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# Phenotyping and Association Analysis of Grain Zinc and Iron Content with Seed Yield in Diverse Local Germplasm of Rice

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## ABSTRACT

**Background:** Rice (*Oryza sativa* L.) feeds more than half of the world population, but it is a poor source of zinc (Zn) and iron (Fe). Therefore, there is a need for Zn and Fe- biofortified rice in the food chain. For this, rice breeding needs to be re-oriented to improve the status of grain Zn and Fe content while increasing the yield potential. Identification of micronutrient-rich donors and their association study with agro-economic traits can pave the way for nutritional and food security.

**Methods:** Zinc and iron content of a set of 92 rice genotypes was estimated in the aliquot of seed extract by using an Inductive Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) to explore donors and to study the degree of association of these micronutrients with agro-economic traits including grain quality traits and seed yield.

**Result:** The top Fe (≥50 ppm) and Zn dense (≥40 ppm) genotypes identified were P44 mutant selection-1, ORCZ 75-3-1, Basudha, Malliphulajhuli, Tikimahsuri and Nikipankhia. P44 mutant Sel.-1 and ORCZ 75-3-1 had good yield potential (44 q $ha^{-1}$). Grains/panicles and the number of effective bearing tillers/hill maintained an appreciably strong positive association with seed yield, while plant height had an inverse relationship. Grain Fe content positively correlated with panicle length. On the other hand, Zn is positively associated with tillering ability and grains/panicle. Grain Fe and Zn revealed strong a *inter se* positive association. Interestingly, grain Fe revealed no association, but grain Zn revealed significant positive relationship with seed yield. This envisaged better scope for genetic enhancement of grain Zn content along with substantial increase in grain Fe without any yield penalty.

**Key words:** Association analysis, Biofortification, Grain iron and zinc content, Rice (*Oryza sativa* L.), Seed yield.

## INTRODUCTION

Rice (*Oryza sativa* L.) is the most important cereal; cooked and consumed as whole grain or other forms by more than half of the world population. But, rice is deficient in a variety of micronutrients including zinc and iron. With the alarming situation of malnutrition, biofortification for micronutrients has become the mainstream breeding programme in the last two decades. In contrast to agronomic fertigation strategy (Barua and Saikia, 2018), biofortification is a sustainable genetic approach (Bouis, 2002) that aims at the genetic enrichment of foodstuffs with vital nutrients (Singh *et al.,* 2017) required for human health. Genetic architectures determining grain iron and zinc content are highly complex and both have appreciably high genotype × environment (G × E) interaction which hinders progress in the development of stable biofortified rice (Naik *et al*., 2020). There is wide variation in iron (6.0-72.0 ppm) (Neelamraju *et al*., 2012) and zinc content (14.0-40.0 ppm) (Martinez *et al*., 2006) in brown rice suggesting tremendous scope for the enrichment of these micronutrients in rice grains. Studying of genetic variation to explore truly exploitable nutrient-dense stable donors as parents and assessing the relationship of iron and zinc with morpho-agronomic traits including seed yield are the initial vital steps to start any biofortification programme. Therefore, in the current study, an attempt was made to identify elite nutrient rich (Fe and Zn) donors and to study the association of grain iron and zinc content with seed yield and ancillary traits in a set of local landraces and high yielding rice genotypes of Odisha (India).

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## MATERIALS AND METHODS

The experimental materials used in the present investigation comprised 92 test genotypes including 54 local landraces of Odisha, 21 improved OUAT and NRRI breeding lines and 17 released varieties of rice. These test entries were transplanted (3-4 seedling per hill after 25 days of sowing) in the field (*Kharif*, 2019) at the Regional Research and Technology Transfer Station, Bhubaneswar located at an altitude of 45 m above sea level (latitude 20.26 and longitude 85.81). The soil was a clay loam type with average pH of 5.8, iron 455 ppm and Zn 0.55 ppm recorded before transplanting. The experiment was laid out in randomized block design (RBD) with three replications at spacing of 20 × 10 cm. The crop was raised following recommended dose of fertilizer (80-40-40 N, $P_2O_5$, $K_2O$ kg $ha^{-1}$), intercultural operations and plant protection measures against diseases and pests.

