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Computation of Grain Quality and Yield Attributes in Biofortified Rice (*Oryza sativa* L.) RILs.

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Abstract

An experiment was conducted for the fifteen biofortified recombinant inbred lines (RILs) of rice including 2 parents, MTU 1010 (P-1) having high iron and moderate zinc content and Karuppunel (P-2) have good zinc and yield, planted in Randomized complete block design (RCBD) at student's instructional farm Kumarganj, Ayodhya during Kharif season 2024-25. Fifteen lines of rice were evaluated during 2024 on the basis of grain quality parameters and yield attributing traits with three replications. The maximum true protein (mg/g) content (10.76) was observed in MK-4 and in MK-25 (10.58). Total starch content (79.79%) in MK-38 and MK-40 (79.71%). The maximum iron was found in MTU 1010 (13.70 ppm) and MK-24 (13.53ppm) and maximum zinc content (ppm) in Karuppunel (37.33ppm) and MK-24 (37.13ppm). Maximum test weight (g) was recorded (34.54g) in MK-24. Maximum grain yield per plant was recorded (35.99g) in MK-27.

INTRODUCTION

Rice (*Oryza sativa*) belongs to family Poaceae. The haploid chromosome number of rice is n=12 (2n=24). Either the species is diploid or tetraploid. Both *Oryza sativa* L. and *Oryza glaberrima* L. are diploid. It is kharif, annual and tropical crop. Rice after wheat is 2nd most important crop covering almost 90% of area across Asia alone (Ghosh *et al*., 2016). It is the primary staple for half of the world population and for more than 70% of the poor. In the Indian scenario it is estimated that the rice demand will be 140 million tonnes in 2025 (Mishra, 2004). By 2030, the world's population is predicted to rise from 6.1 billion to 8.0 billion, and to satisfy the rising demand, rice production needs to rise by 50%. Only by continuing to raise production steadily over time will this anticipated demand be satisfied. The modern period of the World Trade Organization requires that enough rice be produced for both export and self-sufficiency. It is necessary to create the exportable excess of high-quality rice at a competitive price. One of the most challenging health issues in the world, particularly in developing countries, is "hidden hunger," or malnutrition brought on by deficits in certain micronutrients. Globally, micronutrient deficiencies may affect more than three billion people today (Nikhil *et al*., 2022). For women, infants, and children in impoverished countries with limited resources, carbohydrate-rich, cereal-based, monotonous consumption of low-content and poorer bioavailability meals has been recognized as a major contributor to zinc and iron insufficiency (Godfred *et al*., 2020). The goal of the current study was to find early maturing genotypes with high yields. The genotypes were evaluated based on attributes associated with yield and quality metrics. A rise in rice output is essential to ensuring food security. One beneficial chance for selection is to comprehend genetic heterogeneity in the experimental material (Singh *et al*., 2020). The variations among members of a population brought about by genetic makeup and the environment in which they grow are known as phenotypic variability (Sumanth *et al*., 2017). The level of genetic diversity determines how breeding effort aimed at improving quantitative traits is planned and carried out. Thus, the presence of genetic variability with regard to desired qualities and the plant breeder's selection competence are the only factors that can make plant breeding efforts successful (Adhikari *et al*., 2018).

Iron and zinc are essential minerals supporting immune system function, cell growth and development, and protein synthesis (Roohani *et al*., 2013). According to Read *et al*., (2019), zinc also possesses antiviral qualities against a number of virus species that frequently harm people. The zinc requirement increases during pregnancy and the puberty phase of life. Gastrointestinal, central nervous, epidermal, immune, skeletal, and reproductive systems are known to be affected by zinc deficiency (Brown *et al*., 2002). Zinc deficiency is believed to affect over 178 nations worldwide and is one of the main causes of child mortality. The metabolic functions of living things, such as electron transport, deoxyribonucleic acid (DNA) synthesis, and oxygen transport, depend on iron (Abbaspour *et al*., 2014). Thus, the body requires enough consumption of the minerals zinc and iron. In underdeveloped nations, children's health issues are caused by a lack of iron and zinc. Globally, the prevalence of stunting was 29.1%, wasting was 6.3%, and underweight was 13.7% (Ssentongo *et al*., 2021). The human body uses iron and zinc primarily for a variety of metabolic processes. Insufficient consumption of iron and zinc can result in immunological dysfunction mediated by cells, cognitive decline, and, most significantly, growth retardation. Anaemia results from a reduction in the body's circulating haemoglobin and erythrocyte synthesis brought on by an iron shortage. The main and most important foods with significant health benefits are functional food products. The use of supplements seems to be a promising way to avoid and counteract the detrimental effects of deficits in zinc and iron (Abdollahi *et al*., 2019).

