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Nutritional and Mineral Enrichment in Gamma-Irradiated Rice Mutants and their Molecular Characterization Using QTL-Linked Markers

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Abstract: Rice (Oryza sativa L.) remains a major staple for millions in sub-Saharan Africa; however, its nutritional value and resilience to abiotic and biotic stresses are limited in locally adapted varieties. Mutation breeding using gamma irradiation offers a pathway to broaden the genetic base and enrich grain quality traits. In this study, three Nigerian rice genotypes (FARO-44, FARO-52, and FARO-60) were irradiated with Cesium-137 gamma rays at doses ranging from 100–250 Gy and advanced to the M_3 generation. Proximate analysis revealed significant ($P < 0.05$) improvements in moisture, protein, fat, fibre, and carbohydrate content among mutants, with FARO-44-100 (outstanding) consistently outperforming checks in protein (14.34%), carbohydrate (84.99%), and energy value (413.83 $kJ\ kg^{-1}$). Mineral profiling showed enhanced concentrations of calcium (1.09%), phosphorus (1.13%), and potassium (0.96%) in selected mutants, with dose-dependent variability across genotypes. Principal component analysis distinguished high-performing lines such as FARO-44-100 and FARO-52-150, confirming nutritional diversification. Molecular screening with QTL-linked markers revealed irradiation-induced variability in alleles related to abiotic stress (qAG1, qDTY1.1, qPSST6), biotic resistance (Pi9, Xa21), and grain quality (Waxy, GS3, ALK). Virus resistance (RTSV1) and grain filling (GIF1) were consistently present across mutants, while salinity and blast resistance were variable. Together, these findings demonstrate that gamma irradiation can generate nutritionally enriched and genetically diverse rice mutants, offering valuable materials for climate-smart rice improvement.

Keywords: Rice (Oryza sativa L.); Gamma irradiation; Proximate composition; Mineral content; Mutation breeding; Quantitative trait loci (QTL)

Introduction

Rice (Oryza sativa L.) is a principal staple food for over half of the world's population, providing not only calories but also essential macro- and micronutrients (proteins, carbohydrates, lipids, minerals). To meet rising demands and nutritional challenges, breeding rice lines with improved grain quality and stress resilience is critical.

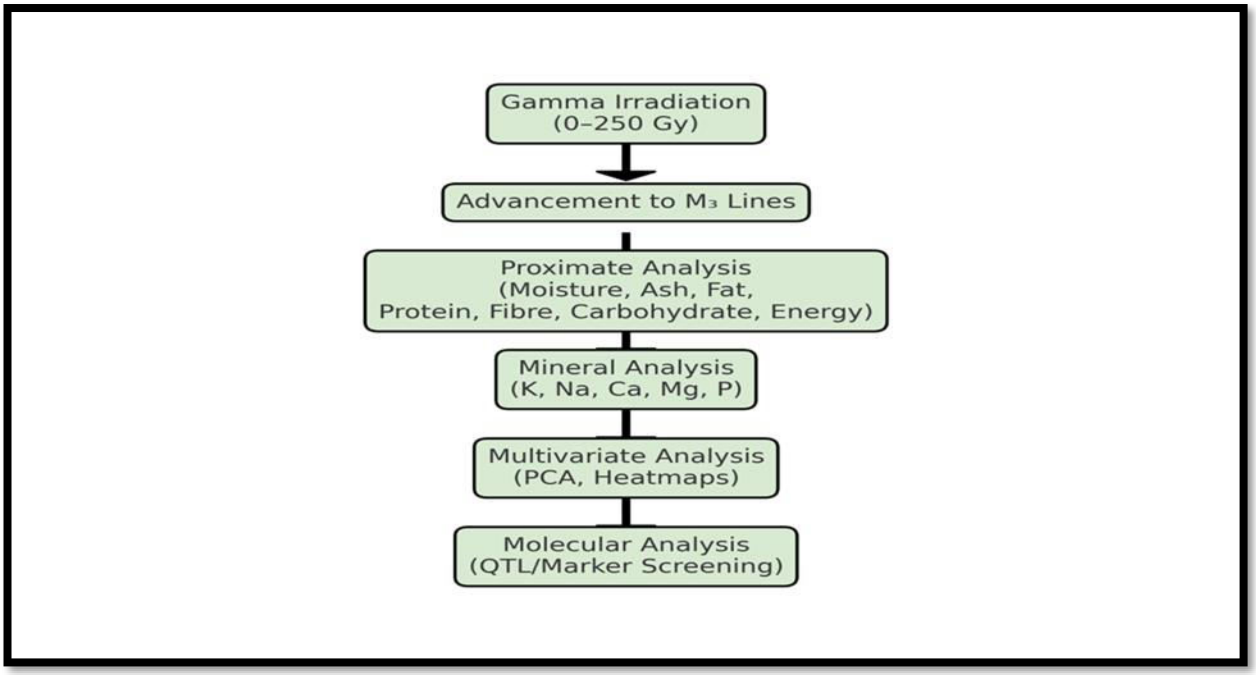
Mutation breeding, particularly using gamma irradiation, has long been a tool for inducing heritable variation outside the constraints of natural recombination. Early work demonstrated that irradiated rice lines could achieve higher protein content than controls. More recently, gamma irradiation has been used to generate improved agronomic variants while enhancing metabolite profiles (Chauhan et al., 2025). The mutational spectrum from cobalt-60 irradiation often includes point mutations, chromosomal breaks, or regulatory changes, enabling the emergence of novel alleles (Kiani et al., 2022). A systematic review of physical mutagens in rice confirms gamma-ray treatment as promising for broadening genetic diversity (Baaduet al., 2023).

