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Assessment of Grain Zinc, Iron and Protein Content in Selected Red Rice (*Oryza Sativa* L.) Mutant Lines

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Abstract

Traditional red rice cultivars grown in coastal regions of Karnataka are popular as food and medicine and specifically in promoting lactation. Effect of zinc, iron and protein deficiency is more in children and lactating women. Analysis of variance evidenced that treatment with gamma rays created significant variability and all the mutant lines exhibited wide range of values for zinc (20-41.5ppm), iron (10-35 ppm), protein content (8-11.9g/100 g) and yield traits. Grain zinc exhibited negative significant association with grain yield per plant, while protein and iron content showed non-significant and negative association with grain yield per plant and very low residual effect of 0.2721 indicated that the traits included in this study explained high percentage of variation in the grain yield and grain zinc, iron and protein content. BMRM15 and BMRM13 mutants showed reduced plant height, early maturity, recorded one percentage increase of protein (9.5% and 10.0%), higher zinc (28 and 25 ppm) and iron content (17 and 13 ppm). These mutant lines can supplement the micronutrient requirement of consumers and also help prevent the micronutrient deficiency.

Key words: Gamma irradiation, Induced variability, Short stature, Early maturity, Fe and Zn Deficiency.

Introduction

In India, red rice occupies a special position since time immemorial (Ahuja *et al*., 2007). Because of their medicinal value and exclusive taste, a number of red grained varieties are cultivated in Kerala, Tamil Nadu, Karnataka and the north eastern states in areas with unfavourable conditions such as deep water, drought, sandy soils, salinity and cold conditions (Chandhni, 2015). A great diversity in cultivars exists within red rice and one such traditionally grown long red kernelled rice in Coastal Karnataka is *Kajejaya,* which is very popular in this region and is consumed as *Kuchalakki* (special processed parboiled rice). Malnutrition is one of the significant problems in the developing countries and imbalanced supply of zinc, iron and protein leads to deficiency affecting the human metabolism (Khan *et al*., 2022 and Galani *et al*., 2022). Rice enriched with iron, zinc, protein along with higher yield and superior agronomic characters can support the health and livelihood of the millions of people who depend on the rice (De Oliveira *et al*., 2020).

Worldwide, there is an increasing emphasis on the relationship between food, nutrition and health and also food production that meets the demands of population and the market (Fukagawa and Ziska 2019). Therefore, strengthening the research and development of rice varieties plays an important role in improving the nutritive values of the grain and in promoting public health. The traditional red rice-

Kajejaya is the most sought after variety in spite of its lower yield and susceptibility to lodging in Coastal region. Hence, it is essential to improve the yield of traditional red rice varieties like *Kajejaya* to meet the food demand with nutritional security.

In this context, an attempt was made to develop an improved variety through mutation breeding. The prime strategy in mutation breeding is to upgrade the well adapted local varieties by altering one or two major traits that limit their productivity or enhance their quality value. Gamma rays are known to influence plant growth and development by including cytological, morphogenetic, biochemical and physiological changes in cells and tissues. The present experiment was conducted to assess the genetic variability induced by gamma rays in selected (M_6) mutant lines of *Kajejaya* for grain zinc, iron, protein content and yield traits.

Materials and Methods

The experiment was conducted during *kharif* 2022 at Zonal Agricultural and Horticultural Research Station, Brahmavar. Initially 200 seeds of the long bold traditional red rice variety *Kajejaya* were exposed to 15kR, 25kR, 35kR and 45kR gamma ray treatment from Cobalt 60 at BARC, Mumbai. Plant to progeny method was followed to forward the individual plants from M_1 to M_2 and semi-dwarf, early and medium bold red grain type were primarily selected and forwarded to M_3 and M_5 generation (106 mutant lines). The material (M_6) for the present study consisted of 5 each superior mutant lines which were selected based on higher grain yield per plant from 15kR, 25kR, 35kR and 45kR gamma treatments, respectively. A total of 20 mutant lines (M_6) with one untreated parent check (*Kajejaya*) laid out in a Randomized Complete Block Design (RCBD) with two replications. The seedlings were raised in wet nursery and twenty-one days old healthy seedlings from each treatment along with the check were transplanted in the well-prepared puddled field. The spacing was maintained with plant to plant spacing of 15 cm within a row and 20cm row to row spacing of 5 meters. The observations were recorded by randomly selecting ten plants in each mutant line. The experimental data was collected on 10 yield attributing traits yield and grain zinc, iron and protein (**Table 1**) were subjected to standard statistical procedure prescribed by Cochran and Cox (1957). The phenotypic coefficients of correlations were made as suggested by Al-Jibouri (1958). The

