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# Characterizing the diversity of grain phytic acid content in Indian rice landraces by multivariate genetic analyses

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**Abstract**

**Phytic acid (PA) acts as chelator of cationic mineral elements iron (Fe) and zinc (Zn) and obstructs their absorption in the human gut. We have evaluated a set of 162 traditional rice landraces for phytic acid phosphorus (PA-P), inorganic phosphorus (Pi) and total phosphorus (TP). Wide variability was observed for PA-P, Pi and TP ranging from 1.12 to 3 mg/g, 0.004 to 0.16 mg/g and 1.17 to 3.04 mg/g respectively. The mineral micronutrients, Fe and Zn were not in correlation with PA-P, Pi and TP implicating the possibility of their independent improvement while PA-P showed a significant positive correlation with TP and significant negative correlation with Pi. Principal component analysis (PCA) identified two principal components PC1 and PC2, explaining 50.6 % and 32.1 % of the total variation, respectively. Cluster analysis grouped the accessions into four clusters. The study has also led to the identification of promising donors such as P1490 and Gowri with low PA content to be utilized in rice biofortification programmes.**

**Key words**: Phytic acid, rice, multivariate analysis, PCA, cluster analysis

## Introduction

Micronutrients are vital for efficient functioning of all the metabolic activities as well as maintaining good health. In the present scenario, micronutrient deficiency has become a major cause for hidden hunger due to inadequate consumption of diets rich in essential minerals and vitamins (White and Broadley 2009). This situation is grimmer in the developing countries where rice-based diet is the major source of the daily calorific requirement of the human body (Kennedy et al. 2003). Moreover, rice is consumed after polishing which removes the essential vitamins and minerals present in the aleurone layer removed in the process of milling. Almost 90% of the grain iron (Fe) and zinc (Zn) are primarily localized in the aleurone layer and therefore removal of aleurone in milling process leaves the grain with very low minerals and vitamins leading to micronutrient deficiency (Perera et al. 2018).

Fe is important component for the synthesis of haemoglobin and myoglobin proteins essential for the oxygen transportation in the body through blood. It is also an important component of enzymes involved in energy metabolism and maintenance of immune system functions (Roeser 1986; Stoltzfus 2001) while Zn acts as a cofactor of various enzymes and performs various biological functions like cell division, cell development, immunity, reproduction and gene expression (Brown et al. 2004). Fe and Zn are commonly viewed together in human nutrition as they are available through common dietary sources and their absorption as well as inhibition is also affected by the similar factors (Lim et al. 2013). Fe deficiency leads to numerous health disorders like high risk of maternal mortality, anaemia, premature child birth, low birth weight, improper cognitive and motor development (Bouis et al. 2003). Whereas Zn deficiency leads to short stature, skeletal abnormality, immunological dysfunction, anorexia, skin disorder, delayed wound healing and hypogonadism (Prasad 1991). Brown rice consumption can overcome the problem of micronutrient deficiency as it is nutritionally superior to white rice (milled rice) (Saleh et al. 2019). It not only has higher Fe and Zn content but also has other essential mineral elements like calcium (Ca),

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manganese (Mn), magnesium (Mg), phosphorus (P), potassium (K) etc. (Saleh et al. 2019). Nonetheless the major hindrance for consumption of brown rice owes to the presence of an anti-nutritional factor phytic acid (PA) chemically known as myo-inositol 1,2,3,4,5,6-hexakisphosphate (Gyani et al. 2020). PA is the major storage form of P in most cereal grains (Basak et al. 2017) and makes up almost 7% of the dry weight or alternatively amounts to 70% of the total stored P in the kernel (Perera et al. 2018). As PA has high negative charge, it chelates multivalent cationic metal ions especially Fe and Zn and makes them insoluble salts which are not absorbed by the gastrointestinal tract, leading to poor bioavailability of these micronutrient metal ions (Graham et al. 2001). Hence it is highly desirable to reduce the seed PA content in order to enhance micro-nutrient bioavailability. Since low PA is genetically governed, identification of sources conferring low seed PA content is essential for crop breeding. However, not many studies are available depicting the PA variability in Indian rice germplasm, particularly aimed at identifying sources for low grain PA content. Hence, in the present study, we have assessed about 162 rice accessions from different parts of India for the PA, inorganic P (Pi), total P (TP), to evaluate the pattern of genetic variability.

## Material and methods

A set of 162 genotypes was used in the evaluation. The genotypes are coded as GP1 to GP162 serially as per details given in the Supplementary Table S1.