However, many mutation breeding studies in rice focus primarily on agronomic traits (height, yield, flowering) and underutilize comprehensive grain quality (proximate, mineral) and molecular characterization. Integrating composition analysis with molecular tagging (QTLs/markers) enables the selection of mutants that combine nutritional improvement with stress tolerance.

Molecular breeding through QTL mapping and marker-assisted selection has matured rapidly. Stable QTLs for abiotic stress (e.g., drought, cold, salinity) have been validated across environments (Raj & Nadarajah, 2022). Breeders are increasingly pyramiding major-effect QTLs, such as QDTY for drought and Sub1A for submergence, to produce multi-stress-tolerant rice lines (Muthu et al., 2020). In functional quality traits, the Waxy locus (amylose) and GS3 (grain size) are frequently used marker targets in marker-assisted breeding. Moreover, submergence tolerance conferred by Sub1A remains a benchmark trait in flood-prone lowlands (Basu et al., 2024).

Given this context, our study aimed to apply gamma irradiation across genotype–dose treatments in three Nigerian rice genotypes, evaluate their proximate and mineral composition, perform multivariate trait analysis, and screen for molecular QTL/marker presence. This integrated approach aims to identify mutant lines that not only enhance nutritional quality but also carry favorable alleles for stress tolerance and grain quality, thereby potentially streamlining selection in breeding programs.

Materials and Methods



Plant material and gamma irradiation treatment

Certified seeds of three Nigerian lowland rice varieties—FARO-44, FARO-52, and FARO-60—were obtained from the National Cereal Research Institute (NCRI), Badeggi, Nigeria.Dry, equilibrated seeds of each variety were irradiated at 100, 150, 200, and 250 Gray (Gy) using a Cesium-137 source at the Centre for Energy Research and Training, Ahmadu Bello University, Zaria. A non-irradiated batch served as the control. Dose selection was informed by earlier radiosensitivity studies in rice (Mohammed et al., 2018).Dose verification was conducted using a calibrated dosimeter

Proximate composition analysis

Proximate composition of milled rice grains was determined according to the standard procedures of AOAC (2019). The moisture content was determined by oven-drying the samples at 105 °C until a constant weight was achieved. In comparison, ash content was determined by incinerating the samples at 550 °C in a muffle furnace. Crude fat was extracted using the Soxhlet apparatus with petroleum ether as the solvent. Crude protein content was estimated by the Kjeldahl method using a nitrogen-to-protein conversion factor of 6.25. Crude fibre was determined by subjecting the samples to sequential acid and alkali digestion. The carbohydrate content was calculated by difference, and the energy value was determined using Atwater conversion factors of 4, 9, and 4 kcal/g for protein, fat, and carbohydrate, respectively. All analyses were performed in triplicate, and the results are expressed as the mean ± standard error (SE). Full raw means, SE, and Tukey group letters are provided in Supplementary Table S1.

Mineral composition analysis

The mineral content of milled rice samples was determined using AOAC (2020) procedures. Approximately 1.0 g of finely ground rice flour was digested with a nitric–perchloric–sulfuric acid mixture (HNO_3:$HClO_4$:H_2SO_4, 650:80:20 v/v/v) until a clear solution was obtained, and then diluted to 500 mL. Calcium (Ca), magnesium (Mg), sodium (Na), and potassium (K) concentrations were quantified using Atomic Absorption Spectrophotometry (AAS; Buck Scientific 210 VGP, East Norwalk, USA) with standard calibration curves prepared from analytical-grade salts. Phosphorus (P) was determined colorimetrically by the molybdate blue method, and absorbance was measured at 660 nm. All analyses were conducted in triplicate, and the results were expressed as a percentage of dry weight (mean ± SE). Raw mineral means, SE, and Tukey group letters are listed in Supplementary Table S2.

Multivariate Statistical Analysis

The proximate composition dataset, comprising seven traits measured across all genotypes and treatment doses, was analyzed using Principal Component Analysis (PCA) to summarize trait variability and explore clustering patterns among mutant lines. Before analysis, the data were standardized to eliminate scale effects. Components with eigenvalues greater than 1 were retained, and biplots were constructed to visualize trait contributions and genotype groupings. All analyses were conducted in R (version 4.3.1) using the FactoMineR and ggplot2 packages for ordination and graphical outputs.

