRODUCTION**

Rice (*Oryza sativa* L.) is one of the oldest domesticated and most important staple food crops in India and also throughout the world. It is the cheapest source of dietary energy, protein and minerals for people but it is a poor source of micronutrients such as iron (Fe) and zinc (Zn) (Bouis and Welch, 2010). Micronutrients deficiency is a global problem and particularly in developing countries Fe and Zn deficiency affects over three billion people (Welch and Graham, 2004) as well as Fe and Zn ranked fifth and sixth, respectively, among the top ten risk factors contributing to trouble of disease (Rani et al., 2019). Global studies identify that approximately half of this anemic situation is due to iron-deficiency anemia (IDA) (Brotanek et al., 2005). Iron deficiency anemia (IDA) is a common type of anemia in which blood lacks adequate healthy red blood cells. Milled grains of popular rice varieties have approximately 2 μg/g iron and 16 μg/g zinc concentration (Trijatmiko et al. 2016). Iron is predominantly existed in the germ and bran layer which on course of milling got removed (Dexter et al., 1998).

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diversity for high micronutrient content in addition to yield and yield attributing traits is a prerequisite for designing an efficient breeding program aiming to improve nutritional quality in rice (Virk et al., 2006). On the other hand, remedies for malnutrition, anaemia and immunity development can be addressed through rice, if desirable genes are incorporated into an elite cultivar. Hence, agriculture must now focus on a new paradigm that will not only produce more food, but also bring us better quality food as well. The present investigation aims at evaluation of recombinant inbred lines for high iron and zinc content with high yielding background in rice genotypes.

**MATERIALS AND METHODS**

A population of 126 F₃ recombinant inbred lines was developed by single-seed decent from a cross between Lemont and Satabdi (both contain moderately zinc-iron). The RIL population were used for field and laboratory experiments (iron and zinc content). Field experiments were conducted in Baruipur, South 24 pgs. (22°51’ latitude north and 88°25’ longitude east) Agricultural Experimental Farm, University of Calcutta under two environments over 2 years during boro season of 2016 and 2017 and laboratory experiments were conducted to estimate iron and zinc at the laboratory of Genetics and Plant Breeding department, Institute of Agricultural Science, University of Calcutta. Yield and yield attributing data viz. Days to maturity, plant height, number of panicle, panicle length, grains per panicle, 1000 grain weight and grain yield per plant (GY) along with grain iron content (Fe), grain zinc content (Zn), were recorded.

Seeds were oven dried at 50°C temperatures for 48 hours to facilitate the dehusking process. The dehusked brown seeds were then ground to make it powder and 0.5 gm of the rice grain powder for each genotype were weighed separately. Then 10 ml of Tri acid mixture (Nitric acid 10: Perchloric acid 4: Sulphuric acid 1) was then added to the flask and placed on a hot plate >80°C to digest for 5-6 hours, after which a white precipitate was remained. Flasks were removed, cooled, diluted with Millipore water and volume made up to 100ml, filtered through Whatman filter paper and stored in 100 ml volumetric flask. Zinc and iron content of rice was estimated using Atomic Absorption Spectrophotometer (Agilent) suggested by Lindsay and Novell (1978). The similarity calculated by Dice coefficient and dendrogram constructed by Ward analysis. Statistical analysis was performed using SPSS software ver. 20.

Some selected elite recombinant inbred lines with high zinc, iron and yield, were subjected to assess the variation of quality traits namely amylose content, gel consistency, alkali spreading value, gelatinization temperature and kernel length and breadth (mm), length/breadth ratio (L/B), kernel length after cooking (KLAC) (mm), kernel elongation ratio (ER). The parameters of rice quality like amylose content and gel consistency were estimated as per the method of Juliano (1971) and Cagampang et al. (1973) respectively. Gelatinization temperature is estimated by the extent of alkali spreading value and was done following the method of Bhattacharya and Sowbhagya (1972). Length and breadth in millimeters was recorded using Dial micrometer. The L/B ratio was calculated by dividing the average length by the average breadth of rice kernel. Elongation ratio was also measured by following the protocol of Azeez and Shafi (1966).