**Table 1.** Descriptive statistics for PA-P, Pi, TP, Zn and Fe contentsbased on 162 rice accessions

| Variable | Min. | Max. | Mean | SD | CV % |
|---|---|---|---|---|---|
| PA-P | 1.12 | 3.00 | 2.80 | 0.26 | 9.23 |
| Pi | 0.00 | 0.16 | 0.04 | 0.02 | 57.09 |
| TP | 1.17 | 3.04 | 2.84 | 0.25 | 8.70 |
| Fe | 6.50 | 23.10 | 12.65 | 3.16 | 24.96 |
| Zn | 13.60 | 38.20 | 22.61 | 5.02 | 22.19 |

PA-P = Phytic acid phosphorous in mg/g; Pi = Inorganic phosphorous in mg/g; TP = Total phosphorous in mg/g; Fe-BR = Fe content of brown rice in µg/g; Zn-BR = Zn content of brown rice in µg/g; SD = Standard deviation; CV = Coefficient of variation in %

The germplasm included local landraces collected from various places across the country, which are being maintained in the Division of Genetics, ICAR-Indian Agricultural Research Institute (ICAR-IARI), New Delhi. All the genotypes were evaluated at ICAR-IARI farm in *Kharif* season of 2018. The seeds of the germplasm were sown on a raised seedbed nursery and 28 days old seedlings were transplanted to the main field, where each genotype was sown in 2 rows of 3 m with a row to row and plant to plant spacing of 20 cm and 15 cm respectively. Recommended agronomic package of practices were followed to raise the seedlings till maturity. Seeds were harvested at maturity, threshed, dried and stored in zip lock bags at room temperature until analysis.

### *Estimation of Pi, PA-P, and TP*

For estimation of phytate and Pi content in seeds, the colorimetric assay given by Lorenz et al. 2007 was used. Ground brown rice seed samples of each genotype weighing 100 mg were put in 2 ml eppendorf tube and 2 ml of 0.65 M HCl was added to each tube. The sample tubes were shaken overnight in a shaker at 120 rpm and were centrifuged at 12000 rpm for 5 minutes.

To estimate PA and Pi, 500 µl of supernatant was transferred to both, a fresh 2 ml eppendorf tube and a 15 ml tube. Phytic acid dodeca-sodium salt was used as PA standard, whereas, $KH_2PO_4$ was used as Pi standard. Both the standards were used in equal volume for estimation. The Pi reagent was freshly prepared by mixing distilled water, ammonium molybdate, ascorbic acid and sulphuric acid in the ratio 2:1:1:1. Inorganic phosphorous was estimated by adding Pi reagent and distilled water in 1:1 ratio. The reaction was left at room temperature for 15 to 20 minutes to develop a blue color and subsequently its optical density was measured at 820 nm using spectrophotometer.

For PA estimation, 1.25 ml of Wade reagent was added to each tube and was left at room temperature for 15 to 20 minutes to develop brown color and subsequently their optical densities were measured at 420 nm using spectrophotometer. Wade reagent was prepared by mixing 0.03 g of ferric chloride, 0.3 g sulfosalicylic acid and 80 ml of distilled water and its pH was adjusted to 3.05 using NaOH on the following day. Finally, the volume was made up to 100 ml by adding distilled water. Phytate P was calculated by multiplying the estimated value of phytate by conversion factor 0.282, as the amount of PA-P present in PA is only 28.2% of PA (Raboy and Dickinson 1984). Whereas, total phosphorus was calculated by adding estimated values of PA-P and Pi.

Statistical analyses were performed using the software packages STAR 2.0.1 (IRRI 2014), R Studio v.1.1.453 and Microsoft Excel.

### *Estimation of genetic diversity*

Principal component analysis (PCA) was performed to obtain principal components (PC) encompassing total variation for PA-P, Pi, TP, Fe and Zn. The data for Fe and Zn was adopted from Bollinedi et al. 2020b. The correlation structure of the component traits was decomposed to components, which accounted for maximum variation progressively on a reducing scale. The components having eigenvalues exceeding one were identified as significant PCs. The factor coordinates of the genotypes were computed for the derived PCs. The factor-variable correlations (factor loadings) were used to compute the contribution of variables to individual PCs. The most influential traits were identified from their relative contribution to the first PC followed by second PC and so on. The contributions of genotypes to PCs were used to scatter them for identifying the genotypes associated with those variables determining the total variability in the data.

The factor coordinates for the genotypes were used for cluster analysis. Only PCs accounting up to 99% of the cumulative variation were used for the clustering process. Clustering was done using K means clustering procedure, an unsupervised algorithm using a set of apriory K values and Euclidean distances. The optimum number of clusters was determined based on elbow method by plotting the inter-cluster deviation against the k-value and determining the lowest k value at which the inter-cluster deviation is minimized. For the K sets, the mean intra-cluster distance, D(k) of the genotypes from the respective cluster centroid was worked out. D(k) will drop to zero as the number of clusters equals to the number of genotypes. By plotting the deviations of D(k) between adjacent K [D'(k)], the K at which D'(k) showed the flattening trend (the elbow) was taken as the optimal K. Cluster statistics of the clusters identified, were worked out.