Molecular Marker Analysis

Genomic DNA was isolated from young leaf tissues of M_3 plants following the CTAB extraction protocol with minor modifications. The purity and concentration of DNA were verified by agarose gel electrophoresis and quantified using a NANO Drop spectrophotometer. A panel of published SSR and gene-specific markers associated with abiotic stress tolerance (qAG1, qDTY1.1, Sub1A, qPSST6), biotic resistance (Pi9, Xa21), and grain quality or yield-related traits (Waxy, GS3, Alk, qZn1.1, GIF1) was employed for PCR amplification. The marker/QTL list, including trait categories and chromosomal positions, is summarized in Supplementary Table S3. Each reaction was set up in a 20 µL volume containing 50 ng of template DNA, 1× PCR buffer, 2.0 MM $MGCL_2$, 200 µM DNTPs, 0.2 µM of each primer, and 1 U of Taq DNA polymerase. Amplified products were separated on 3% agarose gels, and bands were visually scored as present (+), absent (–), partially expressed (+–), or strongly expressed (++).

Data Analysis

Analysis of variance (ANOVA) for proximate and mineral composition data was performed using SAS v.9.4 (SAS Institute, Cary, NC, USA). Mean separation was achieved with Tukey's HSD test at a significance level of $P < 0.05$. To visualize trait associations, heatmaps of standardized values and genotype–trait matrices were constructed in R using the heatmap package. Chromosomal ideograms representing marker positions were drawn using customized matplotlib scripts in Python v.3.10.

Results

Gamma irradiation induced substantial variation in the proximate composition of the three rice genotypes evaluated (Figure 1). Detailed raw values (means ± SE) and multiple-comparison letters for all proximate traits are reported in Supplementary Table S1. Moisture content was markedly reduced in several mutants, with FARO-44-100 Gy recording the lowest value (4.01%), while FARO-52-250 Gy showed the highest (7.95%). Ash content followed a similar pattern of variability, ranging from 0.11% in FARO-60-100Gy to 0.44% in FARO-44-100 Gy. Fat content increased considerably in FARO-44-250 Gy (5.87%), whereas FARO-60-150 Gy exhibited the lowest level (3.21%). Protein concentration varied widely across mutants; the highest value (14.34%) was observed in FARO-44-100 Gy, almost twice that of FARO-44-200 Gy (6.81%). Fibre content was elevated in FARO-44-100 Gy and FARO-52-150 Gy, both exceeding 0.8%, but remained minimal in FARO-60-100 Gy and FARO-60-150 Gy. Carbohydrate content was generally high, with FARO-44-100 Gy (84.99%) outperforming all other genotypes, while the lowest energy values (381.67 kJ kg^{-1}) occurred in FARO-60-150Gy compared with 413.83 kJ kg^{-1} in FARO-44-100 Gy.

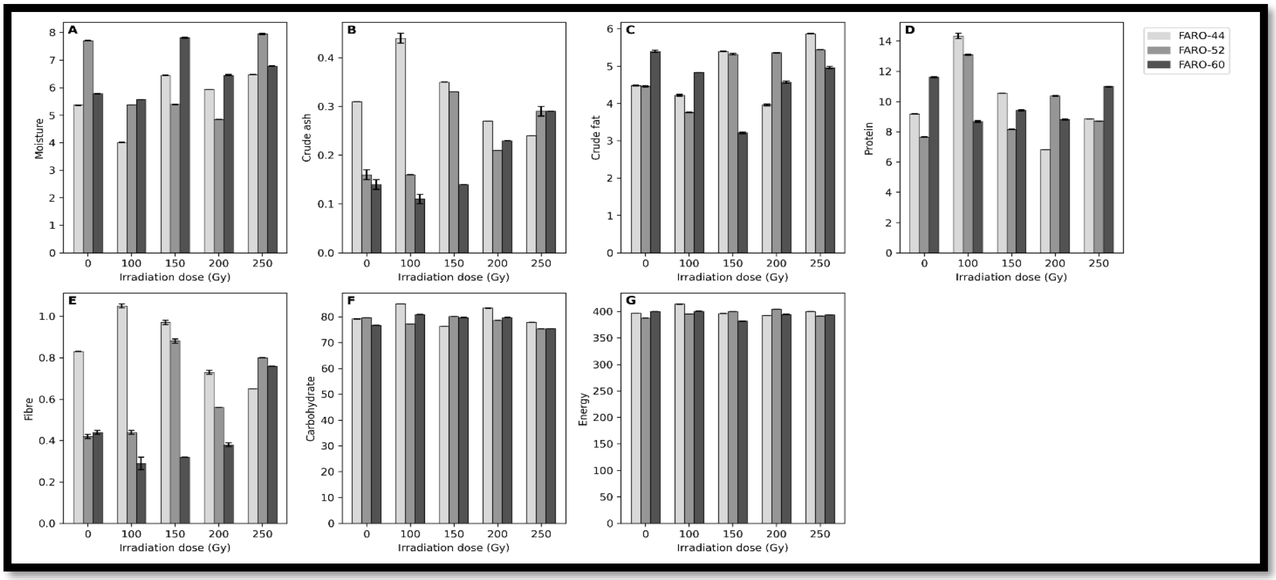


Figure 1. Proximate composition of gamma-irradiated rice mutants.