Table 1: Analysis of Variance for yield components, zinc, iron and protein content in the mutant lines of M_5 generation of red rice variety Kajejaya

Source of Variation	Degrees of freedom	Days to 50% flowering	Days to Maturity	Plant height (cm)	Number of productive tillers per plant	Number of grain per panicle	Test weight (g)	Protein (g/100g)	Iron (ppm)	Zinc (ppm)	Grain yield per plant (g)
Replication	1	0.095	0.0952	6.881	10.500	10.50	0.0688	0.00595	0.857	13.149	1.2758
Mutant lines	19	35.631**	25.4286**	102.181**	18.107**	167.31**	6.8689**	1.18657**	42.907**	47.541**	14.5312**
Error	20	1.445	2.2952	18.581	5.050	30.50	2.3028	0.19895	1.607	1.261	2.5760
CD (5%)		2.5077	3.1602	8.9917	4.6876	11.5201	3.1654	0.9304	2.6444	2.3427	3.3480
CD (1%)		3.4206	4.3107	12.2650	6.3941	15.7139	4.3178	1.2691	3.6071	3.1955	4.5668
** Significance at 1% level, *significance at 5% level											
Mean		76.10	122.29	65.74	18.36	139.93	26.49	10.04	24.64	31.96	29.41
Range	**Min**	72.0	113.0	51.0	12.0	120.0	21.1	8.0	10.0	20.0	23.7
	Max	88.0	129.0	96.0	25.0	159.0	32.2	11.9	35.0	41.5	34.6
PCV (%)		5.65	3.04	11.82	18.53	7.10	8.08	8.29	19.14	15.45	9.94
GCV (%)		5.43	2.78	9.83	13.91	5.91	5.70	7.00	18.44	15.04	8.31
H^2 broad see (%)		92.2	83.44	69.23	56.39	69.16	49.78	71.28	92.78	94.83	69.88
GAM (%)		10.75	5.23	16.86	21.53	10.13	8.29	12.18	36.59	30.19	14.32

phenotypic coefficients of correlation and path coefficients were analyzed by Windstar Version 9.2 from Indostat services.

Quality parameters

Top five superior mutant lines (a total of 20) from each treatment with higher grain yield per plant were analyzed for zinc, iron and protein content including untreated parent check. The protein content was determined by Micro Kjeldhal method, in three steps, namely digestion, distillation and titration. Zinc and iron content of grain samples were assessed through Atomic Absorption Spectrophotometer by feeding the prepared mineral solution to the AAS having appropriate hallow cathode lamps after getting values for standard solutions. The per cent elements concentrations of zinc and iron were calculated in ppm.

Results and Discussion

The mean sum of squares due to mutant lines were highly significant for the ten traits studied, indicating that gamma irradiation generated the variability in the experimental material (**Table 1**). The mutant lines exhibited wide range of values for days to maturity (113-129), plant height (51-96cm), number of productive tillers per plant (12-25), number of grains per panicle (120-159), test weight (21-32.2g), zinc (20-41.5 ppm), iron (10-35 ppm), protein content (8-11.9g/100g) and grain yield per plant (23.7-34.6 g). The observations are evidence that a desirable variation has been generated in the grain quality parameters and also yield component traits in the mutant lines of all treatments.

Moderate phenotypic and genotypic coefficient of variation was exhibited by plant height (11.82 and 9.83), number of productive tillers per plant (18.53 and 13.91), iron (19.14 and 18.44), and zinc content (15.45 and 15.04), (**Table 1**). Similar results were reported by Ullah *et al*., (2023). This result specified the presence of variations and the possibility of improvement of these traits by direct selection when the effect of external environment is considerably low.

High heritability coupled with high GAM was observed for iron (92.78 and 36.59) and zinc content in the grain (94.83 and 30.19). The results were similar with those of Singh *et al*., (2020) and high heritability with moderate GAM was exhibited in days to fifty per cent flowering (92.2 and 10.75), plant height (69.23 and 16.86), number of grains per panicle (69.16 and 10.13), protein content (71.28 and 12.18) and grain yield per plant (69.88 and 14.32). Similar findings were delineated by Demeke *et al.,* (2023). These traits were less affected by the environment and the traits appear to be governed by additive gene action

Table 2: Phenotypic correlation for yield components, zinc, iron and protein in the mutant lines of M_5 generation of red rice variety Kajejaya