## Results

### *Descriptive statistics and association among the traits*

The grain PA-P, Pi and TP contents exhibited significant variation in rice germplasm collection (Table 1). Pi concentration varied from 0.00 (GP357) to 0.16 (GP262) mg/g with a mean of 0.04 ± 0.00 mg/g. PA-P content in BR varied from 1.12 mg/g (GP256) to 3 mg/g (GP237) with a mean of 2.8 ± 0.02 mg/g. Whereas, TP content ranged from 1.17 mg/g (GP256) to 3.04 mg/g (GP237) with a mean value of 2.84 ± 0.02 mg/g. The direction and magnitude of correlation among the traits studied is depicted in Fig. 1. Among the

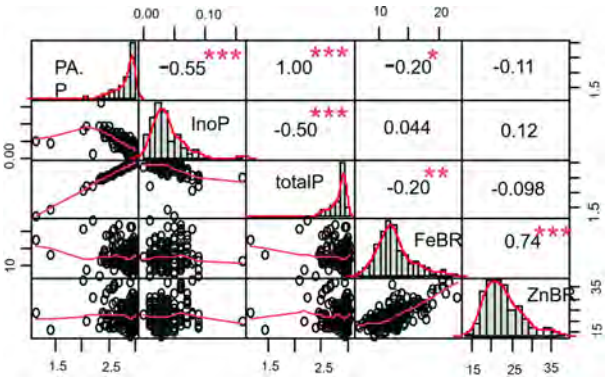


**Fig. 1. Correlogram of PA.P, Pi, TP, Fe and Zn from the rice germplasm set used in the study. The upper diagonal shows the correlation coefficients. The diagonal cells show the histogram of the traits. The nutritional parameters are Phytic acid phosphorous in μg/g (PA.P), inorganic phosphorous in mg/g (Pi), total phosphorous (TP), Fe content of brown rice in μg/g (Fe-BR); Zn content of brown rice in μg/g (Zn-BR). *, **, ***, correlation coefficients are significant at p<0.05, 0.01 and 0.001 respectively**

various parameters, PA-P showed a significant positive correlation with TP (r = 1.00; p < 0.01) and significant negative correlation with Pi (r = –0.55; p < 0.01). We observed absence of correlation between the mineral micronutrients, Fe and Zn with those of PA-P, Pi and TP.

### *Principal component analysis*

Principal component analysis was used to understand how PA-P, Pi, TP, Fe and Zn contributed to the total variability for these traits amongst the 162 rice accessions. The proportion of total variance explained by each PC, arranged in decreasing order of importance is presented in Table 2. The first PCs explained 82.7% of the total phenotypic variation among the accessions. The first PC explained 50.6% of phenotypic variance while the second PC explained 32.1%. Partitioning of eigenvalues of the significant PCs, the factor-variable correlations (factor loadings) indicated that PA-P and TP had positive influence on PC1, while Pi, Fe and Zn had negative influence on it. Whereas, all traits except Pi showed positive influence on PC2.

**Table 2.** Principal components (PCs) extracted for the grain quality traits based on the correlation structure and the factor-variable correlations (factor loadings)

| Parameters | PC1 | PC2 | PC3 | PC4 | PC5 |
|---|---|---|---|---|---|
| **Nutritional quality traits** | | | | | |
| Standard Deviation | 1.59 | 1.21 | 0.79 | 0.48 | 0.01 |
| Proportion of variance | 0.51 | 0.32 | 0.13 | 0.05 | 0.00 |
| Cumulative proportion (%) | 0.51 | 0.83 | 0.95 | 1.00 | 1.00 |
| Eigenvalue | **2.53** | **1.61** | 0.63 | 0.24 | 0.00 |
| **Factor loadings** | | | | | |
| PA-P | 0.93 | 0.29 | 0.22 | 0.04 | 0.01 |
| Pi | -0.68 | -0.26 | 0.68 | 0.10 | 0.00 |
| TP | 0.91 | 0.28 | 0.29 | 0.06 | -0.01 |
| Fe | -0.46 | 0.81 | -0.12 | 0.34 | 0.00 |
| Zn | -0.40 | 0.84 | 0.16 | -0.33 | 0.00 |

PA-P = Phytic acid phosphorous in mg/g; Pi = inorganic phosphorous in mg/g; TP = total phosphorous; Fe = Fe content of brown rice in µg/g; Zn = Zn content of brown rice in µg/g; Eigenvalues in boldface indicate most significant principal components

Eigenvectors, the coefficient of orthogonal transformation and the degree of influence of the variables towards the factors are given in Table 3. Vectors of variable contributions towards major PCs are given in Fig. 2. The contributions are the squared

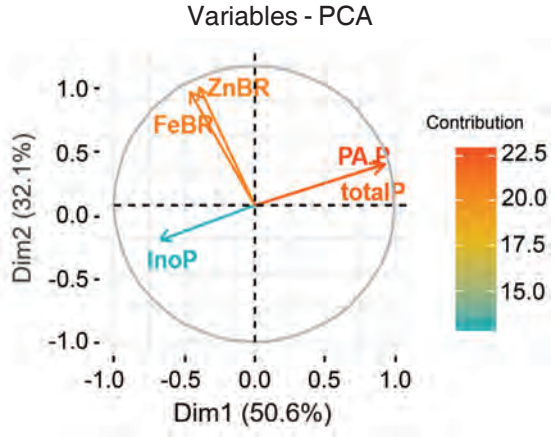


**Fig. 2. Vectors of variable contributions towards major principal components (PCs). The directions of the variable coordinates show the direction of their influence on PCs**

eigenvectors scaled 100 times to bring into % scale. Variable contributions for nutritional traits show high influence of PA-P, TP and Pi towards PC1 with values of 34.22 %, 33.03 % and 18.24 % respectively. Zn was the most contributing trait in PC2 explaining as high as 44.15 % of the variation encompassed by the