**RESULTS AND DISCUSSION**

**Genetic variability in RIL line**

In the present study, genetic variability in a rice population of 126 recombinant inbred lines for yield, yield attributing characters, micronutrient content (Zn and Fe) were estimated. Analysis of Variance (ANOVA) revealed that a considerable variation was present among the RILs at 0.05% level of significance. The genotypic coefficient of variation (54.89) and the phenotypic coefficient of variation (58.98) both were found to be maximum in case of yield per plant and days to maturity. High GCV for no. of panicles suggests that selection can be helpful to improve more number of panicles. In all agromorphic traits, a narrow differences were observed between PCV and GCV suggesting the traits have less environmental influence. Ahmen et al. (2000) also observed similar findings that closeness in GCV and PCV estimates. The heritability estimates ranged from 39.44% to 92.32% (Table 1). A higher estimate of heritability was observed in yield per plant followed by grains per panicle. Thus selection can be advantageous for yield and grains per panicle to genetically improve the traits.

**Fe and Zn content**

In the present study, zinc and iron content showed a wide range of variation among recombinant inbred lines of Lemont X Satabdi, in brown rice. Rice grain zinc concentration showed range from 23.01 ppm to 86.36 ppm and iron concentration ranged from 6.55 ppm to 32.95 ppm. Zn concentration was found highest in line 24 (86.36 ppm), whereas Fe concentration ranked high in line 57 (35.15 ppm). The mean value of Zn and Fe concentration in the RIL population was 41.69 ppm and 18.09 ppm respectively. The result revealed that the high Fe lines (>30ppm) also had high or moderate Zn but the high Zn lines (>50ppm) did not have high Fe. The same observation was observed by Anuradha et al. (2012) in 126 rice germplasm and also from 128 Backcross Intro gression lines (BILs) of BPT5204 x O. Nivara. Depending upon the soil zinc status, concentration of zinc in rice grain may vary but abundant Fe in soil, does not effect in Fe-content of rice grain because of it’s solubility in soil (Gregoria et al., 2000). Fe concentration is known to vary with location but Zn values appear to be more consistent (Chandel et al., 2010).

**Correlation between yield and attributing traits with Fe and Zn**

In this study, highest positive significant correlation was observed between number of filled grains with panicle length and also between yield per plant and number of panicles. No...
Table 1: Comparative Study of phenotypic and genotypic variance, genotypic and phenotypic coefficient of variation, broad sense heritability of 126 Recombinant Inbred lines.

| Characters                  | Source of Variance (ANOVA) | GCV     | PCV     | Heritability (%) |
|-----------------------------|-----------------------------|---------|---------|------------------|
|                             | Replication (2)             |         |         |                  |
|                             | Variety (125)               |         |         |                  |
|                             | Error (250)                 |         |         |                  |
| Days to maturity            | 3.67                        | 38.47** | 1.78    | 54.89            |
| Plant Height                | 40.67                       | 149.56**| 5.34    | 5.27             |
| No. of panicle              | 3.15                        | 19.45** | 1.45    | 18.57            |
| Panicle length              | 1.06                        | 16.45** | 0.88    | 4.56             |
| No. of grains per panicle   | 46.89                       | 208.72**| 10.63   | 13.57            |
| 1000 grain weight           | 109.46                      | 419.56**| 20.41   | 21.89            |
| Yield per plant (gm)        | 4.98                        | 69.325**| 1.09    | 17.45            |
| Zinc content                | 10.25                       | 311.62**| 3.26    | 19.36            |
| Iron content                | 9.56                        | 195.66**| 4.39    | 22.58            |

** significant at 5% probability level.

Table 2: Correlation analysis among pairs of the characters in 126 Recombinant Inbred lines.