In order to address the issues of malnutrition worldwide, a number of national and international organizations, including the FAO, the International Rice Research Institute (IRRI), the Indonesian Centre of Rice Research (ICRR), the Indian Council of Agricultural Research (ICAR), and others in Asia-Africa, are working with partner nations to develop rice cultivars with high Fe and Zn content. (Swamy *et al*., 2021). In context to vitamin shortages, genetically fortifying rice grains is the most viable solution. The process of improving the bioavailability of essential nutrients in food crops through conventional breeding or through modern biotechnology techniques is called as biofortification. Production of rice varieties containing high amounts of bioavailable Fe would improve Fe nutrition in regions where its deficiency is prevalent (Meng *et al*., 2005).

MATERIALS AND METHODS

There were 15 biofortified rice RILs were taken along with parents (MTU 1010 and Karuppunel). An experiment was conducted in the Kharif season 2024 at the Student Instructional Farm (SIF) in Acharya Narendra Deva of Agriculture and Technology University Kumarganj, Ayodhya. The RILs lines have been developed by the crosses of MTU 1010 have high level of iron and moderate level of zinc quantity and Karuppunel have a good zinc and yield and then lines seeds were sown at SIF and for sowing, a raised bed nursery was utilized, then single seedling were transplanted in irrigated conditions. After 25 days, single seedlings were transplanted in the Randomized Complete Block Design (RCBD) trial field. Fifteen rice genotypes were planted with three replication. Every entry was moved onto a plot measuring 1m in length and 1m in width, with 20 cm separating rows, 15 cm separating plants, and 60 cm separating plots. Within replication, the Parents were randomized. Different Observations were recorded, which were Plant Height (cm), Number of tillers per plant, Days to 50 percent flowering, Days to maturity, panicle length (cm), test weight (g) of 1000 grain, biological yield per plant (g), harvest index and grain yield per plant (g) and after harvesting of seeds then quality parameters had been analysed.

Estimation of quality parameters

For the estimation of true protein by using the method of Folin Lowry's (1951). Take a mortar and pestle were used to grind a random sample of seeds from each rice genotype. 0.2M phosphate buffer (pH 7.2) solution was well mixed with 0.50 g of powdered samples. The samples were then centrifuged for 10 minutes at 6000 rpm, and the supernatants were gathered in a new tube. Take test tubes for all genotypes separately, filled a tube with 0.2 ml of supernatant and added distilled water to reach a final amount of 1.0 ml. Then add 5ml of the alkaline copper sulphate solution to each test tube, properly mixed, and incubated for ten minutes. Each tube was filled with 0.5 ml of diluted Folin's reagent, properly mixed, and allowed to sit at room temperature for 30 minutes. The absorbance was measured against a blank at 660 nm. Using the standard curve technique, the true protein content of the rice sample was determined.

The starch content in the grains of rice genotypes was estimated by the modified on the basis of anthrone reagent (Williams *et al*., 1970). Weighed100mg rice flour samples were extracted with hot 70% ethanol, centrifuge at 10000 rpm for 10 minutes to remove soluble sugars then discard supernatant and the residue was washed with 70% hot ethanol 2–3 times and dried to remove the excess of ethanol. Added 5ml of distilled water to the residue and then add 6.5ml of 52% perchloric acid to hydrolyze starch. Shake well and let stand for 20 minutes with occasional mixing, centrifuge and collect the supernatant and repeat the extraction with another 5 ml of perchloric acid, and combine both supernatants then make up the volume to100 ml with double distilled water. Pipette 1 ml of extract into a test tube. Add 4ml of freshly prepared anthrone reagent and mix well and place in a boiling water bath for 10 minutes, cool rapidly in an ice bath and take the absorbance at 620 nm against a reagent blank.