**Table 3.** Eigenvectors of the quality variables and their contributions towards the significant principal components

| Particulars | Eigen vectors | | Contribution (%) | |
|---|---|---|---|---|
| | PC1 | PC2 | PC1 | PC2 |
| PA-P | 0.59 | 0.23 | 34.22 | 5.23 |
| Pi | -0.43 | -0.21 | 18.24 | 4.36 |
| TP | 0.57 | 0.22 | 33.03 | 4.94 |
| Fe | -0.29 | 0.64 | 8.25 | 41.31 |
| Zn | -0.25 | 0.66 | 6.26 | 44.15 |

PA-P = Phytic acid phosphorous in mg/g; Pi = inorganic phosphorous in mg/g; TP = total phosphorous in mg/g; Fe = Fe content of brown rice in µg/g; Zn = Zn content of brown rice in µg/g; Contribution % = Eigenvector$^2$ x 100

PC2, while Fe contributed towards 41.31% of variation to the PC2. Similarly, the influence of genotypes on the PCs was determined from their PC scores, which was used to disperse them (Fig. 3). Here, P1490-03 (GP256) showed maximum influence on PC1, while Mehvan green (GP759) showed greater influence on PC2.

### *Cluster analysis and diversity*

Cluster statistics and diversity parameters obtained are given is Table 4. The elbow points based on the inter-cluster deviation grouped 162 genotypes into 4 clusters on the basis of these five traits (Fig. 4). Cluster 4 was the largest with 66 genotypes (40.74%) followed

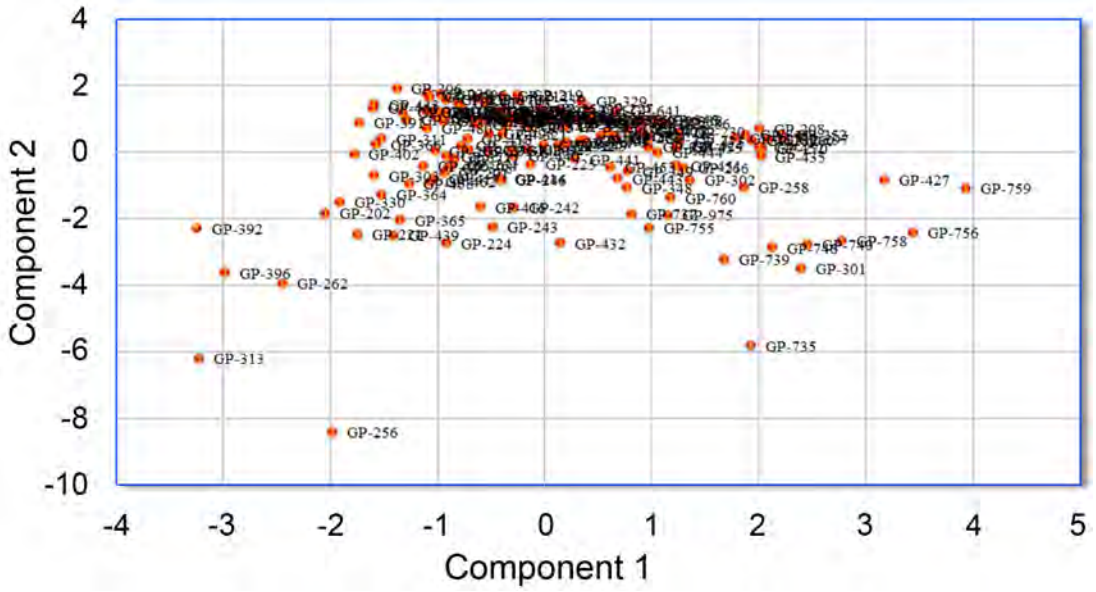


**Fig. 3. Dispersion of genotypes based on their contribution towards major principal components (PCs) for PA.P, Pi, TP, Fe and Zn content. The most divergent genotypes had extreme phenotypes for the most contributing traits towards the respective PCs**