(A) Moisture, (B) Crude Ash, (C) Crude Fat, (D) Protein, (E) Fibre, (F) Carbohydrate, and (G) Energy across FARO-44, FARO-52, and FARO-60 genotype–dose combinations. Bars show mean ± SE; FARO-52 is indicated with diagonal hatching to distinguish the mid-genotype in grayscale. X-axis shows "Mutant line (Genotype–Dose, Gy)". Differences among mutants were significant by ANOVA ($P < 0.05$); detailed means ± SE and multiple-comparison letters are provided in Supplementary Table S1

Mineral composition also revealed significant dose-dependent responses (Figure 2).Data with SE and Tukey letters are presented in Supplementary Table S2; the full ANOVA appears in Supplementary Table S4. Potassium content reached a maximum of 0.96% in FARO-44-100 Gy but was reduced to 0.33% in FARO-60-200 Gy. Sodium was highest in FARO-44-100 Gy (0.22%) but least in FARO-60-0 Gy (0.08%). Calcium levels were enhanced in FARO-44-100 Gy (1.09%) and lowest in FARO-60-0 Gy (0.18%). Magnesium peaked in FARO-60-150 Gy (1.83%), while phosphorus attained its highest concentration in FARO-44-100 Gy (1.13%), nearly five-fold greater than the 0.25% recorded in FARO-52-150.

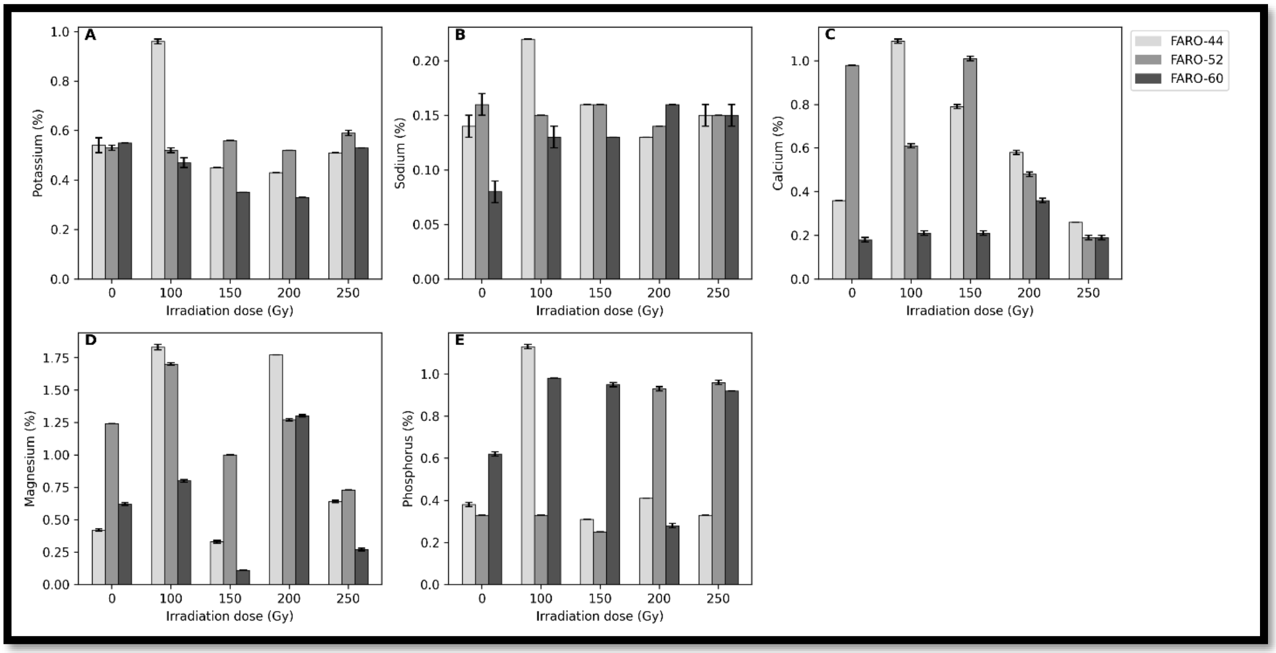


Figure 2. Mineral composition of gamma-irradiated rice mutants.

(A) Potassium (K), (B) Sodium (Na), (C) Calcium (Ca), (D) Magnesium (Mg), and (E) Phosphorus (P). Bars show mean ± SE with the same grayscale/hatch scheme as Fig. 1 and x-axis "Mutant line (Genotype–Dose, Gy)". Group differences were significant by ANOVA ($P < 0.05$); see Supplementary Table S2 for means ± SE.