The amylose content in the grains of rice genotypes was estimated by the modified iodometric method on the basis of amylose - iodine binding mechanism (Juliano *et al*., 1978). In this, rice grain sample of each genotype crushed separately and homogenized with sodium hydroxide and absolute ethanol. Mixture was kept in water bath as per protocol and incubated it 15 min for reaction. The sample was kept off from water bath and 50 ml water was added in gelatinized sample. The diluted samples were put in amber tubes and iodine solution was added with acetic acid. The samples were further diluted with distilled water and kept it at room temperature for 20 min for appearance of blue colour complex. The optical density (OD) of each sample were recorded at 620 nm against blank with the help of benchtop double beam spectrophotometer (Zonotech, ZT-2201). The amylose content in OD was calculated by preparation of standard curve with rice amylose and result showed as g/100 g sample basis.

Estimation of Fe and Zn

Standard procedure was followed for estimation of Fe and Zn contents (George *et al*., 2013).

The 1 gram of sample weighed separately for each genotype, transferred quantitatively into 100 mL pyrex digestion tubes. Ten ml of di-acid mixture (HNO_3+$HClO_4$, 2:1) was added to each tube and allowed to stand overnight until the vigorous reaction phase was over. After preliminary digestion, digestion tubes were placed in a cold-block digester and temperature was raised to 150 °C for 60 minutes until all traces of HNO_3 disappeared. Then, temperature was slowly raised to 235 °C. When white dense fumes of $HClO_4$ appeared, digestion was continued for 30 more minutes, until white precipitates settled down at the bottom of digestion tubes. dH_2O was added in small increments for dissolving the crystals and washing of the tube walls. The contents were filtered through Whatman No. 42 filter paper and 50 mL dilutions were made with dH_2O. Finally, extract was fed to atomic absorption spectrophotometer. The analysis was repeated thrice for each genotype and mean value is presented in results. Fe and Zn concentrations were expressed in parts per million (ppm).

RESULTS AND DISCUSSION The mean performances of 15 rice genotypes for quality parameters and yield attributing traits were presented (Table 1& 2) significant variation within the genotypes for the 9 different characters of yield.

Quality parameters

The protein content in grains was ranged from 5.75 mg/g to 10.76 mg/g. The maximum protein content was found in MK-4 (10.76 mg/g) followed by MK-25 (10.58 mg/g) while minimum in MK-44 (5.75 mg/g) followed by MK-27 (6.28 mg/g). The traditional cultivars had higher protein content as compared to the enhanced ones shows their nutritional value. Total starch content in the grains of rice lines varies 70.36% to 79.79%. The maximum starch content was recorded in MK-38 (79.79%) followed by MK-40 (79.71%). The minimum starch content was recorded in rice genotypes P-2 (70.36%) followed by MK-6 (70.64%). Similar experimental finding has been reported by Kuril *et al*., (2024).

The Maximum Amylose content was estimated in MK-38 (24.87%) followed by P-1 (24.84%). Minimum starch content had been recorded MK-34 (15.94%) preceded by MK-27 (16.99%). The maximum iron content (ppm) was estimated in P-1 (13.70) followed by MK-24 (13.53). The minimum iron content was reported in MK-41 (7.45) followed by MK-44 (8.50). The maximum zinc content (ppm) was recorded in P-2 (37.33) followed by MK-4 (37.13). Minimum zinc content was observed MK-44 (21.73) followed by MK-41 (23.15).

Table No.1 Mean performance of biochemical parameters of 15 rice lines.