| Characters                  | Days to maturity | Plant Height | No. of panicle | Panicle length | No. of filled grains per panicle | 1000 seed weight (g) | Yield per plant (gm) | Zinc content (ppm) | Iron content (ppm) |
|-----------------------------|------------------|--------------|----------------|----------------|----------------------------------|----------------------|---------------------|--------------------|-------------------|
| Days to maturity            | 1                | .076         | -.136          | -.100          | -.032                            | .117                 | -.177               | -.070              | .029              |
| Plant Height                |                  |              | .111           | .173           | .221*                            | .170                 | .170                | .039               | -.093             |
| No. of panicle              |                  |              | 1              | -.025          | .038                             | .086                 | .361**              | .140               | -.113             |
| Panicle length              |                  |              |                |                |                                  | .066                 |                     |                    |                   |
| No. of filled grains per panicle |            |              |                |                |                                  | .066                 |                     |                    |                   |
| 1000 seed weight (g)        |                  |              |                |                |                                  | .066                 |                     |                    |                   |
| Yield per plant (gm)        |                  |              |                |                |                                  | .066                 |                     |                    |                   |
| Zinc content (ppm)          |                  |              |                |                |                                  | .066                 |                     |                    |                   |
| Iron content (ppm)          |                  |              |                |                |                                  | .066                 |                     |                    |                   |

* Significant at 5% level,**Significant at 1% level.

significant positive correlation was observed between grain zinc content and iron content indicating that improvement of iron and zinc content in rice can be attempted separately. In other words, high iron content in rice grain would not result in high zinc content. Yield was not also significantly correlated with iron and zinc in grain (Table 2). Anuradha et al. (2012) reported similar findings in rice. This result signifies that zinc and iron content of grain have no impact in yield and yield attributing traits, so that enhancement of micronutrient content does not result in the enhancement of yield in rice grain or in other words breeding for high iron or zinc can be attempted in high yielding lines.

Genetic divergence

The dendrogram was constructed using the software NTSYS Pc (Ver. 2.20) based on morphological traits and Fe and Zn content to assess divergence. The genotypes were grouped into 3 main clusters (Fig 3). More number of genotypes were in cluster I which composed of five sub-clusters. Cluster II consisted of 41 genotypes dividing into five sub-clusters. Satabdi, one of the parents of RILs grouped to cluster I. Similarly, Cluster II consisted of 41 genotypes with four sub clusters and Lemont i.e another parent of the RILs was in to the cluster II. Cluster III consisted of less number of genotypes i.e. 21 with three sub clusters which occupied intermediate position between two parents. Highest inter-cluster distance was observed between clusters I and III (100.21) suggesting wide diversity between these clusters.

Variability in quality trait

Some selected elite recombinant inbred lines (Table 3) with high zinc, iron and yield, were analysed for quality traits namely amylose content, gel consistency, alkali spreading value gelatinization temperature and elongation ratio to determine improved line in the breeding programs. Quality characteristics of various rice RILs are depicted in Table 4.
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Table 4: Mean performance of some selected promising Recombinant Inbred lines for different cooking quality traits.

