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SCREENING STABLE POTENTIAL RICE MUTANTS FOR GRAIN ZINC AND IRON ACCUMULATION AND YIELD PERFORMANCE

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Abstract

Biofortification of rice through gamma radiation-induce mutagenesis in rice is a well-established approach to achieve sustainable development goals (SDG) 2, SDG 3 and SDG 12. Hence, this approach is considered as effective for both improving the yield and nutritional value of rice. In this regard, a field study was carried out for screening of stable rice mutants for their potential to accumulate high Zn concentrations and performed better yield. To obtain 40 stable rice mutants, 06 rice genotypes (Shua-92, Shadab, NIA-Mehran, IR-8, Sarshar and Shandar) were exposed to gamma rays doses of 100, 150, 200 and 250. The irradiated seeds were sown under the field conditions to obtain potential mutants. The soil used in the study was heavy in texture, calcareous and alkaline in nature, deficient in organic matter and DTPA extractable Zn. The experiment was laid out following RCBD with three replications, and each plot measuring of 8.0 m^2 (4m × 2m). Results revealed that grain yield varied from 3.58±0.11 to 9.75±0.13 kg $plot^{-1}$, Zn concentration 12±0.13 to 38±0.05 mg kg^{-1}while Fe concentration ranged from 3.0±0.11 to 71±0.16 mg kg^{-1}. Phytic acid (PA) varied from 8±0.12 to 15±0.21 mg g^{-1}. Moreover, findings of the AMMI biplot revealed that grain yield and phytic acid exhibited higher interaction, whereas Zn showed relatively stable. Keeping in view the goal of biofortification in rice, best mutants based on multi-trait selection were M24 (Shua-200-4) which was very consistent across traits, M30 (Shadab-200-1-1) was stable and in good micronutrients, M35 (IR8-200-1) with strong Fe and stable, M10 (Shandar-200-4) had good Zn and yield stability and M22 (Shua-100-8) with yield stability and acceptable micronutrients. These findings suggest that both stable and specifically adapted mutants should be considered for breeding programs depending on target environments and nutritional traits.

Key words: Mutation breeding; Rice mutant; Biofortification; Micronutrient; Food security

Introduction

Rice (*Oryza sativa* L.) is one of the important staple food crops worldwide that provides major sources of calories to over half of the world population especially in Asia and some parts of Africa. In addition to its caloric value, rice is the basis of food security and rural economies in most developing economies (Pechdin & Naruepatr, 2025; Tang *et al*., 2023; Mughal & Fontan Sers, 2020). Nevertheless, processed rice grains naturally contain low concentrations of various essential micronutrients, in particular, iron (Fe) and zinc (Zn), which are crucial in human health and development (Kumaravel & Srividya, 2025; Kong *et al*., 2022). These low levels of these micronutrients are also a contributing factor in the hidden hunger from of chronic micronutrient malnutrition affecting over two billion individuals globally (Wairich *et al*., 2022). The most common nutritional disorder in the world is iron deficiency which causes anemia, impaired mental performance, fatigue and poor immunity (Auerbach *et al*., 2025). Zinc deficiency also causes retarded development, late wound healing as well as predisposition to infections (Hussain & Mushtaq, 2025). This is especially acute among the rice-eating populations, where rice supplies 50-80 percent of daily energy but a relatively small proportion of micronutrients necessary to the body (Wairich *et al*., 2022). Consequently, fortification of rice grains with Fe and Zn is a vital measure that is necessary to control micronutrient malnutrition in third world countries.

To deal with this challenge, biofortification, which is an effort for increasing the nutrient densities (minerals) in rice grains, using plant breeding, biotechnology or agronomic methods, has been put into the limelight around the world (Rashid *et al*., 2025). It has been identified as one of the most viable and sustainable ways of reducing hidden hunger (Bouis & Saltzman, 2017). Biofortification of rice can be done by (i) traditional breeding, where a breeder uses existing genetic variation, (ii) induced mutagenesis to create new alleles, (iii) molecular breeding or marker-assisted selection, and (iv) transgenic or genome-editing of metal transporters or storage proteins (Azeem *et al*., 2025; Sandeep & Godi, 2023). Although Fe and Zn play an essential role in human diet, their bioavailability in rice is poor because phytic acid (myo-inositol hexakisphosphate), a significant storage form of phosphorus in cereal grains, exists (Takai *et al*., 2025). Phytic acid binds divalent cation (e.g., Fe^{2+}, Zn^{2+}, Ca^{2+}), and causes insoluble phytate salts which are not readily absorbed in the human intestine (Rahman *et al*., 2025). The concentrations of phytic acid in rice grains are high thereby greatly lowering the nutritional value of the crop despite the good levels of total Fe and Zn concentrations in conventional growing rice varieties (Jangra & Tiwari, 2025; Raboy, 2020).

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The dilemma of breeders is to increase Fe and Zn levels and at the same time decrease phytic acid levels, a complicated issue since these two characteristics tend to be interdependent (Nguyen *et al*., 2025). As an illustration low-phytate mutations can have an influence in seed development and germination, whereas high-Zn traits may be associated with yield penalties (Rohaeni *et al*., 2023; Khan *et al*., 2019). Consequently, there is need to find genotypes or mutants that can be agronomical stable yet attain desirable micronutrient and anti-nutrient contents. Stable rice mutants have proven to have some potential in uncovering genes that control mineral accumulation and storage (Devi *et al*., 2025). Qamar *et al*., (2019), used induced mutagenesis, interbreeding and marker-assisted selection to develop and characterize low-phytate Basmati rice mutants and proved the possibility of integrating classical and molecular breeding techniques. On the same note, (Khan *et al*., 2019) mapped quantitative trait loci (QTLs) in low phytic acid content rice mutants, which determine genomic regions that can be employed in marker-assisted introgression process (Kumar *et al*., 2025; Sahu *et al*., 2024). Further, Rohaeni *et al*., (2023) reported that biofortified rice lines with Fe and Zn tend to have lower phytic acid levels, and this leads to the increased bioavailability of the minerals and no significant yield loss. In addition to this, the high-Zn density and low-phytate features have been reported to significantly improve the bioavailability of Zn in rice grains when paired with the high-Zn density traits (Kaur *et al*., 2025; Wang *et al*., 2021). These results highlight the viability of producing nutritionally improved rice by incorporating strategic application of natural or induced genetic variation and field testing.

The current study is designed with the hypothesis that gamma radiation brings genetic change (mutation) in rice plants. The efforts has made to explore positive mutation in rice with aiming to screen out a group of stable rice mutants for low phytate which is gap in research and high Zn and Fe bioavailability in natural field conditions to test the agronomic performance and nutrition composition. Mutation (irradiation) breeding is more effective than other biofortification approaches to overcome Zn malnutrition in large population especially in rural areas without using additional inputs. This research will focus on selection of potential mutant lines with better yield potential and enhanced Fe and Zn concentrations and lower phytic acid content.