S. No.	Genotype	Protien %	Starch %	Amylose %	Fe (ppm)	Zn (ppm)
1	P-1	9.93	79.71	24.84	13.70	21.73
2	P-2	10.35	70.36	24.69	10.63	37.33
3	MK-3	8.62	73.64	19.42	10.10	32.93
4	MK-4	10.76	75.07	18.17	12.03	37.13
5	MK-6	9.75	70.64	24.24	12.60	33.07
6	MK-15	6.63	76.79	21.04	10.13	32.57
7	MK-24	7.31	71.21	20.82	13.53	31.73
8	MK-25	10.58	72	21.67	10.75	33.20
9	MK-27	6.28	72.5	16.99	11.90	33.20
10	MK-31	6.32	78.36	20.87	9.80	28.60
11	MK-34	7.36	79	15.94	10.53	31.57
12	MK-38	9.57	79.79	24.87	12.30	34.03
13	MK-40	7.6	71.86	18.04	9.20	26.17
14	MK-41	9.46	79.07	22.39	7.45	23.15
15	MK-44	5.75	72.21	21.64	8.50	23.17
	MEAN	**8.42**	**74.81**	**21.04**	**10.88**	**30.64**
	Std. Error	0.45	0.93	0.75	0.46	1.28
	Std. Deviation	1.75	3.60	2.90	1.80	4.96
	LOWEST	5.75	70.36	15.94	7.45	21.73

	HIGHEST	10.76	79.79	24.87	13.70	37.33

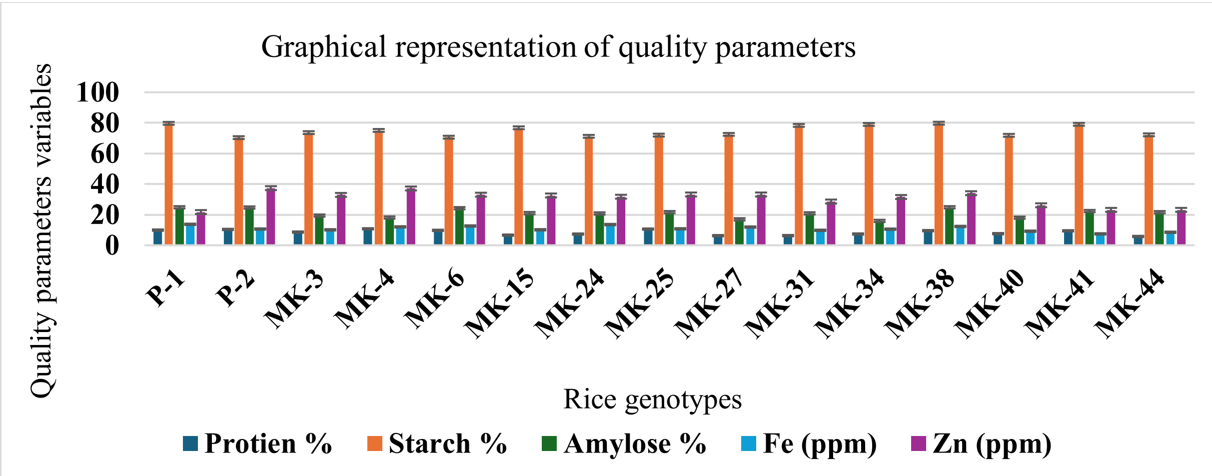

Fig.1. Graphical representation of quality parameters of 15 rice lines.

Yield and yield attributes traits

The rice genotypes had significant variability in growth, yield contributing traits, and yield under normal environmental condition (table 2). The plant height (cm) at physiological maturity ranges from 95.73 to 127.67 cm. According to the current study's findings, under typical climatic circumstances, genotype P-2 had the highest plant height at maturity (127.67cm) whereas genotype MK-31 had the lowest (95.73cm) succeeded by P-1 (100.5cm). Rice genotypes character like plant height, tillers number, days to flowering, panicle length, grains per panicle, test weight, harvest index, biological yield/plant and grain yield/plant are controlled by genetics and environmental factors. significant diversity in agro-morphological traits was reported in rice genotypes by Bardoloi *et al*., (2024). The number of tillers per plant was recorded at flowering stage and varied from 8 to 15.33. The maximum tiller number (count) was noted P-2 (15) and MK-3 (14) while minimum tiller number was observed in MK-34 (8) succeeded by MK-6 (8.67). The days to 50 % flowering ranged from 75.33–91.67 in rice genotypes. The maximum days to 50 % flowering (91.67) was noted in MK-25 while minimum (75.33) in MK-40. Days to physiological maturity of rice genotypes varied from 103.67 to 123.67 days. Highest days to maturity was MK-25 (123.67) followed by P-2 (121.33) while minimum MK-40 (103.67 days) succeeded by MK-38 (105.67 days). Days to 50% flowering is associated with the days to maturity and it is genotypic traits which is varied among rice genotypes. Similar results were also reported by Rathod *et al*., (2017).

