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Genetic variability and association studies of rice (*Oryza sativa* L.) for iron and phosphorus use efficiency under aerobic condition

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Abstract

Two hundred *indica* rice accessions were evaluated under aerobic condition for nine traits to examine the nature and magnitude of variability and character association. Among all traits, Iron use efficiency exhibited highest estimate of PCV and GCV followed by grain yield/plant and leaf iron content. Broad sense heritability was highest for 50 percent flowering followed by leaf phosphorus content and straw yield per plant. Association analysis revealed that iron and phosphorus use efficiency had significant positive association with grain yield. Accessions with Contrasting expressions for yield, iron and phosphorus use efficiency were identified for developing suitable rice genotypes under aerobic cultivation.

Keywords: Aerobic rice, variability, association analysis, iron use efficiency and phosphorus use efficiency

Introduction

Rice (*Oryza sativa* L.) is the most important cereal food crop of the world and about 90 percent of the people of South-East Asia consume rice as staple food. Asia's food security depends largely on irrigated rice fields, which produce three quarters of all rice harvested. But rice is a profligate water user, consuming half of all developed fresh water resources. Aerobic rice is a one of the water saving rice production technology. Growing rice aerobically saves water by eliminating continuous seepage and percolation, reducing evaporation and eliminating wetland preparation Subramanian *et al.* (2008) [13]. Meeting ever increasing demand under water scarcity requires expanding the area of rice under aerobic condition. This method saves up to 50- 60 percent of irrigation water but a concomitant reduction in productivity by up to 40 percent which is certainly not acceptable (George *et al.*, 2002) [6]. The background of these yield failures is not fully understood but the failures could be related to "soil sickness", potentially the combined effect of all telepathy, nutrient depletion, buildup of soil-borne pests and diseases, and soil structural degradation (Ventura and Watanabe, 1978) [23]. The change in soil water regime from flooded to aerobic, the rice plant may encounter more abiotic constraints such as inadequate nutrition. The soil redox potential becomes positive and thus the availability of iron (Fe) and phosphorus (P) decreases. Under aerobic soil condition presence of oxygen and high soil pH the ferrous iron [Fe (II)] is converted to insoluble ferric iron [Fe (III)] and these ferric form of iron is not available for plant metabolic activity (Marschner, 1995) [9]. Similarly, free iron and aluminium oxides present in aerobic soil bind to native and applied P making it unavailable to plants (Yan *et al.*, 2006). Therefore there is a need to improve the plants ability to extract the limiting resources and use them effectively to enhance productivity under aerobic cultivation. This investigation was primarily aimed at assessing the variability for phosphorus and iron uptake efficiency of rice genotypes. Under these considerations, a set of rice germplasm was evaluated for assessing the genetic variability, association among traits and identifying lines with higher productivity under aerobic conditions.

Materials and method

Included in this study were 181 upland *indica* rice germplasm accessions from Central Rice Research Institute Cuttack, India.

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The experiment was conducted in a simple lattice design with two replications at V. C. Farm Mandya, University of Agricultural Sciences, and Bengaluru. Error due to soil heterogeneity was reduced by maintaining uniform blocks and equal spacing between blocks (30 cm) throughout the experiment. Genotypes were raised by direct seeding in the main field at a spacing of 25 cm between the rows and 25 cm between the plants. Recommended cultural operations and plant protection measures were taken up to ensure uniform and healthy crop stand as per package of practices. Five plants were selected at random from each replication for recording observations. The averages of the observations recorded on these five plants were considered for analysis. The observations were recorded for days to 50 percent flowering, straw yield per plant and grain yield per plant. The leaf and grain samples were collected during flowering and at harvest respectively for determining leaf and grain iron and phosphorus contents.

Leaf and grain sample preparation and estimation of iron and phosphorus content

Rice leaf samples were oven dried (75 °C), ball milled and rice grain samples were dehusked, milled. A known weight of (0.50 g) powdered plant samples was taken in 50ml conical flask, 5ml of concentrated HNO₃ was added to flask and kept overnight for predigestion. Then 5ml of di-acid (prepared by using conc. HNO₃ and Perchloric acid in 9:4) was added. The conical flask was kept on hot plate for digestion until the contents turned to snow white residue and diluted to 50 ml using double distilled water and then filtered. From that 5 ml of aliquot was taken for P estimation. The p content in leaf and grain samples was estimated separately by following Vanado-Molybdate method (Jackson, 1973) ^[8] using following formula

$$P \text{ (g/kg)} = \frac{\text{Graph ppm} \times \text{volume of digested sample} \times \text{volume made up}}{\text{Weight of sample} \times \text{aliquot taken} \times 10000}$$

Phosphorus use efficiency was calculated as ratio between the total grain yield per plant and the total phosphorus content of the plant

$$\text{Phosphorus use efficiency} = \frac{\text{Grain yield per plant}}{\text{Total phosphorus in leaf} + \text{total phosphorus in grain}}$$

Orus in leaf + total phosphorus in grain

Estimation of iron (Fe) content in plant samples

Iron content was estimated in the aliquot of sample (leaf) extracts by using Atomic Absorption Spectrophotometer (AAS) at 248.33 nm by feeding suitable dilutions of digested samples to AAS having appropriate hollow cathode lamp after getting values for standards (AOAC, 1980) ^[3]. Whereas, grain iron contents were estimated using Inductively Coupled Plasma Optical Emission Spectrophotometer (ICP-OES) iCAP 6000 series in the Department of Crop Physiology, UAS Bengaluru.