Materials and Methods

Soil physico-chemical characteristics of experimental site: The experiment was done in silty clay loam that was described as having the soil texture, non-saline $EC_{1:2.5}$ (0.68±0.18 dSm^{-1}) and alkaline in nature (pH 7.48±0.21), respectively. The chemical properties refers to poor fertility conditions, which was indicated by a low organic matter content 0.68%, and a low total nitrogen content 0.028% (Khoshnaw & Esmail., 2020). The AB-DTPA method of (Estefan *et al*., 2013) was followed using an atomic absorption spectrophotometer (AAS), and the soil was deficient in AB-DTPA extractable phosphorous (5.92 mg kg^{-1}) and zinc (0.48 mg kg^{-1}), potassium was within physiologically acceptable limits (158 mg kg^{-1}).

Experimental site, design and crop husbandry: A field trial was conducted at the experimental farm of Nuclear Institute of Agriculture (NIA), Tandojam (25^0, 26'0" N Latitude and 68^0 32'0 E Longitude), Sindh Pakistan, during the Kharif season of 2022. The randomized complete block design (RCBD) was arranged by keeping replications in block variable with three replications. Forty stable rice mutants produced from 6 parents (Shua-92, Shadab, NIA-Mehran, IR-8, Sarshar and Shandar) were irradiated with 100, 150, 200 and 250 Gy doses of Gamma rays (Table 1).

Table 1. List of selected rice mutants.

M1	NIA-MEHRAN-100-1	M21	SHUA-100-5
M2	NIA-MEHRAN-100-2	M22	SHUA-100-8
M3	NIA-MEHRAN-100-4	M23	SHUA-200-2
M4	NIA-MEHRAN-200-2	M24	SHUA-200-4
M5	NIA-MEHRAN-200-4	M25	SHADAB-100-1
M6	SHANDAR-100-2	M26	SHADAB-100-3
M7	SHANDAR-100-3	M27	NIA-19/A-100-1
M8	SHANDAR-100-5	M28	NIA-19/A-200-1
M9	SHANDAR-200-1	M29	NIA-19/A-200-2
M10	SHANDAR-200-4	M30	SHADAB-200-1-1
M11	SHANDAR-200-5	M31	SHADAB-200-1-2
M12	SARSHAR-100-3	M32	SHANDAR-200-2/B
M13	SARSHAR-200-1	M33	SHANDAR-200-3
M14	SARSHAR-200-2	M34	NIA-19/A-150-1
M15	SARSHAR-200-3	M35	IR8-200-1
M16	SARSHAR-200-4	M36	NIA-625-150-1-2
M17	SARSHAR-200-5	M37	OD-16/B-2-2(OD-17)
M18	SARSHAR-200-8	M38	NIA-105
M19	SARSHAR-200-9	M39	OD-10/B-2-1 (V6)
M20	SHUA-100-3	M40	OD-16/B-2-1(OD-26)

The selection of mutants was based on low phytate, high Zn and Fe content with high yield performances. The area of experimental plot for each rice mutant was 8.0 m^2 (4m × 2m) with 20 cm plant × plant space was maintained. Randomly 3 plants were selected from each plot for the agronomic recordings and chemical analysis. Triple superphosphate was utilized as phosphorous (60 kg P_2O_5 ha^{-1}) and sulfate of potash was used as the potassium source (50 kg K_2O ha^{-1}) at transplanting time (Panhwar *et al*., 2024). The Zn (8 kg ha^{-1}) was used in the form of Zingro fertilizer after 20 days of transplanting to reduce the antagonistic interactions between the utilized phosphorous and zinc. Urea as a source of N, applied at transplanting, tillering and at the phase of panicle initiation. The experimental field was cleared and smoothed to maintain the uniform distribution of irrigation water, maintaining favorable environment for early seedling establishment and ongoing growth (Abbas *et al*., 2024). The crop was left to grow till full maturity and harvested after turning panicles into yellow showing physiologically mature. The fully matured plants of each experimental plot were harvested carefully with assurance of error-free yield recording and successive analysis.

Growth, yield parameters and nutrient analysis: For the agronomic data and chemical analysis, 3 rice mutant plants from each plot were randomly selected. The height of the plant was measured by using the meter rod from the base of the stem up to the tip of the panicle of the tallest culm and the panicle length was measured from the node to tip of topmost branch. The counting of tillers $hill^{-1}$ was

made to determine the performance of tillers and yield potential (Siddiqui *et al*., 2025). To find out the grain yield of each mutant plot, the grain weight of randomly 3 plants was also combined. A random sample of a hundred grains of each rice mutant plot was weighed on top loading digital balance with high accuracy (Akchaya *et al*., 2025). After dehusking the rice grains were oven-dried at 70℃ for 48 hours to reduce the variability due to moisture. The oven-dried grains were ground to fine powder using stainless-steel grinder for chemical analysis (Abbas *et al*., 2024). To determine the zinc and iron content the ground grain sample was digested using perchloric and nitric acids (wet digestion) utilizing an atomic absorption spectrophotometer (AAS) (Estefan *et al*., 2013). Concentration of phytic acid (PA) was determined utilizing the method of Haug & Lantzsch (1983), also mentioned by (Siddiqui *et al*., 2025). The precision and reproducibility of the results were guaranteed by minimization of experimental error using systematic sampling and standardized analytical techniques.

Statistical analysis

The data were recorded in triplicate and to analysis of variance (ANOVA) was done using Analytical Software Statistix® Version 10.0**,** Tallahassee, FL, USA. Biplots were prepared by using software Meta-R and Microsoft Excel (365 version).

Results and Discussion

Soil physico-chemical properties before transplanting rice: The physico-chemical properties of soil were determined before transplanting rice. Soil samples taken at the depth of 0-20 cm revealed that soil was heavy in texture (Clay loam), non-saline ($EC_{1:2.5}$ 0.68±0.18 dSm^{-1}), alkaline (pH 7.48±0.21), deficient in organic matter (0.68%±0.08), calcareous (6.87%±0.21) in nature and deficient in AB-DTPA extractable Zn (0.62 mg kg^{-1}±0.023), respectively.

Growth and yield attributes: The application of gamma rays (100, 150, 200 and 250 Gy) on seeds of 6 rice varieties (Shua-92, Shadab, NIA-Mehran, IR-8, Sarshar and Shandar) created significantly high mutation in different agronomic and yield traits. This diversification in breeding material helped in selection of potential mutants for zinc biofortification and yield traits.

