

Original Research Article

Enhancing agro morphological traits of three unquetradeitonalrice landraces of Chhattisgarh through Gamma ray-irradiation

Abstract:

Mutation breeding is a simple and effective technique for generating genetic variation within crops. Mutagens such as Gamma radiation induce changes in their DNA that could lead to the development of new traits, such as improved yield or disease resistance. In the present study, Gamma ray mutagenesis was performed on three traditional rice varieties (Gudma dhan, Dawar dhan, and Lohandi dhan) to identify potentially beneficial mutants with desirable agro-morphological agricultural characteristics. The major constraints to address were their tall statures and weak culms, fewer panicles and tillers and late maturity. Using a fixed dose of 300 Gy, 35 mutants were identified in Dawar dhan, 33 in Gudma dhan, and 21 in Lohandi dhan. The selected mutants showed an average reduction in plant height that ranged from 42.69% to 33.11%, as compared to their respective landraces. Also, a substantial reduction (ranging from 3.08%-10.55%) in days to 50% flowering was observed. Importantly, these mutants showed an increase in yield of 38.11% (Dawar dhan), 32.63% (Gudma dhan), and 7.0% (Lohandi dhan). These findings demonstrate the utility of mutation breeding through Gamma radiation in alleviating the key constraints inherent to traditional rice landraces.

Keywords: *Rice landraces, Gamma ray, screening, potential mutants, advancement*

1. Introduction:

India, the second-largest producer of rice globally, is blessed with a wide array of agro-ecological conditions and a rich biodiversity of rice varieties (ref). The state of Chhattisgarh,

known as the "Rice Bowl of India," is particularly famous for its diverse rice cultivars. The Indira Gandhi Krishi Vishwavidyalaya (IGKV) in Raipur, Chhattisgarh, houses a vast collection of around 23,250 rice accessions (Thada et al., 2024) [1]. Various traits, like abiotic and biotic resistance, pigmentation, and medicinal properties have been found in these accessions. Traditional knowledge about these rice varieties was gathered from local farmers and villagers who are being cultivated and consumed these for generation after generation. Preservation and study of these diverse rice varieties, along with their documentation emphasizing traditional knowledge associated with them, are crucial for agricultural research, biodiversity conservation, and the exploration of potential therapeutic applications. The landraces collected by Dr. Ricchharia (Year ?) were classified according to their traits. The medicinal rice landraces were documented along with their pharmacological properties (Table 1). Mutation breeding is a very powerful and effective tool which can help in the development of varieties within a short duration (Stadler, 1928) [2]. Climate change has increased the incidences of biotic and abiotic stresses. Developments of new cultivars or hybrid varieties through conventional breeding methods require a long time but such varieties have narrow genetic bases which make them more susceptible to changing climatic conditions. Landraces are generally have broad genetic bases so, can play a very important role in today's scenario, particularly adapt to varying climatic conditions Hour et al., 2020 [3]. Unfortunately the traditional rice varieties have several undesirable traits like late maturing, uneven panicle maturity, tall stature, lodging susceptibility and low yield. To improve these traits, radiation-induced mutation breeding was exercised as demonstrated by Tiwari et al., 2018 [4]. In the present study, 3 traditional rice landraces of Chhattisgarh, Dawar dhan, Gudma dhan and Lohandi dhan which are popular for their medicinal properties were exposed to 300 Gy of Gamma ray. These landraces are well known to have high iron and zinc contents. Due to such properties, these landraces were included in the radiation-induced

mutation breeding programme to improve the overall **plant architecture and other yield enhancing morphological traits**. The selection of mutants was carried out using changes in morphological traits. **Since, mutations** are mostly recessive, hence **selection practice** in M_2 generation **would be** the most effective for identifying the potentially improved **segregated lines** (Zhu et al., 2017) [5].

2. MATERIAL AND METHODS:

2.1. Mutation breeding: Three traditional medicinal **rice** landraces from Chhattisgarh, **Dawar dhan, Gudma dhan and Lohandi dhan** (Table 2), were subjected to mutation breeding. **Dawar dhan produced medium- bold grain, Gudma dhan short- bold grain while Lohandi dhan produced long- slender grain.** Gamma rays were used to **create** mutations in the paddy seeds. A dosage of 300 Gy (exposure time of 10 m) was given to the seeds in a **Gamma** chamber (GC5000). After irradiation, the seeds **were kept 7 days in isolation** for radioactivity decay. The irradiated M_1 seeds were sown in the Research cum Instructional **Farm under** the Department of Genetics and **Plant Breeding, Indira** Gandhi Krishi Vishwavidyalaya (IGKV), Raipur in the Summer (Rabi) season of 2020-21.