The panicle length of rice genotypes ranging from 19.2 to 26.7 cm. The maximum panicle length was noted in line P-2 (26.7 cm) followed by MK-3 (24.87) while the minimum panicle length was recorded in MK-31 (19.2) succeeded by MK-34 (19.47 cm). The range of Test weight in rice genotypes was observed from 15.73 g to 34.54 g (table 2). The highest test weight was noted in MK-24 (34.54) followed MK-38 (31.51) while MK-25 (15.73) had the lowest succeeded by MK-34 (18.41). Panicle length and test weight were varied among rice genotypes and major contributor for grain yield of rice genotypes. Similar findings were reported by Ahamad *et al*., (2014) and Islam *et al*., (2019).

The range of harvest index in rice genotypes was observed from 21.65 g to 43.83 g. The highest harvest index was noted in MK-6 (43.83) followed MK-15 (38.86) while MK-44 (21.65) had the lowest succeeded by MK-25 (22.77). Biological yield per plant was varied from 53.33 g to 96.67 g. Maximum biological yield per plant was observed in MK-25 (96.67 g) followed by MK-24 (86.67 g) and minimum biological yield per plant in P-1 (53.33g). Grain yield per plant was varied from 19.77 g to 35.99 g. Maximum grain yield per plant was observed in genotypes MK-27 (35.99 g) followed by MK-15 (34.48g) and minimum grain yield per plant in MK-3 (19.77g) succeeded by P-1 (23.22). The grain yield per plant is associated with yield contributing traits of rice genotypes. Effective tillers, panicle length and test weight are major yield contributing traits and determinant yield of rice genotypes under existing environmental condition. Similar results has been found by Kumar *et al*., (2019).

Table No.2. Mean performance of yield and yield attribute traits of rice lines.

S. No.	Genotype	DFF	DMT	PH	NOT	PL	TW	HI	BYP	GYP
1	P-1	88.33	115.33	100.5	14.23	23.7	26.67	33.33	53.33	23.22
2	P-2	91.33	121.33	127.67	15.33	26.7	26.86	27.1	55	24.58
3	MK-3	84.67	114.67	126.17	14.43	24.87	24.74	23.57	78.33	19.77
4	MK-4	82	110	118.5	11.67	20.7	28.51	33.53	55	24.31
5	MK-6	82.67	111.67	114.83	8.67	20.2	18.43	43.83	55	32.46
6	MK-15	78.33	107	121.83	11	21.53	24.36	38.86	68.33	34.48
7	MK-24	78.33	108.33	117.1	10.67	23.67	34.54	30.3	86.67	33.94
8	MK-25	91.67	123.67	102.17	12	20.63	15.73	22.77	96.67	29.49
9	MK-27	79.67	106.67	113.8	9.67	21.3	29.51	35.87	75	35.99
10	MK-31	84.33	112	95.73	10.67	19.2	25.61	34.24	65	29.33
11	MK-34	88.67	116.67	115	8	19.47	18.41	25.72	90	30.06
12	MK-38	75.67	105.67	107.33	12	20.43	31.51	25.98	90	31.19
13	MK-40	75.33	103.67	123.8	13.33	20.1	23.12	31.85	63.33	26.96
14	MK-41	83.33	111.33	111.17	10	23.13	23.54	33.34	63.33	28.15
15	MK-44	81.33	114.33	110.7	8.33	24.17	26.31	21.65	95	27.35
	MEAN	**83.04**	**112.16**	**113.75**	**11.33**	**21.99**	**25.19**	**30.80**	**72.67**	**28.75**
	Std. Error	1.35	1.46	2.43	0.58	0.58	1.30	1.63	4.07	1.17
	Std. Deviation	5.22	5.66	9.42	2.26	2.23	5.02	6.31	15.75	4.54
	C. V.	6.28	7.05	8.28	19.97	10.16	19.92	20.48	21.67	15.78