The data regarding agronomic attributes is given in the tables 2 and 3. The different doses of gamma radiation affected the plant height, the maximum height recorded in mutants M2 (140±1.70 cm), M26 (138±3.78 cm) and M6 (136±1.16 cm), However, lowest height was recorded in mutant M33 (103±1.28 cm). Number of tillers per hill was also significantly affected by gamma rays' application, the highest tiller numbers per hill was observed in mutants M17 (23±1.72) and M3 (22±1.53), while the least in M27 (12±1.73). All the genotypes showed variable significant mutation in thousand grain weight and a key grain yield trait. The highest thousand grain weight was depicted by the mutants M37 (30±0.18 g), M13 (29±0.42 g) and M27 (29±0.32 g). Biological yield is one of the important attributes of any successful and best performing varieties which is probably contributed by plant height and tiller numbers. The highest biomass yield of 36±0.27 kg $plot^{-1}$ was displayed by mutant M16, followed by mutant M15 (35±0.16 kg $plot^{-1}$), however, the least biomass was produced by mutant M40 (17±0.17 kg $plot^{-1}$). The most important attribute amongst all the traits is grain yield (Table 2). End users are mainly interested in the paddy harvest of varieties, this trait is given in the (Table 3). The highest values (9.75±0.13 kg $plot^{-1}$) were shown by mutant M39 followed by M38 (9±0.02 kg $plot^{-1}$) and M13 (8.2±0.04 kg $plot^{-1}$), while the least performance was showed by mutant M30 (3.58±0.11 kg $plot^{-1}$).

Mineral nutrients (Zn and Fe) and phytic acid: The results of this study regarding mineral nutrients zinc (Zn) and iron (Fe) are likely to be involved in designing superior nutritional varieties of rice, which are equipped in supporting biofortification initiatives and solving the regional problem of micronutrient (Zn & Fe) malnutrition. The data is reported in (Table 3. The different doses of gamma radiation significantly affected the mineral nutrient densities of rice genotypes, maximum Zn content was recorded in mutants M13 (38±0.05 mg kg^{-1}), followed by M2 (34±0.35 mg kg^{-1}) and M8 (34±0.12 mg kg^{-1}), while the least Zn were observed by mutant M17 (12±0.16 mg kg^{-1}), respectively. Iron the most important component of human hemoglobin was also significantly affected by gamma rays' application. The highest Fe content of rice grain was observed in mutants M4 (71±0.67 mg kg^{-1}) followed by M27 (69±1.17 mg kg^{-1}), while the least Fe content reported in mutant M31 (31±0.11 mg kg^{-1}), respectively. Phytic acid is also known as phytate, this parameter is crucial factor mineral digestibility in human intestine. Variation in Phytic acid in different mutants was noted after radiation bombardment of different doses. The highest phytic acid showed by mutant M33 (15±0.21 mg g^{-1}) followed by M30 (14±0.12 mg g^{-1}), however, the least phytate content illustrated by mutant M17 (8±0.12 mg g^{-1}).

Additive main effects and multiplicative interaction (AMMI): According to AMMI analysis the environment is one of the dominating sources of variation in yield attributes (grain yield) and mineral and anti-mineral contents (Zn, Fe and phytic acid), which contributes larger sum of squares significant F-value, whereas genotype effects are also highly significant among 40 rice mutants under study. The mutants with environment interaction was significant, indicating differential genotypic variation and response to varied environmental conditions which in turn justify the use of AMMI modeling.

Based on AMMI biplot interpretation, genotypes located near the origin with favorable positioning were considered stable and high performing genotypes. For grain yield, mutants M11, M24, and M22 were identified as superior (Fig. 1). Zinc content showed stability across genotypes, with M19 and M10 performing consistently (Fig. 2). Iron content highlighted M35 and M13 as stable performers (Fig. 3), while low and stable phytic acid content was observed in M39 and M30 (Fig. 4). Multi-trait evaluation identified M24, M30, and M35 as promising candidates for biofortification programs. Each AMMI biplot showed X-axis (IPCA1 / Factor 1) depicted major portion of GE interaction, Y-axis (IPCA2 / Factor 2) pertained secondary interaction, points (M1–M40) showed genotypes (mutants), polygon (dashed blue) were identified as vertex genotypes (most responsive/extreme) and green vectors mentioned the environments or repeats.

Table 2. Agronomic parameters of selected rice mutants.