Calculation

$$\text{Iron content (ppm)} = \frac{\text{Graph ppm} \times \text{volume of digested sample}}{\text{Weight of sample}}$$

Iron use efficiency was calculated as ratio between the total grain yield per plant and the total iron content of the plant.

$$\text{Iron use efficiency} = \frac{\text{Grain yield per plant}}{\text{Total iron in leaf} + \text{total iron in grain}}$$

Statistical analysis: Computed average values over replications and years was used for statistical analysis. Rank correlation of performance of accessions in two years was performed for testing the stability of accessions over years for days to 50% flowering (flw), leaf iron content (FeL), grain iron content (FeG), leaf phosphorus content (PL), grain phosphorus content (PG), straw weight per plant (SW), iron use efficiency (FeUE), phosphorus use efficiency (PUE) and grain yield per plant (GY). Highly significant rank correlation indicated absence of significant G × E interactions and hence data pooled over two years was used for further statistical analysis.

Components of variability and Correlation coefficients:

The analysis of variance (ANOVA) was carried out to dissect total variability of the entries into sources attributable to genotype. Variability parameters such as phenotypic coefficient of variability (PCV) and Genotypic coefficient of variability (GCV) were estimated following Burton and De Vane (1953) ^[4]. Heritability in broad sense and genetic advance as percent mean was estimated by using the formula of Johnson *et al.* (1955) ^[7]. Correlation coefficients were estimated as per formulae suggested by Al-Jiberari *et al.* (1958).

Results and Discussion

Analysis of Variance

The analysis of variance for nine traits including grain yield and its related traits in the present set of rice accessions revealed significant differences for all the traits (Table 1). This suggested that there is an inherent genetic difference among the genotypes. Similar finding for various traits in the rice genotypes were also reported by many rice workers Singh *et al.* (2007) ^[11] Sangam Kumar Singh *et al.* (2011) ^[12] and Ratnakar *et al.* (2016 & 2017) ^[18]. Considerable range of variation was observed for all the traits under study indicating enough scope for bringing about improvement in the desirable direction.

Analysis of variance by itself is not enough and conclusive to explain all the inherent genotypic divergence in the collections. This is revealed by determining the total genetic variability inherent in the genotypes obtained after due partitioning of the phenotypic variance the phenotypic variation of traits is attributable to genotype and environment assuming absence of interaction between them. The variation due to genotype can only be managed to suit to end-user needs. To compare variation of the accessions across eight productive traits at phenotypic and genotypic levels, the phenotypic and genotypic variations were standardized to make them unit-free and expressed as phenotypic coefficient of variability (PCV) and genotypic coefficient of variability (GCV). Days to 50% flowering was less variable while Iron use efficiency noticed high variability both at phenotypic and genotypic levels compared to other traits among the germplasm accessions as indicated by its PCV and GCV estimates in relation to other traits. Similar findings were also reported by several rice workers namely Anandrao *et al.* (2011) ^[2], Prajapati *et al.* (2011) ^[16], Sravan *et al.* (2011) ^[19]

and Sarma *et al.* (2012) ^[20] and Ratnakar *et al.* (2016) ^[17]. However, substantial range in the expression of germplasm lines for all the traits offer ample scope for selection of desirable lines for further use in genetic enhancement. Comparatively limited influence of weather variables on the expression of germplasm lines for most of the traits including grain yield, iron and phosphorus use efficiency as suggested by narrow difference between PCV and GCV has clearly reflected in higher broad-sense heritability (Table 1). The coefficient of variation at genotypic and phenotypic levels explain only the extent of variability in different traits, but this variation fails to explain the amount of heritable portion. In this situation, heritability in broad sense has an important role in the determining the heritable portion of variation. Knowledge of heritability of a trait is an essential measure to breeder in choosing suitable genotypes to employ in improving the trait under specified situation. The results in the experiment revealed higher heritability estimate for all the characters. High heritability indicates less influence of environment and is governed by additive gene effects. For the character with low heritability, selection may be considerably difficult or virtually impractical due to the masking effect of environment on genotypic effect [Sedek *et al.* (2009)] ^[21]. In a comparable study, Vange (2009) ^[22] Pandey and John Anurag (2010) ^[14] and Ratnakar *et al.* (2017) ^[18] also reported high heritability for grain yield per plant and filled grain per panicle. The broad-sense heritability does not indicate relative magnitude of additive (fixable) and non-additive (non-fixable) genetic variation [Chahal and Ghosal (2002)] ^[5]. Since rice is

self-pollinated crop, the germplasm represents a mixture of pure-lines. The genetic component of variability among rice germplasm accessions is therefore attributable to additive and additive-based epistatic interaction of genes controlling different traits. Hence, the broad-sense heritability estimates for different traits in the present study also represent narrow-sense heritability estimates. Fairly higher broad-sense heritability (also narrow-sense heritability for reasons explained) suggested effectiveness of selection for the traits under study as is also indicated by higher predicted genetic advance. Higher predicted genetic advance could be realized as the genetic variation among the germplasm accessions is solely attributable to genes acting additively, which cause greater resemblance between selected parents and their progeny. Maximum genetic gain per cycle could be realized even with simple pure-lines selection. Present study, all the character including the FeUE, PUE and grain yield recorded high heritability accompanied with high genetic advance indicated selection of parents for hybridization based on these traits would be highly effective.