Observations were recorded on seven agro-morphological traits including seed yield and nine quality traits including zinc and iron content of brown rice. Basing on grain length and grain length (L)/Breadth(B) ratio, rice genotypes were classified into seven grain types as per Govindaswamy (1985). Fine ground samples of brown rice of each of the genotypes in three replicates were digested by di-acid mixture of nitric acid (HNO3): and perchloric acid ($HClO_4$) as per Jahan *et al.* (2013). Fe and Zn content were estimated using Inductive Coupled Plasma-Optical Emission Spectrophotometer (ICP-OES) at 238.2nm and 206.2nm wavelength respectively (Fig 1) at Central Instrumentation Facility (CIF), OUAT, Bhubaneswar.

Analysis of variance and covariance were done as per Singh and Choudhury (1985) using sample means of various traits under study. Estimates of the correlation coefficient for each pair of characters were computed following as per Al-Jibouri *et al.* (1958) and the significance of correlation coefficients was tested by the t- test at n -2 degrees of freedom.

## RESULTS AND DISCUSSION

### Micronutrient content and seed yield

Modern high yielding rice varieties are deficient in Fe and Zn. The breeding target is approximately fixed at 13-15 ppm for iron (Bouis *et al.,* 2011) and 28-30 ppm for zinc (Shahzad *et al.*, 2014) biofortification for polished rice. About 57% of iron and 40% of Zn are lossed during milling and polishing of rice grains (Hansen *et al.*, 2009). In the present study, grain Fe and Zn content ranged from 8.3-52.15 ppm and .3.0-52.7 ppm respectively in brown rice. Most of the local landraces showed rich source of above minerals as Anandan *et al.* (2011) also reported. The top Fe dense (≥40 ppm) genotypes identified were Tikimahsuri (52.15 ppm), Jabaphulla (52.15 ppm), Kala Kusuna (52.1 ppm), OR CZ 75-3-1(51.95 ppm), P 44 mutant selection-1(51.9 ppm), CR 2327-23(51.4 ppm), Budhidhan (51.15ppm) and Kalamakhi (50.15 ppm) (Fig 2). Interestingly, Basudha, Malliphulajhuli, Tikimahsuri and Nikipankhia also revealed higher grain Zn content (>40.0 ppm) in addition to iron. These lowland land races recorded very low seed yield (20.2-27.9 q $ha^{-1}$) except Nikipankhia (34.0 qtl $ha^{-1}$). Some landraces (Roy and Sharma, 2014), basmati types (Brar *et al.*, 2011) and wild rice (Banerjee *et al.*, 2010) are also reported to retain high grain Fe and Zn content but japonica rice harbour the least (Anuradha *et al.*, 2012a). The iron and zinc dense genotypes identified in this study may serve as potential donors for biofortification in rice.

### Inter-relationship of morpho-agronomic traits with seed yield

Grains/panicle and effective bearing tillers(EBT)/hill followed by panicle length and days to maturity exhibited positive significance relationship with seed yield but plant height maintained inverse relationship with tillers per plant (0.555**), grains per panicle (0.440**) and seed yield (-0.529**) and indicating the fact that semi-dwarf plant types are associated to high tillering and higher number of grains per panicle leading to perform better for seed yield than intermediate to tall plant types (Table 1). This corroborates the findings of Nagesh *et al.* (2013) and Bekele *et al.*, (2013). Besides, panicle length associated favourably with ear bearing tillers, grains per panicle and grain weight as also reported by Sri Devi *et al*. (2019). Thus, such desirable panicle feature is likely to result high productivity.

### Inter se relationship with physical quality traits

Yield has shown positive correlation with grain length, grain length/breadth, grain type, kernel length, kernel length/ breadth and zinc content. But productivity was shown to be negatively associated with kernel breadth (Table 1). This indicates that increase in yield in rice may be achieved by selecting long grain with slender kernel types. Besides, kernel length was shown to have a favourable association with panicle length, effective bearing tillers/hill and grains/ panicle indicating the possibility of increasing productivity through correlated selection response based on the above component traits. Chouhan (1996) reported significant positive association of kernel length and kernel L/B ratio but a negative association with kernel breadth.