Mutant No.	Plant height (cm)	Tillers hill^{-1}	Thousand grain weight (g)	Biological yield plot^{-1} (kg)
M1	111 ± 1.57 g-k	13 ± 1.00 a-c	26 ± 0.47 c-h	29 ± 0.08 g-l
M2	140 ± 1.70 a	20 ± 1.36 a-c	23 ± 0.28 j-o	28 ± 0.17 i-n
M3	105 ± 1.20 jk	22 ± 1.53 ab	27 ± 0.45 b-f	28 ± 0.67 k-p
M4	108 ± 1.79 i-k	14 ± 1.26 a-c	28 ± 0.57 a-c	30 ± 1.15 f-j
M5	105 ± 1.35 jk	19 ± 0.55 a-c	21 ± 0.49 op	30 ± 0.43 f-j
M6	136 ± 1.16 ab	16 ± 1.16 a-c	22 ± 0.50 n-p	28 ± 0.35 j-o
M7	107 ± 0.79 jk	16 ± 0.58 a-c	26 ±0.15 c-h	30 ± 0.23 g-k
M8	112 ± 2.33 f-k	18 ± 0.87 a-c	27 ± 0.18 a-e	27 ± 0.27 k-p
M9	129 ± 2.89 a-c	17 ± 1.58 a-c	22 ± 0.50 op	34 ± 0.14 a-c
M10	106 ± 1.24 jk	19 ± 2.37 a-c	25 ± 0.28 f-k	31 ± 0.26 e-h
M11	113 ± 2.73 e-k	15 ± 1.86 a-c	24 ± 0.24 h-n	26 ± 0.15 p-r
M12	108 ± 1.59 i-k	20 ± 2.11 a-c	23 ± 0.11 k-p	27 ± 0.13 l-q
M13	116 ± 0.86 d-j	16 ± 1.77 a-c	29 ± 0.20 a	33 ± 0.08 c-e
M14	115 ± 3.11 e-k	17 ± 2.03 a-c	22 ± 0.39 op	28 ± 0.14 j-o
M15	128 ± 2.15 a-d	14 ± 0.93 a-c	22 ± 0.43 n-p	35 ± 0.16 a-c
M16	121 ± 1.30 c-h	20 ± 1.50 a-c	25 ± 0.17 f-m	36 ± 0.27 a
M17	124 ± 1.03 b-f	23 ± 1.72 a	21 ± 0.2 1p	35 ± 0.22 ab
M18	125 ± 1.16 b-e	19 ± 2.27 a-c	21 ± 0.24 op	32 ± 0.10 d-f
M19	113 ± 1.72 e-k	14 ± 1.00 a-c	28 ± 0.40 a-d	25 ± 0.09 qr
M20	107 ± 1.59 jk	17 ± 1.96 a-c	25 ± 0.14 f-k	26 ± 0.09 n-q
M21	115 ± 1.96 e-k	14 ± 0.75 a-c	27 ± 0.12 b-f	29 ± 0.25 h-l
M22	115 ± 1.62 e-k	19 ± 1.93 a-c	25 ± 0.24 e-j	29 ± 0.09 g-l
M23	122 ± 2.67 c-g	18 ± 2.76 a-c	29 ± 0.42 ab	30 ± 0.33 f-j
M24	113 ± 1.84 e-k	13 ± 1.61 a-c	27 ± 0.12 a-e	29 ± 0.12 i-m
M25	109 ±3.48 h-k	16 ± 2.15 a-c	26 ± 0.14 d-h	24 ± 0.20 rs
M26	122 ± 2.45 c-g	19 ± 1.08 a-c	23 ± 0.23 i-o	27 ± 0.33 k-p
M27	122 ± 5.28 c-g	12 ± 0.73 c	29 ± 0.32 ab	31 ± 0.08 e-g
M28	138 ± 3.78 a	16 ± 1.96 a-c	25 ± 0.08 f-l	33 ± 0.16 b-d
M29	116 ± 3.20 e-k	20 ± 1.50 a-c	23 ± 0.34 l-p	30 ± 0.15 g-l
M30	109 ± 2.66 h-k	18 ± 1.81 a-c	26 ± 0.36 e-i	29 ± 0.20 g-l
M31	113 ± 1.79 e-k	13 ± 0.81 a-c	27 ± 0.29 b-g	30 ± 0.24 f-j
M32	108 ± 1.93 i-k	15 ± 0.71 a-c	26 ± 0.14 e-j	27 ± 0.10 m-q
M33	103 ± 1.28 k	12 ± 1.33 c	22 ± 0.09 m-p	25 ± 0.14 qr
M34	115 ± 2.58 e-k	14 ± 0.66 a-c	24 ± 0.23 g-m	30 ± 0.22 f-i
M35	108 ± 1.12 i-k	13 ± 0.60 bc	27 ± 0.37 b-f	26 ± 0.17 o-r
M36	136 ± 3.73 ab	14 ± 2.38 a-c	26 ± 0.25 c-h	29 ± 0.14 g-l
M37	113 ± 2.63 e-k	16 ± 2.18 a-c	30 ± 0.1 8a	23 ± 0.14 s
M38	109 ± 1.33 h-k	20 ± 1.96 a-c	29 ± 0.38 ab	25 ± 0.15 p-r
M39	120 ± 0.80 c-i	19 ± 0.84 a-c	27 ± 0.19 a-e	25 ± 0.26 p-r
M40	114 ± 1.59 e-k	14 ± 1.74 a-c	29 ± 0.17 a	17 ± 0.14 t

The grain yield of genotypes for the AMMI depicted that IPCA1 explains 53.94%, IPCA2 explains 46.06% that is strong GE interaction captured, mutants like M3, M26, M38 lie far from origin which depicted the highly interactive (unstable), mutants near origin (e.g., M11, M24, M22) were displayed stable yield performers and polygon vertices (e.g., M3, M6, M21, M26) showed specifically adapted genotypes (Fig. 1). The AMMI for Zn described that IPCA1 was 62.2%, IPCA2 was 37.8%, and many mutants clustered near origin and had less GE interaction. Some mutants (e.g., 3, 16) far from origin and were considered as Zn-responsive but unstable (Fig. 2). The AMMI for iron content in rice grains showed IPCA1 was 65.17%, and IPCA 2 was 34.83% (Fig. 3). Greater dispersal observed compared to Zn which was higher GE interaction. Mutants like M3 and M37 were vertex genotypes and showed as highly responsive, however, some stable mutants M13 and M35 were near to center. Phytic acid is anti-nutritional organic ligand, hence the AMMI for phytic acid content in rice grains showed IPCA1 of 62.24%, and IPCA 2 was 37.76% (Fig. 4). Greater spread depicted the strong GE interaction. Mutants like M1 and M38 were highly interactive mutants and mutants M39 and M30 were observed near to origin.

Table 3. Grain yield, mineral nutrient (Zn & Fe) contents and phytic acid of selected rice mutants.