	DF	DM	PH	PT	NG	TW	Pr	Fe	Zn	GY
DF	**1****									
DM	-0.160	**1****								
PH	0.154	0.345*	**1****							
PT	-0.036	0.183	0.243	**1****						
NG	-0.106	0.091	0.008	0.459**	**1****					
TW	0.040	-0.085	-0.169	0.499**	0.279	**1****				
Pr	0.017	-0.004	-0.365*	-0.168	-0.265	0.216	**1****			
Fe	-0.111	0.068	-0.537**	-0.301	-0.077	0.194	0.414**	**1****		
Zn	0.218	-0.476**	-0.377*	-0.352*	-0.401**	-0.059	0.290	0.188	**1****	
GY	-0.167	0.054	0.285	0.685**	0.672**	0.348*	-0.304	-0.204	-0.364*	**1****

DF: Days to 50 per cent flowering; **DM**: Days to maturity; **PH** : Plant height (cm); **NG** : Number of grains per panicle; **PT** : Number of productive tillers; **TW** : Test weight (g); **Fe** : Iron (ppm); **Pr** : Protein (g/100g); **Zn** : Zinc (ppm); GY :Grain yield per plant suggesting ample scope for genetic improvement through selection.

The results of correlation coefficient are presented in **Table 2**. Grain yield per plant (g) exhibited positive and significant association with productive tillers per plant, number of grains per panicle and test weight. These results are in collaboration with Srihari *et al*., (2023) and Suman *et al*., (2006) for number of grains per panicle; Monalisa *et al*., (2006) for number of productive tillers per plant; Gholipoor *et al*., (1998) and Habib *et al.,* (2007) for test-weight. Productive tillers per plant, grains per panicle and test-weight are useful in increasing the grain yield. Therefore, the selection of these traits will be beneficial in the process of yield improvement.

There was a positive correlation between grain iron, protein and zinc content results are in accordance with Jeom Ho *et al*., (2008). Grain zinc exhibited negative significant association with grain yield per plant while protein and iron content showed non-significant and negative association with grain yield per plant. These results are accordance with Nagesh *et al*., (2012) and Kanatti *et al*., (2009). This negative association may be the result of pleiotropy or linkage. Therefore, a cautious selection of these component nutritive traits is imperative for the concurrent development.

The path coefficient analysis was computed to estimate the contribution of individual traits to grain yield. In this study, the phenotypic direct and indirect effect of different traits on grain yield is presented in **(Table 3)**. The results of path coefficient analysis revealed that among the traits number of grains per panicle showed highest the direct and positive effect on grain yield, followed by productive tillers per plant, plant height, test weight, iron and zinc content the results were on par with Bagudam *et al*., (2018), indicating the effectiveness of direct selection for these traits in improvement of grain yield per plant. Days to maturity had highest negative direct effect followed by days to fifty per cent flowering and protein content. Similar results were found for number of productive tillers per plant by Kole *et al.,* (2008); for number of grains per panicle by Yogameenakshi *et al.,* (2004) and Panwar *et al.,* (2007) for test weight by Habib *et al.,* (2007) towards grain yield.

Table 3: Phenotypic path coefficient analysis for yield components, zinc, iron and protein in the mutant lines of M_5 generation of red rice variety Kajejaya

	DF	DM	PH	PT	NG	TW	Pr	Fe	Zn	r Values
DF	**-0.18277**	0.03505	0.05642	-0.01699	-0.05002	0.00066	-0.00095	-0.01931	0.01002	**-0.16789**
DM	0.02937	**-0.21809**	0.12654	0.08519	0.04271	-0.0014	0.00023	0.01187	-0.02182	**0.0546**
PH	-0.02817	-0.07537	**0.36614**	0.11331	0.00375	-0.00278	0.01972	-0.0934	-0.0173	**0.2859**
PT	0.00667	-0.03991	0.08912	**0.4655**	0.21561	0.00818	0.00908	-0.05242	-0.01613	**0.6857**
NG	0.0195	-0.01987	0.00293	0.2141	**0.46882**	0.00457	0.0143	-0.01338	-0.01838	**0.67259**
TW	-0.00735	0.0186	-0.06224	0.23273	0.13094	**0.01637**	-0.01168	0.03374	-0.00271	**0.3484**
Pr	-0.00322	0.00092	-0.13393	-0.0784	-0.12438	0.00355	**-0.05392**	0.07209	0.01329	**-0.304**
Fe	0.02031	-0.0149	-0.19676	-0.14041	-0.0361	0.00318	-0.02236	**0.17381**	0.00864	**-0.20459**
Zn	-0.03997	0.1039	-0.13829	-0.16392	-0.18809	-0.00097	-0.01564	0.03278	**0.0458**	**-0.3644**

Residual effect= 0.2721

DF: Days to 50 per cent flowering; DM: Days to maturity; PH : Plant height (cm); NG: Number of grains per panicle; PT: Number of productive tillers; TW: Test weight (g); Fe: Iron (ppm); Pr: Protein (g/100g); Zn: Zinc (ppm); GY: Grain yield per plant.