| Genotypes | AC %  | GC    | ASV  | GT          | KLBC | KBBC | L/B Ratio | KLAC | ER  |
|-----------|-------|-------|------|-------------|------|------|-----------|------|-----|
| Line 57   | 21.88 | 53.67 | 4.33 | Intermediate| 6.00 | 1.99 | 3.02      | 10.67| 1.78|
| Line 97   | 25.59 | 41.67 | 4.33 | High Intermediate| 6.27 | 2.07 | 3.03      | 13.33| 2.13|
| Line 120  | 18.94 | 52.22 | 3.33 | High Intermediate| 7.33 | 2.33 | 3.14      | 11.00| 1.50|
| Line 99   | 16.52 | 68.11 | 3.22 | High Intermediate| 6.60 | 2.03 | 3.25      | 12.56| 1.90|
| Line 48   | 20.24 | 46.00 | 3.86 | Intermediate| 6.07 | 2.20 | 2.76      | 10.00| 1.65|
| Line 124  | 15.16 | 92.00 | 2.40 | High Intermediate| 6.60 | 2.17 | 3.05      | 11.83| 1.79|
| Line 24   | 21.09 | 46.44 | 3.50 | High Intermediate| 6.50 | 1.87 | 3.48      | 9.33 | 1.44|
| Line 9    | 21.59 | 60.22 | 4.67 | High Intermediate| 6.07 | 2.43 | 2.49      | 10.67| 1.76|
| Line 6    | 20.79 | 54.00 | 4.33 | Intermediate| 6.00 | 2.37 | 2.54      | 9.33 | 1.56|
| Line 23   | 21.42 | 56.00 | 3.83 | High Intermediate| 7.03 | 2.37 | 2.97      | 9.33 | 1.33|
| Line 29   | 18.60 | 65.67 | 2.17 | High            | 6.50 | 1.50 | 4.33      | 9.67 | 1.49|
| Line 125  | 20.57 | 51.67 | 2.33 | High            | 6.50 | 2.03 | 3.20      | 8.33 | 1.28|
| Line 35   | 18.99 | 68.56 | 2.92 | High Intermediate| 6.07 | 1.90 | 3.19      | 10.17| 1.68|
| Line 105  | 15.41 | 76.67 | 5.00 | Intermediate    | 6.27 | 2.27 | 2.76      | 8.83 | 1.41|
| Line 16   | 17.63 | 65.67 | 2.93 | High Intermediate| 6.10 | 2.07 | 2.95      | 13.00| 2.13|
| Line 103  | 17.10 | 70.33 | 4.67 | Intermediate    | 6.93 | 2.10 | 3.30      | 10.33| 1.49|
| Line 58   | 15.64 | 79.78 | 4.33 | Intermediate    | 6.00 | 2.47 | 2.43      | 11.17| 1.86|
| Line 114  | 17.98 | 64.00 | 4.30 | Intermediate    | 6.03 | 2.27 | 2.66      | 8.67 | 1.44|
| Line 66   | 12.25 | 80.00 | 2.13 | Intermediate    | 7.10 | 1.73 | 4.10      | 11.33| 1.60|
| Line 75   | 15.13 | 65.67 | 2.33 | High            | 7.10 | 2.07 | 3.44      | 10.17| 1.43|
| Lemont    | 17.19 | 76.33 | 3.50 | High Intermediate| 5.83 | 1.83 | 3.25      | 10.72| 1.84|
| Satadb1   | 21.55 | 65   | 3.46 | High Intermediate| 6   | 1.67 | 3.67      | 10.14| 1.69|

AC= Amylose content %, GC= Gel Consistency, ASV= Alkali spreading value, GT= Gelatinization temperature, KLBC= Kernel length before cooking, KBBC= Kernel breadth before cooking, L/B= Length/breadth ratio, KLAC= Kernel length after cooking, ER= Kernel elongation ratio.

Fig 1: Bar graph depicting the comparison of Amylose content % (AC), Gel Consistency (GC), Alkali spreading value (ASV).

Fig 2: Comparison of kernel length before cooking (KLBC), kernel breadth before cooking (KBBC), length/breadth ratio, kernel length after cooking (KLAC), kernel elongation ratio (ER).
Chemical properties like amylose content, gel consistency and gelatinization temperature (GT) are also three important characters that influence eating parameters and highly dependent on amount of starch present in grain. There was significant difference found in the amylose content of different RILs. The variation of amylose content obtained in RIL was in the range of 12.25-25.59%.