Principal component analysis (Figure 3) summarized the variation in proximate traits, with the first two principal components explaining 67.3% of the total variance (PC1 = 43.5%; PC2 = 23.8%). PC1 was driven by positive associations with moisture and protein, contrasting with negative associations with carbohydrate, effectively separating FARO-44-100 from other lines. PC2 was primarily defined by fat and fibre, distinguishing FARO-44-150 Gy and FARO-52-250 Gy as nutritionally distinct clusters. This multivariate approach highlights the combined contribution of proximate traits in differentiating irradiated mutants and underscores the unique profiles of FARO-44-100 Gy and FARO-52-150 Gy.The standardized trait matrix used for PCA is provided in Supplementary Table S1.

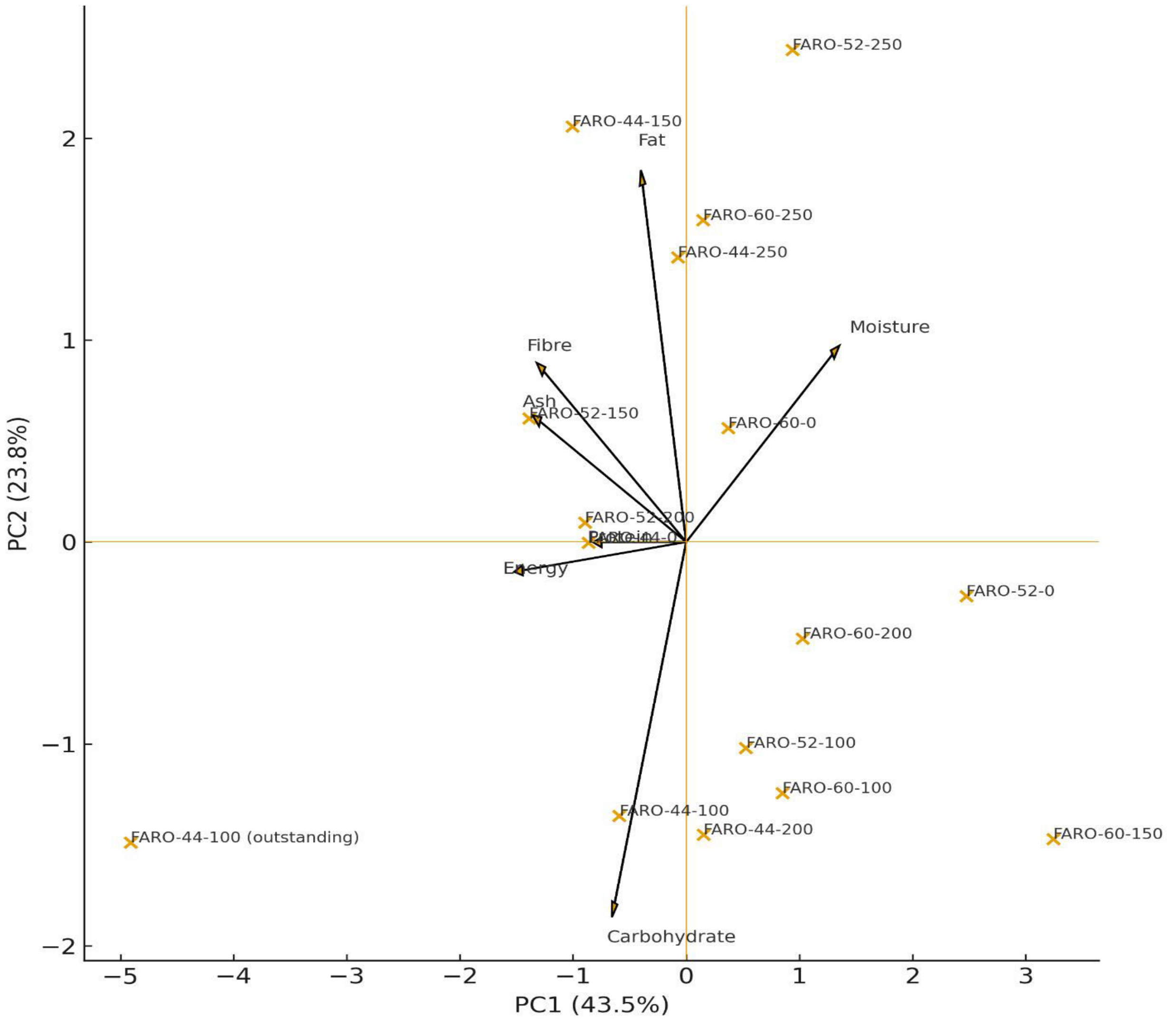


Figure 3. Principal component analysis (PCA) of proximate traits

Biplot of genotype–dose combinations (scores) and trait loadings (arrows). PC1 and PC2 explain the variance shown on the axes (PC1 ≈ 43.5%, PC2 ≈ 23.8% in the current plot). Positive PC1 loadings align mainly with Moisture/Protein/etc, as shown, separating lines such as FARO-44-100 (outstanding) and FARO-60-150 from other treatments.