Association studies indicated that FeUE, PUE and straw weight had significant positive association with grain yield. Among the characters phosphorus content in leaf had significant positive association with phosphorus content in grain and Iron use efficiency. However iron content in leaf and phosphorus use efficiency had significant positive association with each other indicating that there is mutual beneficial mechanism between iron content in leaf and phosphorus use efficiency (Table 2).

Table 1: Estimates of variability parameters for nine traits in 200 accessions of rice

Parameters		Days to 50% flowering	Plant height (cm)	Leaf Phosphorus content (g/kg)	Grain phosphorus content (g/kg)	Phosphorus use efficiency	Leaf Iron content (ppm)	Grain Iron content (ppm)	Iron use efficiency	Straw yield per plant (g)	Grain yield per plant (g)
Range	Min	70	51.80	1.23	2.09	0.08	71	16.67	0.989	7.10	3.70
	Max	130	144.2	3.63	4.37	0.26	414	76.6	12.35	50.39	39.54
Mean		97.70	81.40	2.32	3.10	0.17	157.33	36.26	4.97	21.32	16.90
SEm(±)		3.98	0.93	0.212	0.18	0.03	2.80	0.57	0.14	0.33	0.33
CV (%)		0.612	1.88	3.06	2.57	0.06	4.27	3.40	0.09	8.23	9.05
CD @ 5% level		1.56	3.09	1.42	1.55	0.12	13.66	2.32	1.23	3.46	3.02
PCV (%)		10.94	17.63	18.1	11.8	26.31	33.04	31.1	48.47	31.58	40.43
GCV (%)		10.87	17.34	17.58	11.23	24.15	32.75	29.52	45.92	29.58	38.61
Heritability (%)		98.96	96.81	94.33	90.65	84.29	85.30	90.20	89.77	91.48	91.22
Genetic advance(% of mean)		22.29	35.14	35.18	22.03	45.69	46.90	57.80	89.73	58.13	75.99
Rank Correlation between two year performance											
Rank correlation coefficients		0.61	0.83	0.85	0.86	0.88	0.77	0.92	0.89	0.68	0.49

Table 2: Correlation coefficients for nine traits among rice genotypes

Characters	FeL	FeG	PL	PG	SW	FeUE	PUE	GY
Flw	0.05	0.04	0.06	-0.03	0.01	0.09	0.12	0.17*
FeL		-0.01	-0.14	-0.01	-0.02	-0.64***	0.04	-0.06
FeG			0.14	0.01	0.11	-0.17*	-0.06	0.02
PL				0.29**	-0.22*	0.20*	-0.33**	-0.02
PG					-0.06	0.08	-0.33**	0.07
SW						-0.24*	-0.2	0.52***
FeUE							0.55***	0.45***
PUE								0.55***

Flw- 50% flowering, FeL- Leaf Iron content (ppm) FeG- Grain Iron content (ppm) PL- Leaf Phosphorus content (g/kg), PG- Grain Phosphorus content (g/kg), SW- Straw yield per plant (g), FeUE- Iron use efficiency, PUE- Phosphorus use efficiency, GY-Grain yield per plant (g)

Identification of promising donor genotypes among rice accessions for high iron and phosphorus content and use efficiency.

After thorough evaluation of 200 rice accessions in two consecutive years several genotypes were identified as promising donor lines for high iron, phosphorus and their use efficiency. Among them JBT- 36 / 79 had high grain iron (75.37 ppm) and high phosphorus content (4.27%) which served as promising donor parent for introgression breeding programmes. In aerobic ecosystem substantial yield decline had been noticed by several researchers (Ventura and Watanabe, 1978; George *et al.*, 2002 and Peng *et al.*, 2006) [6, 23, 15]. Reduction in growth and yield caused by several factors, one such factor is low nutrient use efficiency especially iron and phosphorus. So identification of high iron and phosphorus use efficiency with high yielding genotypes will justifies the present investigation. With this direction few promising donor lines were identified for high iron and phosphorus use efficiency. Among them AC-39020 and PS-360 had both high iron and high phosphorus use efficiency with high yield. These genotypes were also identified as root donor parents for introgression breeding by Mohan Kumar (2010) [10] under aerobic condition. Hence these genotypes serve as trait donor parents for high root, high iron and phosphorus use efficiencies with high grain yield under aerobic condition.

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