Mutant No.	Grain yield plot^{-1} (kg)	Zinc content (mg kg^{-1})	Iron content (mg kg^{-1})	Phytic acid (mg g^{-1})
M1	8.01 ± 0.09 b-d	23 ± 0.12 j-l	25 ± 0.33 m-o	12 ± 0.16 f-k
M2	6.00 ± 0.10 h-n	34 ± 0.35 b	64 ± 0.64 b	12 ± 0.18 e-k
M3	5.56 ± 0.28 j-p	31 ± 0.10 cd	43 ± 0.87 f	13 ± 0.39 b-d
M4	6.85 ± 0.17 e-i	26 ± 0.21 i	71 ± 0.67 a	13 ± 0.21 b-e
M5	5.65 ± 0.18 j-p	29 ± 0.11ef	58 ± 0.53 c	15 ± 0.13 ab
M6	6.60 ± 0.26 f-j	22 ± 0.27 k-m	14 ± 0.38 rs	13 ± 0.19 c-h
M7	7.00 ± 0.18 d-h	30 ± 0.15 de	54 ± 0.45 cd	14 ± 0.13 a-c
M8	6.15 ± 0.16 g-m	34 ± 0.12 b	38 ± 0.23 gh	11 ± 0.26 g-m
M9	8.03 ± 0.06 b-d	27 ± 0.12 gh	36 ± 0.25 g-i	15 ± 0.40 a
M10	7.15 ± 0.13 c-g	22 ± 0.10 l-n	39 ± 0.37 g	11 ± 0.14 g-l
M11	5.10 ± 0.07 m-q	23 ± 0.21 j-l	65 ± 0.21 b	13 ± 0.30 b-f
M12	6.20 ± 0.07 g-l	20 ± 0.05 op	30 ± 0.22 kl	11 ± 0.15 g-l
M13	8.20 ± 0.04 bc	38 ± 0.05 a	53 ± 0.16 d	11 ± 0.11 g-m
M14	4.45 ± 0.06 q-s	22 ± 0.05 l-n	28 ± 0.24 lm	14 ± 0.28 ab
M15	6.35 ± 0.12 g-l	19 ± 0.13 q-r	35 ± 0.34 h-j	11 ± 0.17 j-o
M16	7.20 ± 0.22 c-g	13 ± 0.51 u	19 ± 0.44 pq	9 ± 0.34 o-q
M17	7.10 ± 0.11 d-g	12 ± 0.16 u	32 ± 0.30 jk	8 ± 0.12 q
M18	8.03 ± 0.09 b-d	22 ± 0.07 l-n	24 ± 0.29 no	11 ± 0.18 g-m
M19	4.90 ± 0.03 o-q	22 ± 0.16 k-m	38 ± 0.13 g-i	12 ± 0.09 d-j
M20	6.75 ± 0.08 e-i	19 ± 0.05 p-r	25 ± 0.07 mn	13 ± 0.25 c-g
M21	6.82 ± 0.19 e-i	22 ± 0.22 kl	25 ± 0.38 mn	9 ± 0.17 m-q
M22	7.80 ± 0.07 c-e	21 ± 0.09 m-o	19 ± 0.26 pq	10 ± 0.10 l-q
M23	7.60 ± 0.28 c-f	18 ± 0.21 r-t	35 ± 0.89 h-j	10 ± 0.25 k-p
M24	5.50 ± 0.04 k-q	18 ± 0.08 rs	16 ± 0.29 qr	10 ± 0.07 k-p
M25	3.75 ± 0.07 rs	17 ± 0.11 t	32 ± 0.42 jk	11 ± 0.17 g-l
M26	5.55 ± 0.20 j-p	28 ± 0.14 fg	48 ± 0.75 e	9 ± 0.16 n-q
M27	6.55 ± 0.19 f-k	28 ± 0.21 g	69 ± 1.17 a	11 ± 0.26 i-n
M28	6.85 ± 0.14 e-i	19 ± 0.09 rs	35 ± 0.51 ij	8 ± 0.08 q
M29	4.65 ± 0.11 p-r	28 ± 0.09 g	47 ± 0.29 ef	13 ± 0.12 b-f
M30	3.58 ± 0.11 s	29 ± 0.15 ef	24 ± 0.25 no	14 ± 0.12 b-d
M31	5.90 ± 0.24 i-o	20 ± 0.19 o-q	3 ± 0.11 v	11 ± 0.09 g-l
M32	6.20 ± 0.04 g-l	26 ± 0.15 hi	32 ± 0.23 jk	11 ± 0.23 j-o
M33	6.00 ± 0.09 h-n	37 ± 0.26 a	46 ± 0.51 ef	15 ± 0.21 a
M34	6.60 ± 0.11 f-j	17 ± 0.09 t	15 ± 0.36 r	12 ± 0.30 d-j
M35	5.31 ± 0.06 l-q	21 ± 0.07 no	28 ± 0.15 lm	12 ± 0.08 e-k
M36	5.83 ± 0.09 i-o	24 ± 0.16 j	22 ± 0.35 op	11 ± 0.23 g-m
M37	6.77 ± 0.10 e-i	23 ± 0.09 jk	13 ± 0.28 rs	11 ± 0.26 j-n
M38	9.02 ± 0.14 ab	30 ± 0.08 e	11 ± 0.82 st	11 ± 0.52 h-n
M39	9.75 ± 0.13 a	17 ± 0.19 st	7 ± 0.16 u	9 ± 0.07 pq
M40	5.02 ± 0.12 n-q	32 ± 0.11 c	10 ± 0.21 tu	12 ± 0.15 d-i

Discussion

The present study was conducted to explore potential rice mutants developed through mutation for Zn and Fe biofortification using different gamma radiation levels. This study is more helpful for achieving the food security, improved nutrition of human beings and sustainable agriculture to SDG 2 (Zero hunger). Mineral nutrients (Zn & Fe) are crucial for human health to meet requirement of SDG 3 (Good health and wellbeing for all). Agriculture industry/sector is responsible to produce enough for human consumption for meeting SDG 12. Nuclear radiation is ideal source of mutation in rice, hence application of nuclear energy may be a vital source for creating ideal attributes or traits in rice genotypes such mineral (e.g. Zn, Fe) nutrient densities in grains, high yielding, better rice quality. This study showed variable impact and mutations in mutants for height, number of tillers, thousand grain weight, biomass yield and grain yield. Out of total (40) mutants 04 mutants showed lower height (<106cm), 30 mutants showed medium height (106 to 126 cm) and 06 mutants exhibited tall height (>126cm). Mutant M2 showed highest plant height and lowest by mutant M33 as mentioned in (Table 2). It was noticed that gamma radiation had great effects on rice growth, especially plant height, tillers, thousand grain weight biomass yield, grain yield and mineral nutrient

contents. High radiation doses (200 Gy and 250 Gy) showed decreased germination with high mutation while low radiation doses (100 Gy and 150 Gy) exhibited the highest growth and height (43.9 cm, 59.4 cm) and high chlorophyll content observed during growth phase (Danu, *et al*., 2025). The long-term exposure caused DNA damage and oxidative stress (Choi *et al*., 2021). Also, high radiation doses (150 Gy and 200 Gy) affect rice plant tissues, thus inhibiting plant height, growth reduction, physiological imbalances such reduced chlorophyll (Dash & Kujur, 2024). Radiation doses showed highly significant mutation in tiller hill^{-1}, 06 mutants contributed less tillers, 31 mutants contributed medium number of tillers, while 03 mutants exhibited high number of tillers. In this study mutants M17 illustrated maximum number of tillers (23±1.72) and M3 least (22±1.53). Danu *et al*., (2025) reported that gamma radiation significantly affected the tillers hill^{-1}. In another study, Abdelnour-Esquivel *et al*., (2020) showed that low doses of radiation could stimulate rice growth by increasing number of tillers. High radiation doses showed contrasting results to low radiation doses (Wahyudi & Indrawanis, 2025). The nuclear radiation brought mutation in chlorophyll content, growth and grain weight at low gamma radiation (Danu *et al*., 2025, Herlina & Julsento, 2024). Biomass yield and grain yield of rice are important traits to improve livelihood of end users. Both the parameters were significantly affected by different radiation doses as illustrated by many mutants on the identified and selected in this study. Based on the findings of this study 05 mutants exhibited low biomass, 30 mutants exhibited medium and 05 mutants showed high. The highest biomass was produced by mutant M16 (36±0.27 kg plot^{-1}), Grain yield of mutants revealed that 06 mutants illustrated low grain yield, 28 mutants produced medium and rest of 06 mutants showed high grain yield, while the highest grain yield per plot was observed in mutant M39 (9.75±0.13 kg plot^{-1}). Similar findings have been reported under low radiation (100 Gy and 150 Gy) showing positive mutation resulting in increased biomass yield, grain yield and physiological traits (Wahyudi & Indrawanis 2025, Herlina & Julsento, 2024, Abdelnour-Esquivel *et al*., 2020).

The present study was conducted to explore rice mutants having capability of accumulating higher Zn and Fe densities in rice grains. The gamma irradiation created induced mutation in genomics of rice genotypes, mutants on the basis of ideal traits for higher accumulation of mineral nutrients (Zn & Fe) were selected. Realizing the highest and profound impact of micronutrient mineral in human health for overcoming the malnutrition, this proved worth significant tool for opening window of mineral biofortification (Zn and Fe) in rice. The phytate content of rice grain is considered as negative trait for biofortification; therefore, the selection criterion may be on basis of low phytate, high Zn, Fe contents and stability in yield. The mutant M13 had high Zn but moderate PA, while M17 had low PA but lower Zn. The combination of high Zn and low PA in M13 is more beneficial for human nutrition in comparison to combination of lower Zn and low PA (M17).