Molecular analysis further revealed irradiation-induced variability in key stress and quality-related loci (Figure 4). The QTL/marker set, trait categories, and chromosomal positions underlying Fig. 4A–B are summarized in Supplementary Table S3.The chromosome ideogram (Figure 4A) mapped multiple QTLs associated with abiotic stress tolerance (qAG1, qDTY1.1, qPSST6), biotic resistance (Pi9, Xa21), and grain quality (Waxy, Alk, GS3, qZn1.1). The presence–absence matrix (Figure 4B) showed differential retention of these loci among the 16 mutant lines. Notably, S1, S5, S14, and S16 combined multiple favorable alleles for abiotic stress tolerance, whereas S13 lacked several resistance loci. Virus resistance (RTSV1) was consistently detected across all lines, while blast resistance was weak or partial in most cases.

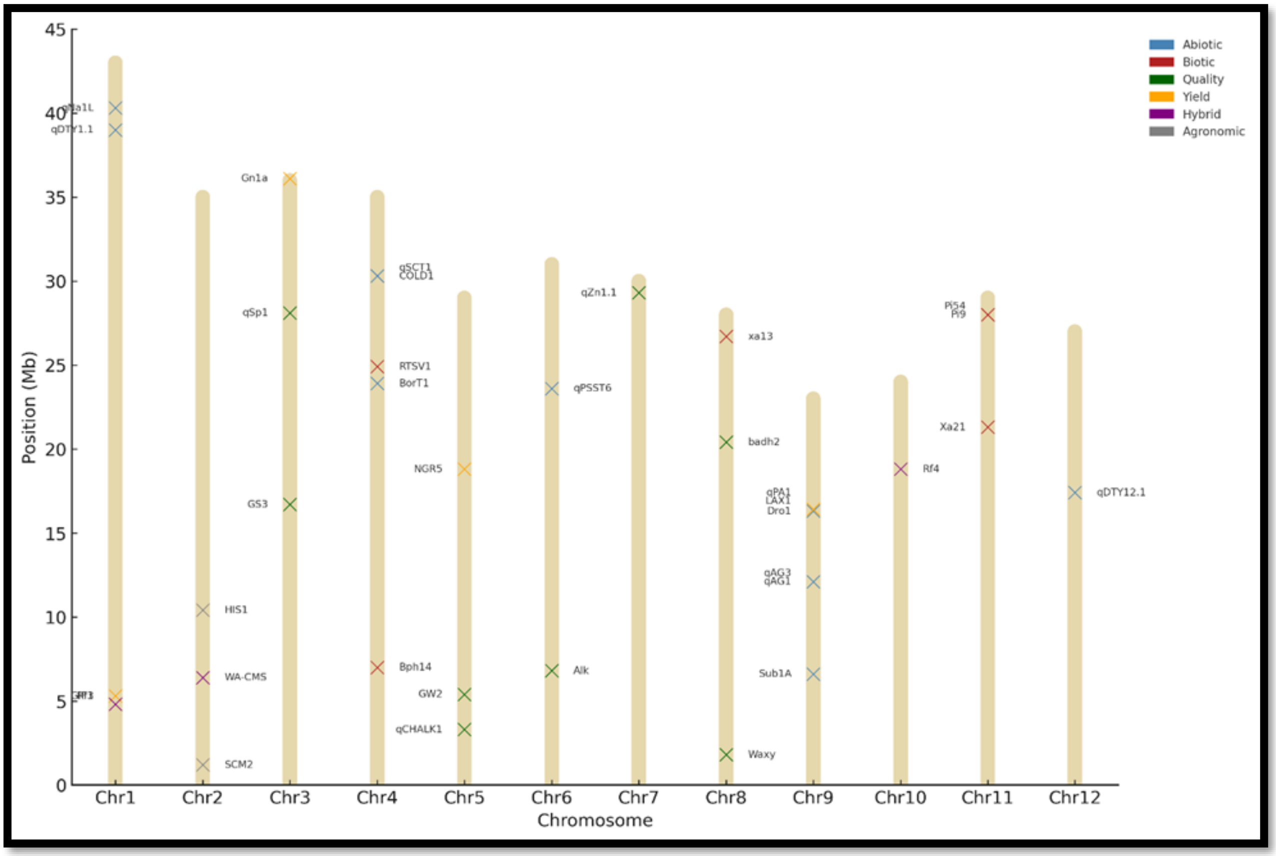
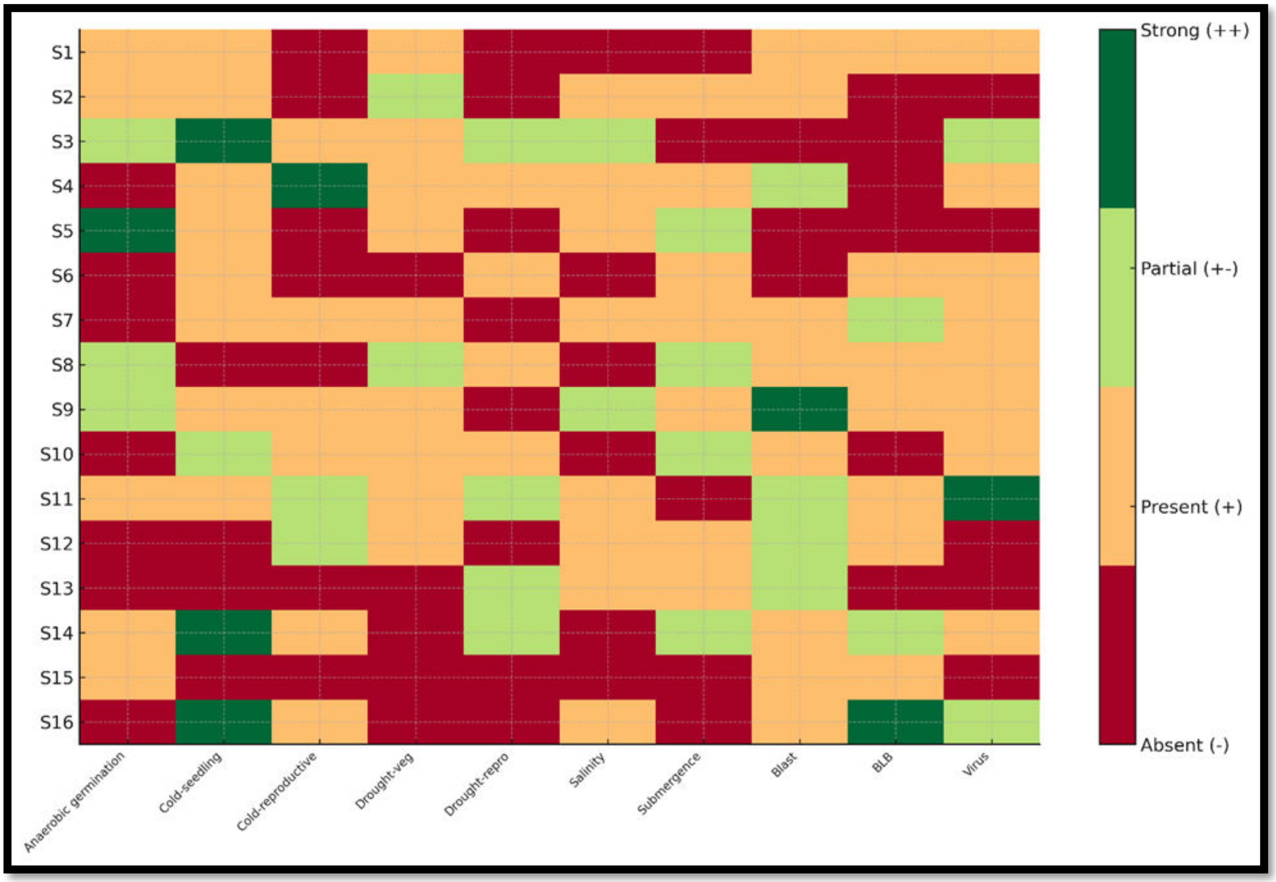