### Correlation with grain Fe and Zn content

Grain iron and Zinc content determine the success of biofortification in rice. The present study revealed no relationship between grain iron content and seed yield but grain zinc content maintained a significant positive

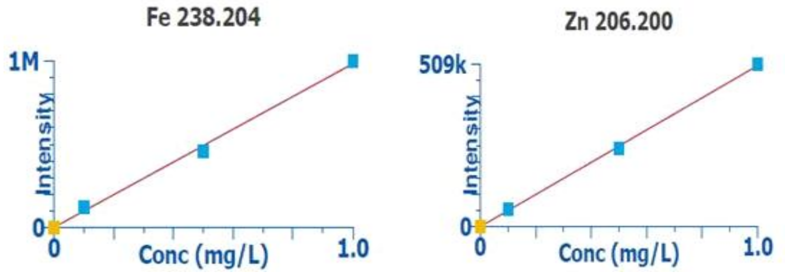
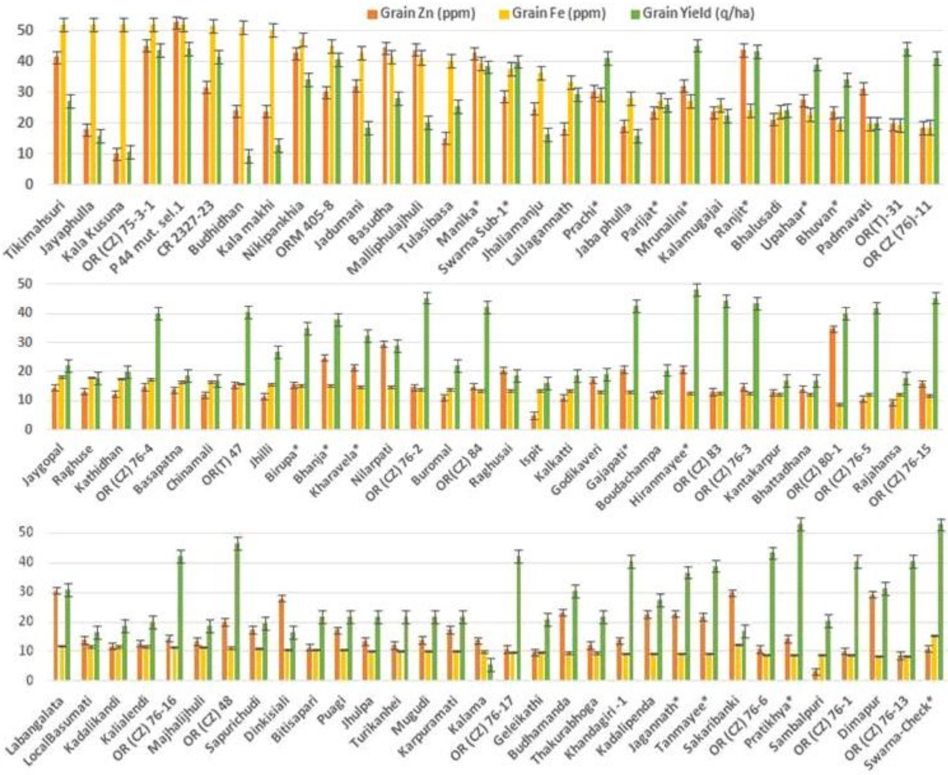



**Fig 1:** Calibration of standard curve for grain Fe and Zn content using ICP-OES.




*-Released rice varieties, OR and CR code-OUAT and NRRI breeding lines, Rest are all local landraces of Odisha