Radiation doses created mutation in Zn contents. Categorization of Zn in rice grain revealed that 05 mutants illustrated low Zn (<16 mg kg^{-1}), 29 mutants exhibited medium Zn (18 to 30 mg kg^{-1}) and 06 mutants showed high Zn concentrations (>30 mg kg^{-1}), rice mutant M13 revealed the highest Zn content (38±0.05 mg kg^{-1}). Iron content of the rice grain varied significantly in rice mutants with application of different gamma levels. Seven mutants contained low Fe (<3 mg kg^{-1}), 26 mutants displayed medium Fe (3 to 35 mg kg^{-1}), while 07 mutants exhibited high Fe (>35 mg kg^{-1}) content in rice grains. The mutant M4 (71±0.67 mg kg^{-1}) was the best performer mutant in terms of Fe content. Zinc and Fe contents of rice grain are directly proportional to chlorophyll content, oxidative stress, DNA damage, and organic compounds in rice. Hence, low doses of gamma radiation (100 Gy and 150 Gy) created positive mutation for enhancing mineral nutrient content directly and indirectly in rice grains (Danu *et al*., 2025; Wahyudi, & Indrawanis 2025; Herlina & Julsento, 2024; Abdelnour-Esquivel, *et al*., 2020). Phytic acid an anti-mineral organic compound, which chelates mineral nutrients (Zn & Fe) in digestibility of human intestine. Variability in phytic acid contents of different mutants was noted due to different radiation doses. The 07 mutants showed low phytate (<10 mg g^{-1}), 26 mutants exhibited medium phytate (10 to13 mg g^{-1}) and 07 mutants illustrated high (>13 mg g^{-1}) phytate content in rice grains. The highest phytic acid was showed by mutant M33 (15±0.21 mg g^{-1}). Application of low radiation doses (100 Gy and 150 Gy) was used by Danu *et al*., (2025), Wahyudi, & Indrawanis (2025), Herlina & Julsento, (2024), Abdelnour-Esquivel *et al*., (2020), they reported that high radiation doses (200 Gy and 250 Gy) showed positive mutation and reduced physiological traits and phytate content (Wahyudi, & Indrawanis 2025, Herlina & Julsento, 2024, Bughio *et al*., 2021, Abdelnour-Esquivel, *et al*., 2020).

Additive Main effects and Multiplicative Interaction (AMMI) biplot interpretation revealed that grain yield showed substantial GE interaction, with IPCA1 exhibiting 53.94% of variation. Mutants located near the origin showed stability, while those at polygon vertex exhibited specific adaptability and higher interaction. Best performers and stable 05 mutants were M11, M24, M22, M12 and M10 (Fig. 1). Zinc content exhibited moderate GE interaction, with most genotypes clustering near the origin, indicating relative stability across environments. Mutants M19, M10, M32, M25, and M5 were revealed as best mutants on the basis of Zn content. Iron content was more environmentally sensitive compared to Zn (Fig.2). Therefore, selection based on stable plus moderately Fe content genotypes may be successful. Best mutants based on Fe content were M15, M13, M16, M31 and M30, respectively (Fig. 3). Phytate content revealed the strong genotype × environment (GE) interaction, highlighting environmental sensitivity and need for careful selection of low phytate genotypes. Best low phytic acid (PA) mutants were M39, M30, M24, M35 and M26 (Fig. 4). The following is the comparative interpretation of the mutants (Table 4).

Limitations: The limitations of resources and lack of funds occurred during the study, and we were unable to conduct field experiments in different ecological zones of Pakistan. Due to limited resources, we planned study in single environment but in three locations of climatic conditions of Tando Jam.

Table 4. Comparative interpretation.

Trait	G × E interaction	Stability	Breeding implication
Grain yield	High	Moderate	Select stable and high yielding
Zinc (Zn)	Low-moderate	High	Broad adaptation possible
Iron (Fe)	Moderate-high	Moderate	Select environment-specific
Phytic acid (PA)	High	Low	Careful selection needed

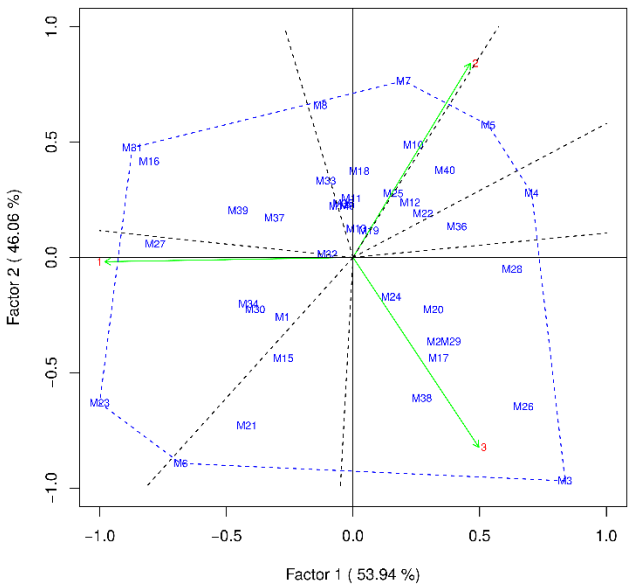


Fig. 1. AMMI biplot for grain yield performance of rice mutants.

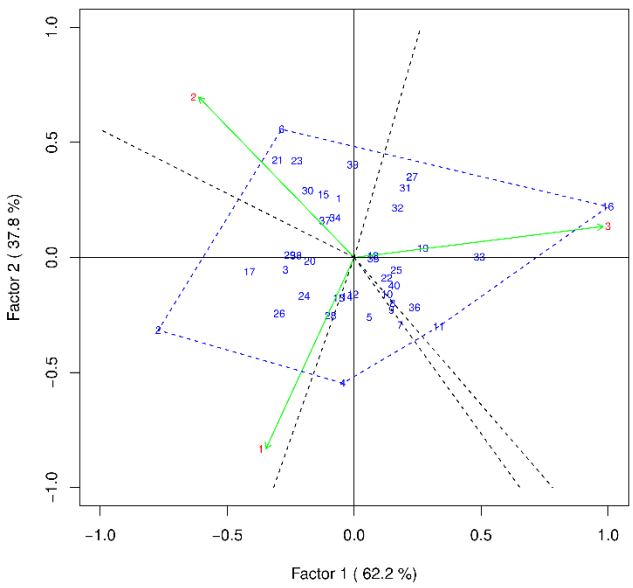


Fig. 2. AMMI biplot for Zn content of rice mutants.