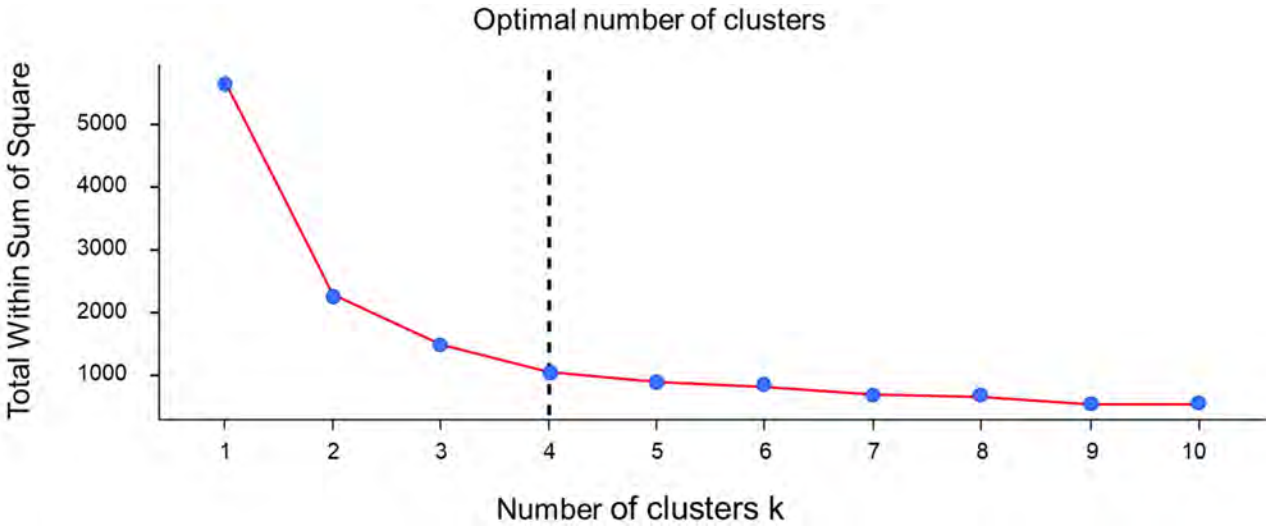


**Fig. 4. Determination of optimum clusters by K-means clustering using the elbow method**

by cluster 1 with 56 genotypes (34.57%), cluster 3 with 30 genotypes and cluster 2 with 10 genotypes (6.17%). Cluster 4 exhibited the highest average inter-cluster distance from cluster 1 (19.48). Comparing the cluster means, Cluster 4 exhibited the highest mean for Zn (34.88) followed by Fe (19.86) and PA-P (18.42) while the highest mean value for PA-P was observed in cluster 3 (2.85). The distribution of Pi to all the three clusters was almost similar. Cluster1 showed lowest mean for PA-P (2.55) while highest mean for Fe (19.86) and Zn (34.88).

Patterning of diversity of germplasm set for the traits, the distribution of diverse genotypes in the clusters was at a level of 0.88 evenness for these traits. However, Shannon-Weiner diversity index of these traits was 1.22.

## Discussion

The exploration of genetic variation present in germplasm is prerequisite before their utilization in the improvement of desirable traits during a breeding program. The rice accessions showed large genetic

**Table 4.** Cluster statistics and diversity indices for grain quality traits based on principal component scores of genotypes

| Particulars | Nutritional traits | | | |
|---|---|---|---|---|
| | Cluster1 | Cluster2 | Cluster3 | Cluster4 |
| No. of genotypes | 56 | 10 | 30 | 66 |
| Proportion% | 34.57 | 6.17 | 18.52 | 40.74 |
| Dissimilarity | $d_{(1-2)}$=8.40 | $d_{(2-3)}$=7.51 | $d_{(3-4)}$=7.68 | |
| | $d_{(1-3)}$=14.62 | $d_{(2-4)}$=11.81 | | |
| | $d_{(1-4)}$=19.48 | | | |
| Evenness | 0.88 | | | |
| Shannon-Weiner DI | 1.22 | | | |
| PA.P | 2.55 | 2.79 | 2.86 | 2.79 |
| InoP | 0.06 | 0.04 | 0.03 | 0.04 |
| totalP | 2.61 | 2.83 | 2.89 | 2.83 |
| FeBR | 19.86 | 15.36 | 12.40 | 10.53 |
| ZnBR | 34.88 | 27.80 | 22.84 | 18.20 |

PA-P = Phytic acid phosphorous in mg/g; Pi = inorganic phosphorous in mg/g; TP = total phosphorous in mg/g; Fe = Fe content of brown rice in µg/g; Zn = Zn content of brown rice in µg/g; DI = diversity index