	LOWEST	75.33	103.67	95.73	8	19.2	15.73	21.65	53.33	19.77
	HIGHEST	91.67	123.67	127.67	15.33	26.7	34.54	43.83	96.67	35.99

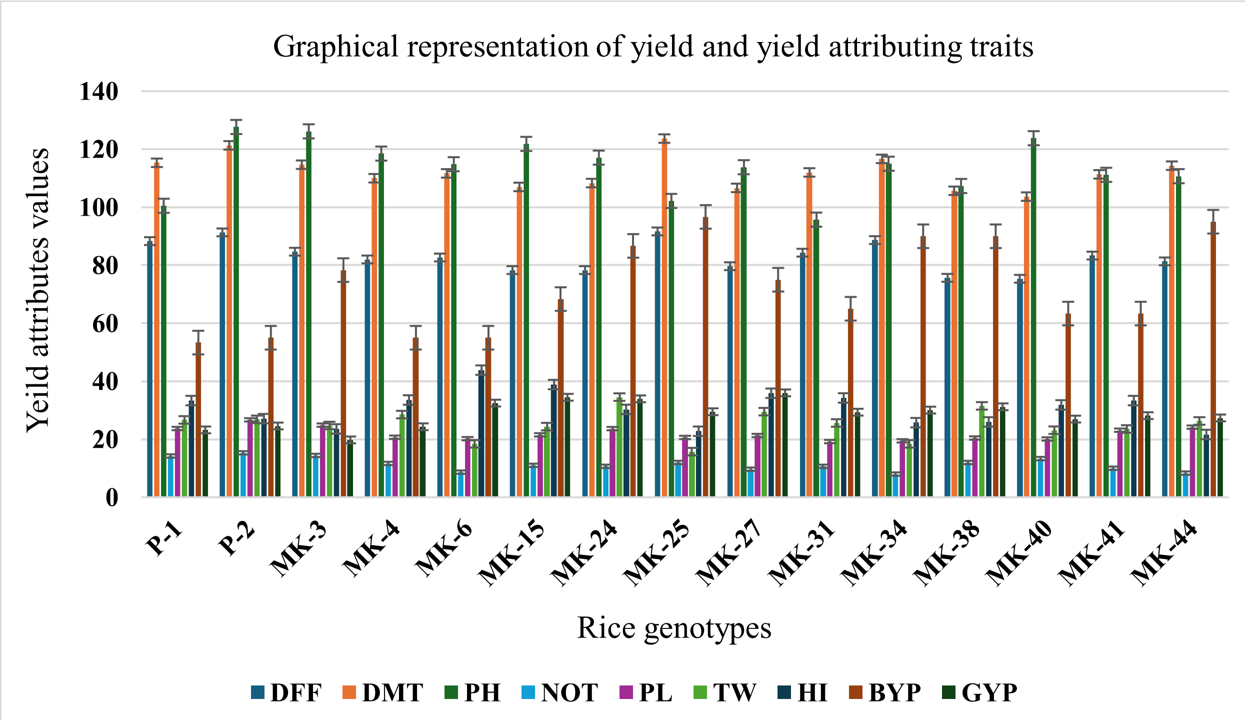

Fig.2 Graphical representation of yield and yield attributing traits.

Note: DFF -Days to 50% flowering, DMT- Days to physiological maturity, PH-Plant height, NOT- Number of tillers, PL-Panicle length, TW-Test weight, HI- Harvest Index, BYP-Biological yield per plant, GYP-Grain yield per plant.

CONCLUSION

From this study, it can be concluded that sufficient variability exists in most of the parameters. There were three lines MK-24, MK-27 and MK-38 out of 15 lines has been found with better qualities of nutritional parameter and with good yield. These lines are better for the future prospects to overcome the problems of malnutrition.

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