In the present study, very low residual effect of 0.2721 indicated that the traits included in this study explained high percentage of variation in the grain yield. The highest indirect positive effect on grain yield was exhibited by productive tillers per plant through test weight, followed by number of grains per panicle, plant height and days to maturity, thus selection based on number of productive tillers per plant, number of grains per panicle, plant height and test weight would be most effective, since test weight, number of productive tillers per plant and number of grains per panicle were had maximum direct effect as well as indirect effect on other characters *via* these traits.

Out of 20 mutant lines, two productive mutant lines ranked top, based on early maturity and reduced plant height as compared to check (**Table 4**). BMRM15and BMRM13 mutant lines mature early (114 and 117 days), which showed reduced plant height (84 and 80 cm), exhibited more number of productive tillers per plant (19 and 18), number of grains per panicle (141.5 and 140.5), test-weight (29.5 and 28.6 g) and grain yield per plant (30.5 and 30.6 g) as compared to the check. Mutations could create novel and unique variations as natural variability could not have provided the alleles for desired traits and application of radiation for induction of mutation must have resulted in direct development of 89% of mutant varieties in rice (Velmurugan *et al.,* 2010).

Table 4: Mean performance of high zinc, iron and protein content productive mutant lines of each treatment based on higher grain yield in M_6 generation of red rice variety Kajejaya

Gamma rays treatment	Mutant lines	Days to maturity	Plant height (cm)	Number of productive tillers per plant	Number of grains per panicle	Test weight (g)	Protein (%)	Zinc (ppm)	Iron (ppm)	Grain yield per plant (g)
35kR	BMRM15	114.0	84.0	19	121.5	29.5	9.5	28.0	17.0	30.5
45kR	BMRM13	117.0	80.0	18	120.5	28.6	10.0	25.0	13.0	30.6
Check- Kajejaya		120-125	90-95	12	115	24.25	8	20	10	24

Rice is the main staple food playing an important role in meeting calorie needs of the people hence, there is an ever-increasing consumer demand for rice for its functional nutritive quality. In present investigation, protein content in BMRM15 was 9.5% and BMRM13 recorded about 10.0%. In India, 80% of children under five years of age are under nourished, for whom the recommended intake of protein is 13-19 g/day/ child. As recommended calorie intake is 1000-1400 cal/day/ child (200-300g of rice) out of which 150-450 calories are to be supplied by protein, even 1% increase in grain protein content would add significant amount of protein to the diet. The OsASN1 OX rice plants produced grains with increased N and protein contents without yield reduction compared to wild-type (WT) rice (Lee 2020).

These two mutant lines also showed higher zinc (28 and 25ppm) and iron (Fe) content (17 and 13ppm). Zhang *et al*., (2012) and Bashir *et al.,* (2013) reported that, the OsVIT2 is a vacuolar localized transporter, which plays an important role in the vacuolar sequestration of Fe to regulate Fe homeostasis. It has been demonstrated that the disruption of *OsVIT2* results in an increase in Fe and Zn concentration in rice grains. Kandwal *et al.,* (2022) and Cheng *et al.,* (2007) noted that *naat*1 mutation exhibited strong stimulation of the Fe(II) acquisition system and leads to a significantly higher concentration of iron in both brown and polished (*naat*1) rice grown under water logged field conditions. BMRM15 and BMRM13 mutant Lines should be tested in different environmental condition for further confirmation of protein, iron and zinc content and can be efficiently used as donor in varietal improvement programmes to develop healthier rice varieties with high yield potential.

Conclusion

Biofortification is the only feasible way of reaching the malnourished population with vital nutrients and to produce nutrient packed rice grains in a sustainable way. Present investigation results documented that gamma ray treatment induced notable variability in mutant lines not only for yield attributing traits but also on grain zinc, iron and protein content. BMRM15and BMRM13 mutant lines can be used as parents in a breeding program targeting semi-dwarf and short duration characters. These two mutant lines should be evaluated in multi-location trial to assess their performance in a wide range of environments for nutritional quality and productivity.

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