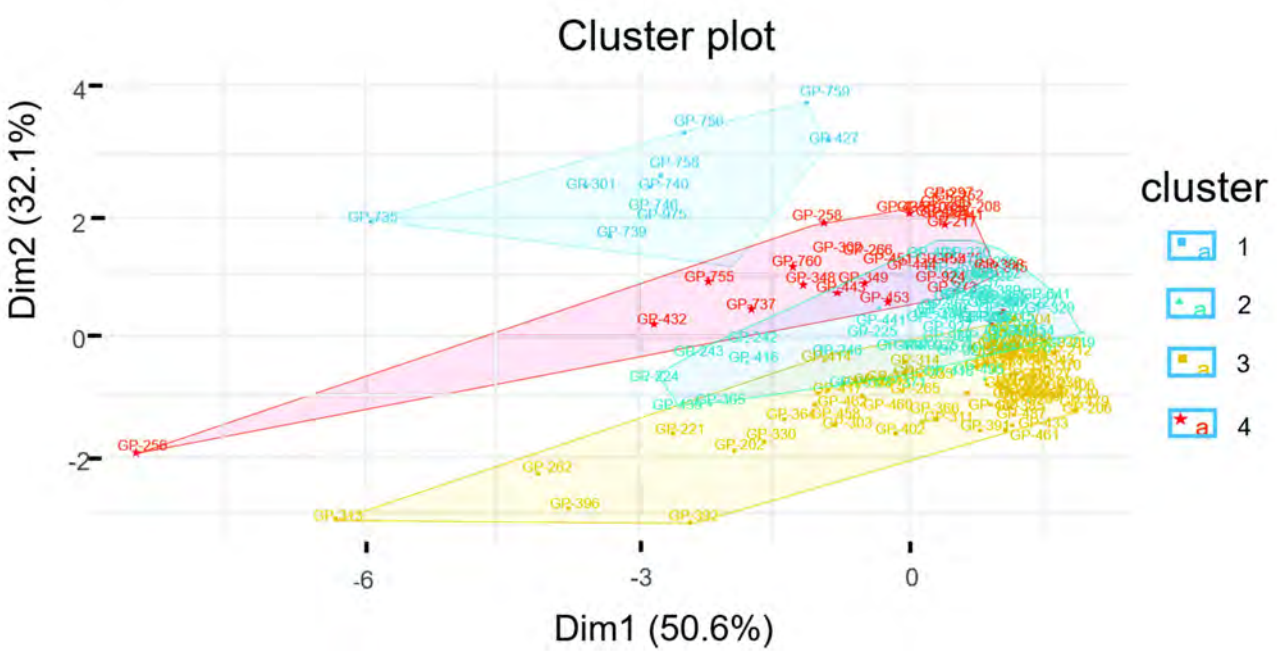


**Fig. 5. Cluster-wise distribution of genotypes for PA.P, Pi, TP, Fe and Zn content based on principal component analysis**

variation for PA-P, Pi, TP, Fe and Zn content pertaining to their wide geographic distribution and genotypic variation (Bollinedi et al. 2020). Significant variation for these traits was also observed in previous studies (Welch and Graham 2004). The genotypes showing high Fe and Zn content as well as low PA content such as GP 256 which could directly be used as parent in breeding program for transferring these traits into elite breeding lines.

Until now, there are only few reports on assessing the natural variation for low PA trait in rice (Liu et al.

2007; Wang et al. 2011; Lee et al. 2014). Effective and efficient utilization of the natural variation in rice germplasm using conventional and marker aided breeding will complement development of rice varieties with high bioavailability of Fe and Zn. In one study, phytic acid content in grains of 72 japonica rice cultivars ranged from 6.85 mg/g for Xiu 217 to 10.3 mg/g 103% for Huai 9746, with a mean of 8.3 mg/g (Liu et al. 2004). In another study, brown rice (BR) of 68 Chinese japonica cultivars were analyzed for PA by colorimetric method, whereas Fe and Zn content by the atomic absorption spectrophotometer, which could estimate PA content to the level of 12.6 mg/g while Fe and Zn having an average content of 7.4 ug/g and 19.1 ug/g respectively (Lee et al. 1997). Further, variation in 30 boro rice cultivars was observed for PA content ranging from 18.2 to 32.36 mg/g with a mean of 24.21 mg/g (Lee et al. 2014). In yet another study, Liang et al. 2007 found variation in Chinese rice cultivars ranging from 7.2 to 11.9 g/kg with a mean of 9.6 g/kg. The finding was comparable to Chinese japonica rice (6.9 to 10.3 g/kg; mean of 8.7 g/kg) and Korean rice (8.6 to 17.6 g/kg; mean of 12.6 g/kg) (Lee et al. 1997; Liu et al. 2005; Liang et al. 2007). However, in six japonica rice genotypes showed variation for PA in BR ranging from 3.68 to 5.07 mg/g (Su et al. 2014), whereas, three indica rice cultivars showed PA content from 3.99 mg/g to 7.34 mg/g (Wang et al. 2011). More recently, variation in the PA contents of 69 WRC (World Rice Core collection) accessions found to be in the range of 8.24-17.41 mg/g (Perera et al. 2019). In the present study a high variation for PA and TP was found ranging from 3.98 to 10.65 mg/g. the study has identified the genotypes. P1490-03 (GP 256), Gouri (GP 313), with low PA that can be effectively utilized as donors in biofortification programmes for enhancing the mineral bio-availability 3.98 to 4.97 mg/g.

The study depicted wide variation for PA and TP content which is a reflection on the effect of various allelic combinations of the genes governing PA content in rice. Though previous studies reported few QTLs affecting PA in rice grain, a comprehensive molecular mechanism underlying PA content still remains elusive. The variations in PA and TP content observed in the accessions used in the study indicated that this germplasm set constituted a valuable genetic resources for allele mining studies to identify various allelic variants at different loci. It is pertinent here to emphasize that the genotypic constituents in the present assembly included several landraces those are seldom cultivated on commercial scale.

Correlation analysis among the phenotypic traits is useful for understanding their relationships. Studies showing high positive correlation of Zn with PA-P content ($r = 0.5$; $<0.01$) (Liang el al. 2007) to no correlation between PA and mineral elements (Su et al. 2014; Perera et al. 2019) have been reported earlier. The present study also revealed absence of significant correlation between the mineral micronutrients (Fe and Zn) and PA which is an indication that reduction of PA as well as increase in the Fe and Zn can be done simultaneously in breeding programs.