**Fig 2.** Grain yield and micronutrient content (of brown rice) of a set of 92 diverse germplasm.

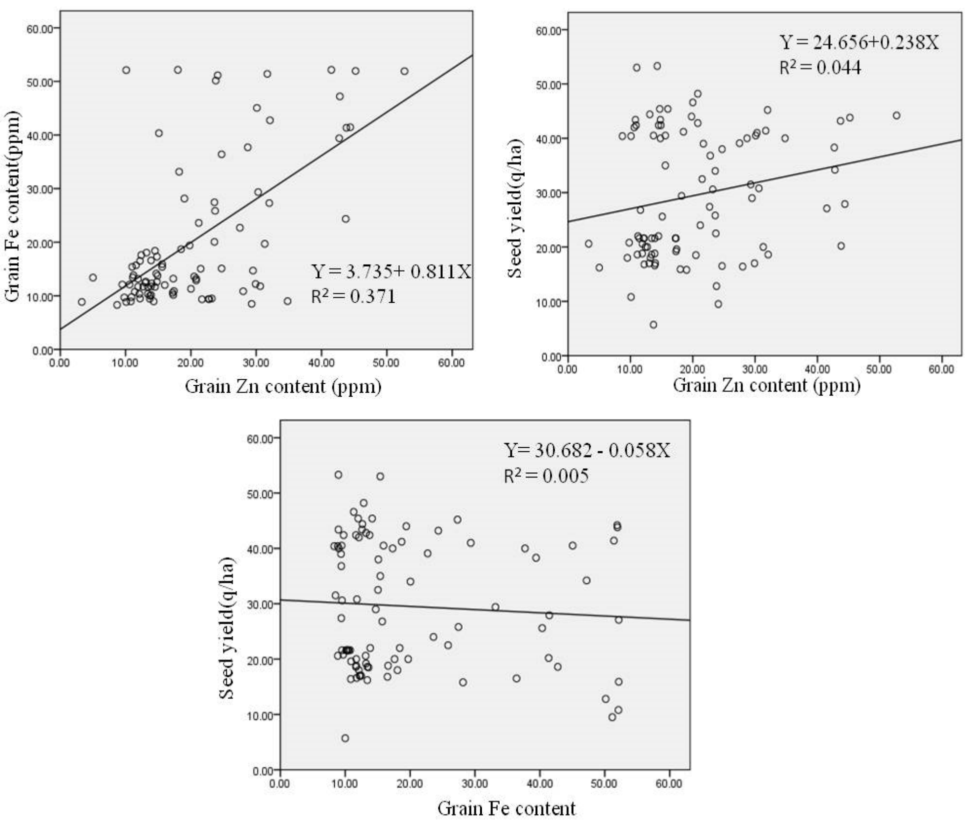



**Fig 3:** Relationship of grain Zn, grain Fe content and seed yield in rice.

**Table 1:** Genetic relationship among agro-economic traits and quality features and micronutrient content in rice.

| Characters | Days to maturity | Plant height (cm) | Panicle length (cm) | EBT /hill | Grains /panicle | 1000-GW (g) | GL (mm) | GB (mm) | GL /GB | Grain type score | KL (mm) | KB (mm) | KL /KB | Zn (mg/kg) | Fe (mg/kg) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Plant height | 0.040 | | | | | | | | | | | | | | |
| Panicle length | 0.070 | 0.077 | | | | | | | | | | | | | |
| EBT/m$^2$ | 0.035 | -0.555** | 0.317** | | | | | | | | | | | | |
| Grains/panicle | 0.236* | -0.440** | 0.468** | 0.595** | | | | | | | | | | | |
| Grain weight | 0-.112 | 0.148 | 0.290** | -0.067 | -0.102 | | | | | | | | | | |
| Grain length | -0.139 | -0.150 | 0.108 | 0.156 | 0.311** | -0.149 | | | | | | | | | |
| Grain breadth | 0.048 | 0.133 | -0.174 | -0.105 | -0.169 | 0.071 | -0.188 | | | | | | | | |
| Grain length/Grain breadth | -0.146 | -0.191 | 0.184 | 0.151 | 0.368** | -0.135 | 0.815** | -0.558** | | | | | | | |
| Grain type | -0.153 | -0.155 | 0.218* | 0.059 | 0.328** | -0.121 | 0.755** | -0.502** | 0.882** | | | | | | |
| Kernel length | -0.073 | -0.175 | 0.210* | 0.215* | 0.285** | -0.118 | 0.830** | -0.181 | 0.638** | 0.588** | | | | | |
| Kernel breadth | -0.030 | 0.226* | -0.242* | -0.171 | -0.359** | -0.056 | -0.303** | 0.634** | -0.631** | -0.589** | -0.226* | | | | |
| Kernel length/Kernel breadth | -0.025 | -0.267** | 0.291** | 0.241* | 0.441** | -0.043 | 0.728** | -0.541** | 0.850** | 0.781** | 0.746** | -0.798** | | | |
| Zn content | 0.149 | -0.310** | 0.192 | 0.290** | 0.250* | -0.013 | -0.192 | -0.056 | -0.157 | -0.052 | -0.016 | -0.067 | 0.013 | | |
| Fe content | -0.052 | -0.136 | 0.216* | 0.107 | 0.004 | 0.057 | -.204 | -0.117 | -0.057 | -0.027 | -0.071 | -0.127 | 0.012 | 0.609** | |
| Seed yield/plant | 0.226* | -0.529** | 0.403** | 0.705** | 0.894** | -0.115 | .386** | -0.145 | 0.405** | 0.320** | 0.347** | -0.369** | 0.481** | 0.209* | -0.068 |