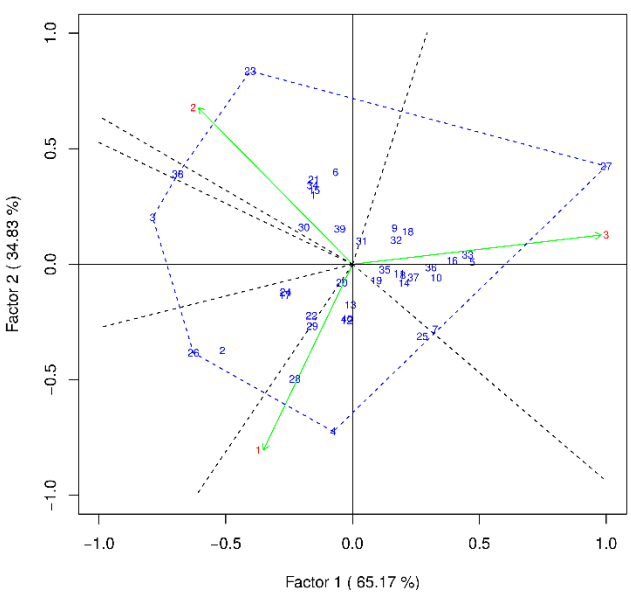


Fig. 3. AMMI biplot for Fe contents in rice grains.

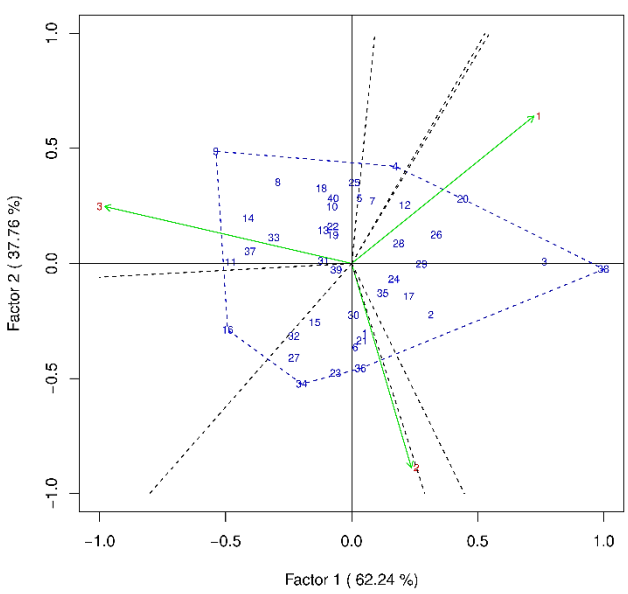


Fig. 4. AMMI biplot for phytic acid contents of rice mutants.

Conclusion

The findings of grain yield and phytic acid exhibited significant interaction while Zn content was observed stable trait in genotypes. Best performing rice mutant selected based on multi-traits was M24 (Shua-200-4) which was very consistent across traits. Mutant M30 (Shadab-200-1-1) was stable and good in micronutrients, M35 (IR8-200-1) with strong Fe and stable in yield, M10 (Shandar-200-4) with good Zn and yield stability and M22 (Shua-100-8) with yield stability with acceptable micronutrients. These mutants are recommended on the basis of high Zn/Fe with low PA and yield stability. These findings suggest that both stable and specifically adapted mutants should be considered for breeding programs depending on target environments and nutritional traits. Moreover, potential molecular follow-up studies (marker-assisted introgression, transporter gene analysis) are suggested to strengthen the breeding pipeline.

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References

Abbas, M., N. Depar, J.A. Shah, J. Afzal and N.A. Sial. 2024. Soil zinc availability alters the contribution of pre-anthesis and post-anthesis uptake in rice grain zinc loading. *J. Agric. Biol.*, 2(2): 111-119.

Abdelnour-Esquivel, A., J. Perez, M. Rojas, W. Vargas and A. Gatica Arias. 2020. Use of gamma radiation to induce mutations in rice (*Oryza sativa* L.) and the selection of lines with tolerance to salinity and drought. *In Vitro Cell. Dev. Biol. Plant*, 56(1): 88-97.

Akchaya, K., T. Sampathkumar, J. Bhuvaneswari, S.K. Natarajan, S. Thenmozhi and S. Manibharathi. 2025. Impact of foliar nutrient application on growth and yield of drip fertigated aerobic rice. *Plant Sci. Today*, 12(2): 1-10. https://doi.org/10.14719/pst.6480

Auerbach, M., T.G. DeLoughery and J.S. Tirnauer. 2025. Iron deficiency in adults: a review. *JAMA*, 333(20): 1813-1823.

Azeem, A., S. Khan, M. Zia Ul Haq, S. Shafiq, M.T. Aslam, S. Munir and M. Sikandar Zaman. 2025. Biofortification strategies for enhancing crop nutritional value: a review of methods, challenges, and future directions. *Discov. Plants*, 2(1): 185.

Bouis, H.E. and A. Saltzman. 2017. Improving nutrition through biofortification: A review of evidence from HarvestPlus, 2003-2016. *Glob. Food Secur.*, 12: 49-58.

Bughio, Z.R., S.M. Bhatti, N. Depar, I. Rajpar, M.S. Sarki and H.R. Bughio. 2021. Enhancement of zinc concentration and bioavailability in rice grains by zinc application methods. *Pak. J. Agri. Sci*., 58(5):1483-1490.

Choi, H.I., S.M. Han, Y.D. Jo, M.J. Hong, S.H. Kim and J.B. Kim. 2021. Effects of acute and chronic gamma irradiation on the cell biology and physiology of rice plants. *Plants*, 10(3): 1-14. https://doi.org/10.3390/plants10030439

Danu, L., H. Siti, S. Yusida, A. Ika, S. Putri and S. Yusmaidar. 2025. The effect of gamma ray radiation on the growth of local rice plants (*Oryza sativa* L.) Silumat variety. *Jurnal Agronomi Tanaman Tropika*, 7(2): 671-674.

Dash, S. and M. Kujur. 2024. Impact of gamma irradiation on biochemical and physiological characteristics of black rice. *Curr. Agric. Res. J.*, 11(3): 813-825. https://doi.org/10.12944/carj.11.3.12

Devi, A., K. Kumar and M.A. Khah. 2025. The potential of mutation breeding for ensuring sustainable food security. *Int. J. Adv. Res. Sci. Technol.,* 14(3): 1533-1541.

Estefan, G., R. Sommer and J. Ryan. 2013. Methods of soil, plant, and water analysis: A Manual for the West Asia and North Africa Region, *3rd ed. International Center for Agricultural Research in the Dry Areas (ICARDA),* pp. 1-243.