Principal component analysis reflects the importance of largest contributor to the total variation at each axis of differentiation. Here 82.7 % of the variation is explained by two axes of variation *i.e*. PC1and PC2, the two canonical vectors representing primary and secondary axes of differentiation respectively. In PC1, PA-P and TP were major contributors of variation, therefore, these traits can be used for selection to obtain desirable genotypes with low PA content. Dispersion of genotypes based on their contribution towards PC1 and PC2 for PA-P, Pi, TP, Fe and Zn content showed that the most divergent genotypes P1490-03 (GP 256), Gouri (GP 313) to have low PA content. These accessions can be utilized as donors in rice biofortification programs for enhancing the micronutrient status of rice endosperm. The landrace Mehvan (green), Kew and PRR 109 was grouped in cluster 1 with the highest concentration of grain Fe and Zn. Such varieties can be crossed with the genotypes from cluster 1 (GP 256) and cluster 3 (GP 313) to combine low PA trait. Transgressive segregants from these crosses having low PA, high Fe and Zn can be identified and forwarded for varietal development. Mapping populations can be developed using these crosses to map the genomic regions governing low PA and high Fe and Zn.

In conclusion, the study analyzed the extent of genetic variability and diversity for PA-P, Pi, TP, Fe and Zn in the primary gene pool of rice and identified promising donors for enhancing the nutritional status of rice grain.

**Authors contribution**

Conceptualization of research (AKS, HB); Designing of the experiments (HB, PCG); Contribution of experimental materials (HB, PKB, GKS, PKB); Execution of field/lab experiments and data collection (PCG, HB, MN); Analysis of data and interpretation

(PCG, HB, KKV, RKE); Preparation of manuscript (PCG, HB, GKS, KKV).

**Declaration**

The authors declare no conflict of interest.

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**Supplementary Table S1.** List of germplasm used in the study with their genotype codes

| No. | Code | Genotype | No. | Code | Genotype | No. | Code | Genotype | No. | Code | Genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | GP-201 | CR 2461-9 | 42 | GP-273 | BJ-1 | 83 | GP-360 | Pant dhan 15 | 124 | GP-441 | DHMAS-70G-164-29 |
| 2 | GP-202 | UPRI-2003-45 | 43 | GP-282 | VL-88-97-1-7 | 84 | GP-361 | JGL_11727 | 125 | GP-443 | Ranbir Basmati |
| 3 | GP-203 | PNR381 | 44 | GP-284 | Kamlesh | 85 | GP-362 | Mahanadi | 126 | GP-444 | T23 |
| 4 | GP-204 | Punjab Mehak1 | 45 | GP-286 | IR-78908 | 86 | GP-364 | Pant Sugandh Dhan15 | 127 | GP-447 | ASD 19 |
| 5 | GP-205 | PNR 381 | 46 | GP-292 | B6144-MR-6-0-0 | 87 | GP-365 | Bhuman San | 128 | GP-450 | BPT 5204 |
| 6 | GP-206 | Jayati | 47 | GP-297 | HPR 2104 | 88 | GP-366 | JR 75 | 129 | GP-451 | IC-2127 |
| 7 | GP-208 | CT 10006-7-2M-5-1 P3-M | 48 | GP-301 | SahPasand | 89 | GP-370 | CO-37 | 130 | GP-452 | VOH-PCR-3113 |
| 8 | GP-210 | Chandrahasini | 49 | GP-302 | Chimbalate Basmati | 90 | GP-371 | Sumati | 131 | GP-453 | TYPE-3 |
| 9 | GP-211 | HPR 2143 | 50 | GP-303 | PMK-1 | 91 | GP-372 | SAF-1221-83 | 132 | GP-454 | Nagina 12 |
| 10 | GP-212 | MAS 946-1 | 51 | GP-311 | SitwaDhan | 92 | GP-373 | P 1447 | 133 | GP-455 | Sonasal(small Grain) |
| 11 | GP-217 | PRR 126 (NPP- 27/ KH-2010) | 52 | GP-313 | Gouri | 93 | GP-374 | P1447-00-5-1(P1324x AJAY) | 134 | GP-458 | KharaMunga |
| 12 | GP-219 | Ajay | 53 | GP-314 | Ananga | 94 | GP-385 | HUR 105 | 135 | GP-460 | ADT 39 |
| 13 | GP-221 | RNRM 7 | 54 | GP-315 | Pant dhan 4 | 95 | GP-387 | Manaswini | 136 | GP-461 | Haldimuri |
| 14 | GP-224 | Phalguna | 55 | GP-321 | OYR 69 | 96 | GP-389 | RR 8585 | 137 | GP-462 | MTU 7029 |
| 15 | GP-225 | VLT-6 | 56 | GP-323 | CR 2499 | 97 | GP-391 | Kudrat-3 | 138 | GP-464 | PDKV- Chinoor-2 |
| 16 | GP-227 | RIL-10 | 57 | GP-324 | Pusa 1460 | 98 | GP-392 | RAU 3061 | 139 | GP-468 | Swarna Sub 1 |
| 17 | GP-228 | Pant dhan 19 | 58 | GP-326 | UPRI-2003-24 | 99 | GP-396 | NDR 625 | 140 | GP-475 | Pusa-33 |
| 18 | GP-229 | NDR- 8015-1 | 59 | GP-327 | Poornima | 100 | GP-398 | JGL-3828 | 141 | GP-479 | Pratikshya |
| 19 | GP-230 | Pusa Sugandh 3 | 60 | GP-328 | UPRI-2003-18 | 101 | GP-400 | Muskan | 142 | GP-480 | Jhulhat |
| 20 | GP-232 | Pant sankar dhan-3 | 61 | GP-329 | Narendra UsarDhan III | 102 | GP-402 | MR-219 | 143 | GP-483 | MTU 1001(Vijetha) |
| 21 | GP-233 | Samanta | 62 | GP-330 | NDR 359 | 103 | GP-403 | UPRI-2003-15 | 144 | GP-487 | Bameshwari |
| 22 | GP-234 | Tapaswani | 63 | GP-331 | NDR 97 | 104 | GP-405 | Sharbati | 145 | GP-495 | Ramachandi |
| 23 | GP-237 | HPR 2083 | 64 | GP-333 | Bhadrakali | 105 | GP-406 | HKR-200-57-1 | 146 | GP-641 | Apo |
| 24 | GP-239 | Birupa | 65 | GP-334 | Shiva | 106 | GP-407 | WGL-23985 | 147 | GP-735 | Begum |
| 25 | GP-242 | Bhubana | 66 | GP-975 | Chittimutyalu | 107 | GP-411 | Raja Vaellu | 148 | GP-737 | Buta Baber |
| 26 | GP-243 | Pant dhan 10 | 67 | GP-338 | IR 77384-12-35-3-6-7-2-B | 108 | GP-412 | CR-246-16 | 149 | GP-739 | Baber |