Figure 4. Molecular features of the mutant panel.

(A)*Chromosome ideogram mapping representative QTLs/genes by physical position (Mb) across the 12 rice chromosomes. Colors denote trait categories: Abiotic (blue), Biotic (red), Grain quality (green), Yield (orange), Hybrid (purple), Agronomic (gray).*



(B)*Presence/absence matrix for stress- and disease-related QTLs across lines S1–S16. Color scale encodes Absent (–), Present (+), Partial (+–), and Strong (++). The heatmap summarizes Supplementary Table S3 and highlights lines carrying multiple favorable alleles for abiotic stress and disease resistance.*

Discussion

Proximate and mineral responses to gamma irradiation

Gamma irradiation generated apparent, dose-dependent shifts in proximate composition across FARO-44/52/60 lines. Reductions in moisture alongside increases or rebalancing of protein and fat in specific mutants (e.g., high protein in FARO-44-100 (outstanding) and high fat in FARO-44-250) are consistent with reports that γ-irradiation can lower moisture and modulate macromolecules through starch–protein–lipid rearrangements and mild radiolysis of water and polysaccharides (Reddy & Viswanath, 2019). Studies on irradiated rice flours also show dose-dependent effects on amylose content and gelatinization, as well as on functional properties, supporting our observation that proximate traits separate mutants clearly on PC1–PC2 (Fig. 3) (Razaak*et al.,* 2021).