relationship with seed yield (Table 1) indicating the possibility of the role of Zn than Fe for the increase in sink size in rice. Linear regression analysis shown in Fig 3 further confirms such relationship. This supports the findings of Ajmera *et al.* (2017) and Sri Devi *et al.* (2019) though other workers opined inverse relationship (Shivani *et al.*, 2019 and Inabangan-Asilo *et al.*, 2019) or even no association (Pandey *et al.*, 2018) of both Fe and Zn with seed yield. However, Moreno-Moyano *et al.* (2016) made it clear that over-accumulation beyond 23 µg Fe/g and 40µg Zn/g of rice grain (in os-NAS/IR 64 transgenic progenies) had significant negative impact in a number of agro-morphological traits affecting grain yield.

Our study revealed significant positive association of grain Fe- content with zinc (r=0.609) possibly due to co-location of QTLs for Fe and Zn in chromosome 7 and chromosome 12 in brown rice (Anuradha *et al.*, 2012b). This corroborates the findings of Maganti *et al.* (2019) and Inabangan-Asilo *et al.*(2019). The linear regression shown in Fig 2 further confirms such strong relationship between the above micronutrients. This means that the varieties which have efficient transport of zinc would have also higher translocation of iron and loading of the same in the grain. However, no such strong relationship can be realized between these trace elements in polished rice (Sala *et al.*, 2013) possibly due to loss of the major fraction of Fe during milling compared to Zinc, as rice bran contains 55% and 34% Fe and Zn respectively.

Grain zinc content positively correlated with effective bearing tillers and grains/panicle which might be a cause for its erstwhile mentioned positive significant association with seed yield. On the other hand, iron content was shown to have significant positive correlation with panicle length indicating possibility of increasing iron content by selecting long panicle types. Grain and kernel dimension (length, breadth and length/breadth) revealed no correlation with either iron or zinc content indicating their no interference with genetic enhancement for Fe and Zn. In contrast, Anuradha *et al.* (2012a) revealed strong negative association of Zn concentration with grain elongation in a set of 126 germplasm lines and Bekele *et al.* (2013) showed positive correlation of grain Zn concentration with grain weight and grain length.

## CONCLUSION

Conventional breeding essentially exploits the available genetic variation present either in cultivated or wild germplasm. The high Zn and Fe donors identified in this investigation can serve as source of valuable QTLs and/or important candidate genes for biofortification breeding in rice. Maturity duration, panicle length, tiller number and grains/panicle had significant positive association with seed yield but plant height had inverse relationship with yield. Grain Fe content revealed no association but grain zinc maintained significant positive relationship with seed yield.

Since, grain Fe and Zn has inherent strong *inter se* positive association; selection of high Zn plants may be emphasized which would result simultaneous increase in grain Fe without any yield penalty.

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