Haug, W. and H.J. Lantzsch. 1983. Sensitive method for the rapid determination of phytate in cereals and cereal products. *J. Sci. Food Agric.*, 34(12): 1423-1426.

Herlina, H. and H.P. Julsento. 2024. Dosis iradiasi sinar gamma terhadap pertumbuhan padi Mentik Wangi generasi M7. *J. Agrium*, 21(2): 171-177.

Hussain, S. and N. Mushtaq. 2025. Understanding zinc and its importance in human health. In: *Strategies to Alleviate Human Zinc Deficiency*, pp. 1-18. IGI Global Scientific Publishing.

Jangra, A. and S. Tiwari. 2025. Empowering nutrition: Biofortification to combat hidden hunger. In: *Advances in Agri-Food Systems: Volume II*, pp. 285-305. Springer Nature Singapore, Singapore.

Kaur, J.P., P. Alagappan, S. Singh, L. Kashyap, I. Dhaliwal, A.K. Padhy and V.S. Sohu. 2025. Genetic reduction of phytic acid and enhancement of micronutrient content in wheat (*Triticum aestivum* L.) to improve micronutrient bioavailability. *Euphytica*., 221(11): 180.

Kavitha Kumaravel, S.S.S. and N. Srividya. 2025. Evaluation of bioprocess-based technique for iron and zinc fortification in red rice genotypes. *Foods*, 14(18): 3162.

Khan, M.S.S., R. Basnet, S.A. Islam and Q. Shu. 2019. Mutational analysis of OsPLDα1 reveals its involvement in phytic acid biosynthesis in rice grains. *J. Agric. Food Chem.*, 67(41): 11436-11443.

Khoshnaw, M.R. and A.O. Esmail. 2020. Comparison between organic matter content of main soil orders in Kurdistan region using two different methods. *Iraq J. Agric. Sci.*, 51: 1-8.

Kong, D., S.A. Khan, H. Wu, Y. Liu and H.-Q. Ling. 2022. Biofortification of iron and zinc in rice and wheat. *J. Integr. Plant Biol.*, 64(6): 1157-1167.

Kumar, R., K. Maloa, D.S. Lagoriyab, A.K. Singh and A. Tutlani. 2025. Advancing nutritional quality through genome editing for biofortification: A comprehensive review. *J. Adv. Biol. Biotechnol.*, 28(10): 744-760.

Mughal, M. and C. Fontan Sers. 2020. Cereal production, undernourishment, and food insecurity in South Asia. *Rev. Dev. Econ.*, 24(2): 524-545.

Nguyen, T.D., A.A.T. Johnson, E. Lombi, C.L. Doolette, E. Smith and S.J. Watts-Williams. 2025. Arbuscular mycorrhizal fungal inoculation increases the bioavailability of zinc and iron in wheat grain. *Plants People Planet*, 1-13. https://doi.org/10.1002/ppp3.70051

Panhwar, Q.A., A. Ali and N. Depar. 2024. Influence of various organic nutrient management technologies on microbial abundance, growth, and yield of rice (*Oryza sativa* L.) under field conditions. *Mehran Univ. Res. J. Eng. Technol.*, 43(4): 69-81.

Pechdin, W. and J. Naruepatr. 2025. Toward rice export competitiveness, community development, and farmer well-being: Evidence from Thailand. *Multidiscip. Sci. J.*, 7(9): 2025443.

Qamar, Z.U., A. Hameed, M. Ashraf, M. Rizwan and M. Akhtar. 2019. Development and molecular characterization of low phytate basmati rice through induced mutagenesis, hybridization, backcross, and marker assisted breeding. *Front. Plant Sci.*, 10: 1525.

Raboy, V. 2020. Low-phytic-acid crops: Observations based on four decades of research. *Plants*, 9(2): 140.

Rahman, M.M., D.Y. Seo, M.M. Rahman, C.Y. Huang, A. Ogawa, T. Beaurepère and J.S. Jeon. 2025. Toward nutrient-rich rice: Biofortification through mineral accumulation and low phytic acid content. *J. Agric. Food Chem.*, 73(38): 24262-24277.

Rashid, A., M.A. Zeshan, N. Ghafoor, H. Bashir, F. Mehmood, M.A. Hameed and M.U. Ghani. 2025. Biofortification of wheat, rice, and maize with zinc: Combating hidden hunger. *Plant Environ.*, 6(01): 1-23.

Rohaeni, W.R., S.U. Trikoesoemaningtyas, M. Ghulamahdi, W.B. Suwarno and H. Aswidinnoor. 2023. Phytic acid content in biofortified rice lines and its association with micronutrient content and grain yield. *SABRAO J. Breed. Genet.*, 55(5): 1629-1640.

Sahu, A., R. Verma, U. Gupta, S. Kashyap and I. Sanyal. 2024. An overview of targeted genome editing strategies for reducing the biosynthesis of phytic acid: An anti-nutrient in crop plants. *Mol. Biotechnol.*, 66(1): 11-25.

Sandeep, T.S.R. and S. Godi. 2023. Insights into the biofortification of zinc and iron in rice. *Nutr. Food Sci. Int. J.*, 12(1): 1-7.

Siddiqui, M.A., S.M. Bhatti, I. Rajpar and N. Depar. 2025. Comparative efficacy of Zn-EDTA and $ZnSO_4$ in mitigating zinc deficiency in wheat for sustainable production. *Plant Prot.*, 9: 251-260. https://doi.org/10.33804/pp.009.02.5552

Takai, T., H. Saito, J. Marui, A.Z. Oo, K. Vilayheuang, S. Phongchanmixay and H. Asai. 2025. Detection and characterization of quantitative trait loci for phytic acid in grains toward improved black rice in northern Laos. *Front. Sustain. Food Syst.*, 9: 1620644.

Tang, Z., Z. Chen, Y. Gao, R. Xue, Z. Geng, Q. Bu, Y. Wang, X. Chen, Y. Jiang and F. Chen. 2023. A strategy for the acquisition and analysis of image-based phenome in rice during the whole growth period. *Plant Phenom.*, 5: 0058.

Wahyudi and E. Indrawanis. 2025. Response of total number of tillers, plant height, and dry straw weight of Jangguik rice genotypes (*Oryza sativa*) with gamma ray irradiation treatment. *Jurnal Agronomi Tanaman Tropika (JUATIKA)*, 7(1): 289-293. https://doi.org/10.36378/juatika.v7i1.4126

Wairich, A., F.K. Ricachenevsky and S. Lee. 2022. A tale of two metals: Biofortification of rice grains with iron and zinc. *Front. Plant Sci.*, 13: 944624.

Wang, Y., Y. Meng, Y. Ma, L. Liu, D. Wu, X. Shu, L. Pan and Q. Lai. 2021. Combination of high Zn density and low phytic acid for improving Zn bioavailability in rice (*Oryza sativa* L.). *Rice*, 14: 1-12.