The maximum amylose content derived in the present study was in line 97 which constituted cluster I with parent Satabdi (Ac content- 21.55%), a widely popular check indica type variety on the contrary minimum % of amylose content was present in line 66 belonging to cluster II with parent Lemont which a tropical japonica variety. Amylose is the linear fraction of starch prevalent, in the non-glutinous varieties. It is relatively resistant to digestion (Oko et al., 2012) and determines the hardness or softness, cohesiveness, tenderness, colour of cooked rice. Intermediate amylose content (20-25%) is favoured by Indian consumers. Similar to our study, Nirmala devi et al. 2009 earlier reported that the AC of rice ranged between 14.06 - 27.82.

Gel consistency measures gel viscosity of milled rice i.e. tendency of the cooked rice to get harden after cooling. Lower gel consistency is associated with harder cooked rice and is evident in high-amylose rice. In this experiment, gel consistency ranged from 41.67 to 92%. Maximum genotypes belonged to more than 60% range and were categorized as soft. This indicates the tendency of cooked rice of the studied materials were mostly soft on cooling.

Varetil distinctions are clearly obtained by ASV test,
Correlation between different quality parameters

There was significant and positive correlation found between some cooking parameters (Table 5). A significantly negative correlation was observed between amylose content with gel consistency suggesting that those varieties with high amylose content will result in hard and shorter length of gel than varieties with low amylose content due to retrogradation behavior of amylose during the cooling of gel (Rani et al., 2006). The same finding was reported earlier by Khatun et al., 2003. The varieties with low gelatinization temperature and high ASV generally had high amylose content. So, elongation ratio and alkali spreading value amylose content has a very significant positive correlation. The positive significant relationship between amylose and ASV is also reported by Jennings et al. (1979) and Nayak et al. (2003). Most of the physico-chemical characteristics were significantly correlated with each other which indicate that efforts aimed at selecting rice varieties with improved cooking quality traits would warrant a consider good quality of the rice grain. In addition to that, the yield parameter is the most vital concern in any study which is a complex character. It was found that different quality parameters were positively correlated with yield, but none of these had any significance. It may suggest that quality parameters had less influence on improving yield potential but quality rice with high yield potential will always attract the premium customers and market as well. So, both the traits can be improved concurrently for a successful future breeding program.

**CONCLUSION**

The present investigation showed the wide variability in recombinant inbred lines of Lemont X Satabdi, in iron zinc content with yield attributing and quality traits. Some superior lines were identified namely line 57, 97, 120, 48, 99, 124 those carried high Fe content in grain more than 30 ppm and lines 24, 6, 9, 23, 29, 125 were high Zn containing lines with more than 50 ppm. It was also found that high Fe containing lines in grains also possessed high zinc content though the reverse was not true. The range of variation of zinc in rice grain was more than Fe content in grain. The high yielding lines containing high zinc content along with moderately high Fe can be advised for multi locational trials for further confirmation of performance in different agro-climatic zones. Interestingly the lines 9, 6, 48 and 57 were recorded to be high yielding which performed better yield or as per with the check variety Satabdi (24.28 gm) with high zinc and Fe content in grain and also possessed all the good quality traits. So these varieties may be used in further improvement in quality attributes for varietal development program and popularized among the farmers.

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**REFERENCES**

Ahman, E., Allen, H., Beaton, G., Benoist, B., Flores, B., Gilespe, S., Robeneck, S. and Viteri, F. (2000). Nutrition through the life cycle-4th Report on the World Nutrition Situation: ACC/SCN in collaboration with IFPRI, Geneva, Switzerland, 23-27.

Anuradha, K., Agarwal, S., Batchu, A.K., Babu, A.P., Swamy, B.P.M., Longvah, T. and Sarla, N. (2012). Evaluating rice germplasm for iron and zinc concentration in brown rice and seed dimensions. Journal of Phytopathology, 4(1): 19-25.

Azeex, M.A. and Shafi, M. (1966). Quality in rice. Department of

| Zinc  | Iron  | AC    | GC    | ER    | ASV  | Yield |
|-------|-------|-------|-------|-------|------|-------|
| .098  | -.145 | .044  | .061  | -.405 | .026 | 1     |

* Significant at the 5% level.

** Significant at the 1% level.