**Supplementary Table S1.** Contd.

| No. | Code | Genotype | No. | Code | Genotype | No. | Code | Genotype | No. | Code | Genotype |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 27 | GP-246 | OYR 128 | 68 | GP-339 | Narendra Usar Dhan II | 109 | GP-413 | Phunchi | 150 | GP-740 | Baber Safed |
| 28 | GP-249 | OYC 183 | 69 | GP-340 | SKAU 220 | 110 | GP-414 | Pusa Basmati 1121 | 151 | GP-746 | Gull Baber |
| 29 | GP-252 | BJ-1(Red Kl, Purple awns) | 70 | GP-341 | PRR 105 (NPP-Kh-2010) | 111 | GP-416 | Improved Sabarmati | 152 | GP-755 | Kaw Qudder |
| 30 | GP-253 | Chandana | 71 | GP-343 | PRR 117 (NPP- 94/ KH-2010) | 112 | GP-417 | TomphaKhau | 153 | GP-756 | Kew |
| 31 | GP-254 | Urvashi | 72 | GP-345 | PRR 103 (NPP-43/ Kh-2010) | 113 | GP-418 | CN-1268-7 | 154 | GP-758 | Mehvan (purple) |
| 32 | GP-255 | Pant dhan 16 | 73 | GP-347 | PRR 123 (NPP-56/ KH-2010) | 114 | GP-424 | Pusa 1301 | 155 | GP-759 | Mehvan (green) |
| 33 | GP-256 | P1490-03- | 74 | GP-348 | PR 114(NPP-15/ kh-2010) | 115 | GP-425 | Kanak | 156 | GP-760 | Mir Zug |
| 34 | GP-257 | CSR 27 | 75 | GP-349 | PRR 120 (NPP-71/ KH-2010) | 116 | GP-427 | PRR 109 (NPP-29/ Kh-2010) | 157 | GP-923 | CO 50 |
| 35 | GP-258 | China 988 | 76 | GP-352 | PRR 115 (NPP-85/ kh-2010) | 117 | GP-428 | PRR 110 (NPP-57 /Kh-2010) | 158 | GP-924 | CO 51 |
| 36 | GP-260 | DV 85 | 77 | GP-353 | PRR 104 (NPP-75/ Kh-2010) | 118 | GP-431 | PRR 122 (NPP-97/ KH-2010) | 159 | GP-925 | Arupathaam Kuruvai |
| 37 | GP-262 | IRAT 240(IREM950) | 78 | GP-355 | PRR 121 (NPP- 70/ KH-2010) | 119 | GP-432 | PRR 118 (NPP- 93/ KH-2010) | 160 | GP-927 | Improved Samba Mahsuri |
| 38 | GP-264 | Selected Sabarmati | 79 | GP-356 | Pant dhan 18 | 120 | GP-433 | HUR-36 | 161 | GP-929 | SabarSurabhit (RAU 3036) |
| 39 | GP-265 | MTU 1010 | 80 | GP-357 | Indravati | 121 | GP-435 | RR 166-645 | 162 | GP-932 | North Andaman 2 |
| 40 | GP-266 | Kataktara | 81 | GP-358 | Pant Sugandh Dhan 17 | 122 | GP-439 | Nirri | | | |
| 41 | GP-270 | Kasturi | 82 | GP-359 | HUR-200-57-1 | 123 | GP-440 | C 22 | | | |