Mineral profiles varied among lines (notably higher Ca and P in some FARO-44 mutants, and high Mg in FARO-60-150). While reports on direct mineral shifts after γ-irradiation are fewer in number than those on proximate quality, mutation breeding reviews document that doses in the 100–300 Gy range frequently induce selection-worthy physiological and biochemical variants, including grain quality attributes that may indirectly co-vary with mineral loading (Baadu*et al*., 2023). Our results add to this evidence by quantifying significant, genotype-specific mineral differences.

Multivariate structure of quality traits

The PCA biplot (Fig. 3) captured more than 65% of the variance on the first two axes, with moisture/protein versuscarbohydrate as the dominant contrast. This observation aligns with previous findings that irradiation and post-irradiation selection can alter the starch–protein balance and, consequently, the carbohydrate–energy axis of rice quality (Reddy & Viswanath, 2019). The distinct positioning of FARO-44-100 (outstanding) and FARO-52-250 highlights how different doses and genetic backgrounds produce contrasting nutritional "signatures" that are practically useful for quality-oriented selection.

Molecular landscape and relevance to stress/quality breeding

The chromosome ideogram (Fig. 4A) and genotype×trait heatmap (Fig. 4B) show that several stress- and quality-related loci are retained or newly revealed in the mutant panel. The presence of qDTY1.1 and related qDTYs is noteworthy, given their long-demonstrated, background-independent contributions to reproductive-stage drought tolerance and yield stability in diverse germplasm and breeding products (Vikram *et al*., 2011). The detection of Sub1A in a subset of lines aligns with its canonical role in submergence tolerance and the broad body of work that translated SUB1 from discovery to mega-varieties via MAS (Waadt*et al*., 2022). For cold tolerance, the COLD1/qSCT1 phenotype is consistent with recent gene/QTL reports at the seedling stage, reinforcing the plausibility of the signals observed in our FARO backgrounds (Xiao *et al.,* 2018).

On grain quality, our molecular signals at Waxy (Wx) and GS3 match their known functional roles—Wx as the primary determinant of endosperm amylose via GBSSI splicing/abundance, and GS3 as a key controller of grain length/size—providing a genetic context for the proximate differences among lines (Gu *et al.,* 2023). The limited detection of the badh2 (aroma) marker in our panel is also unsurprising, as aroma is often absent outside targeted aromatic backgrounds or requires specific mutant alleles; mutation breeding can introduce such alleles, but with variable frequency (Hasibuzzaman*et al.,*2025).

Collectively, the molecular profiles indicate that gamma irradiation generated useful allelic mosaics, as some mutants combine favorable abiotic-tolerance markers (e.g., DRO1/QDTY loci and Sub1A) with high-quality alleles (e.g., GS3/Wx). This aligns with wider experience that physical mutagens are effective for broadening allelic diversity for both agronomic resilience and grain quality targets (Baadu*et al.,* 2023).

Implications, Limitations, and Future Directions

From an applied breeding standpoint, the FARO-44-100 (outstanding) line remains the most nutritionally enhanced mutant, combining high protein, carbohydrate, and energy values with elevated Ca and P concentrations. In parallel, several FARO-60 and FARO-52 derivatives exhibit molecular signatures for abiotic-stress tolerance, suggesting their suitability for integration into quality- and resilience-focused improvement programs.

A key limitation of this study is the absence of field-level yield evaluation and validation of abiotic stress. Nevertheless, prior research demonstrates that pyramiding QDTY and SUB1A loci in elite rice backgrounds confers substantial drought- and submergence-tolerance advantages, providing a clear trajectory for these mutants. Consequently, the next phase should involve marker-assisted forward selection followed by multi-environment testing under drought, submergence, and iron (Fe) toxicity stress conditions, coupled with standard yield trials (Dhawan *et al*., 2021).

Future investigations should further quantify amylose content and gelatinization temperature to substantiate the quality enhancements implied by Wx-related allelic variation and proximate shifts. Additionally, cold-tolerance assays are recommended when the COLD1 and qSCT1 loci are detected. Testing in Fe-rich, waterlogged lowland soils is particularly critical, given the prevalence of Fe toxicity in African rice ecologies and its impact on yield and nutrient balance (Razaak*et al*., 2021).

Conclusion

Gamma irradiation induced stable nutritional and genetic variation in Nigerian rice, enhancing protein, energy, and mineral content, particularly in FARO-44-100 and FARO-52-150. Distinct clustering of superior mutants and irradiation-driven polymorphisms linked to *GIF1* and *RTSV1* confirm its dual role in quality enrichment and allelic diversification. These mutants serve as promising pre-breeding materials for developing climate-smart, nutrient-dense rice varieties suited to sub-Saharan Africa.

Conflict of Interest

The authors declare that they have no known financial or personal conflicts of interest that could have influenced the research presented in this study.

Data Availability Statement

The datasets generated and analyzed during the current study are available from the corresponding author on reasonable request. Due to institutional data management policies, the data are not publicly archived; however, they can be shared for academic and non-commercial purposes upon request.

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