2.2. Cultivation of M_1 and M_2 generations: **Lohandi dhan** was the tallest with an average height of 158 cm, followed by **Dawar dhan** with a height of 156 cm and **Gudma dhan** had a height of 154 cm. **Required amounts of seeds of the landraces** were procured from R.H. Richharia medium-term germplasm storage, IGKV (Raipur). All the required cultural and agronomic properties were adopted to cultivate the M_1 generation. Mother panicle from each plant was harvested and stored separately **to sow** in the subsequent **seasons**.

The seeds harvested **from** M_1 generation were sown in Kharif 2021, in the Research **Farm** of IGKV using panicle to row method. Seeds from each plant were sown in separate rows. The row-to-row and plant-to-plant **distances were** maintained at 20 cm x 20 cm. Around 10,987 plants were **raised** for Gudma dhan, 11,256 for **Lohandi dhan** and 11,105 plants of Dawar.

dhan. In addition, untreated seeds of landraces were sown alongside to act as controls or checks. All the required agronomic practices were adopted for the cultivation of the M₂ generation.

2.3. Screening of mutants: Rigorous screening was carried out in the M₂ generation to identify desirable mutants considering the following criteria:

1. Albino mutants were rejected as they could not survive for more than a few days.
2. Plants with reduced height as compared to their parents
3. Plants with a higher number of tillers
4. Plants with more number of effective panicles
5. Plants with early flowering
6. Plants with early maturity
7. Plants with evenly maturing panicles

2.4. Bagging, tagging and numbering: The panicles of selected mutants were covered with butter paper bags to prevent the seeds from shattering. The selected mutants were tagged in the following manner:

- 1). The first line indicated the name of the parent variety
- 2). The second line represented the generation of mutants
- 3). The third line indicated the mutant number. The number started with one with a prefix P and the numbering progressed with screening and selection.
- 4). Lastly, the tags denoted the special characters, the mutants were displayed like early flowering, early maturity etc.

For Example: A mutant of Dawar dhan identified in 2nd generation, 5th in line was tagged as:

DawardhanM₂, P5

Early flowering

2.5. Mutation frequency: The mutagenic frequency of induced mutations was estimated using the progenies in the M₂ population with the following formula as given by Gual, 1964 :

$$\text{Mutation frequency} = \frac{\text{Number of mutated progenies}}{\text{Total progenies in M}_2 \text{ population}}$$

The observations for plant height (cm), panicle length (cm), flag leaf length (cm), flag leaf width (cm), number of tillers per plant, effective number of tillers per plant and grain yield per plant (g) were recorded to assess the amplitude of variability induced due to radiation-induced **mutation**.

2.6. Statistical analysis: All the data **analyses were** carried out using MS Excel and Rstudio.

Table 1: Details of medicinal properties of landraces **according to** Dr. R.H. Ricchharia **(Year ?)**

Sr. No.	Name of rice Landrace	Medicinal properties
1.	Gudma dhan	<ul style="list-style-type: none">• For addressing Anemia which is also called "hele" in halbi language• For addressing breathlessness (Asthma) which is also called as "dhaki" in halbi language• For controlling blood sugar in case of diabetes
2.	Lohandi dhan	Used to enhance the immunity and good nutritional value
3.	Dawar dhan	Safe pregnancy and easy removal of placenta in cows with good nutritional values

4.	Layacha	Rice is eaten by females for expecting delivery of a healthy infant.
5.	Maharaji	Rice is eaten by females for overcoming postpartum weakness
6.	Gathuwan	Rice is known for reducing intensity of joint pains in humans
7.	Soth	It is useful for people suffering from cold.
8.	Sarai phool	Rice is known for removing weakness in humans.
9.	Karhani	This rice is known to be used for patients suffering from paralysis.
10.	Resari	Rice is fed to cattle for overcoming weakness after being overcooked in water in a semi solid form.
11.	Chepti-gurmatiya	Rice is believed to be useful for people suffering from diabetes.

Table 2: Passport **information** of landraces used in the experiment.

Entry	Genotype	CGR No.	Location			
			Village	Block	District	Province
1.	Dawar dhan	CGR-725	Pidapal	Kanker	Bastar	C.G.
2.	Gudma dhan	G-1042	Faraspal	Gidam	Dantewada	C.G.
3.	Lohandi dhan	CGR-249	Chilhari	Manpur	Shahadol	M.P.

Table 3: Agro-morphological details for Dawar dhan, Gudma dhan and Lohandi dhan

Landrace	Days to 50% flowering	Plant height (cm)	Panicle length (cm)	Flag leaf length (cm)	Flag leaf width (cm)	Total tillers	Effective tillers	Yield/ plant (g)
Dawar dhan	110	156	28.6	31.5	1.1	9	9	9.5
Gudma dhan	91	124	31.3	29.2	1.6	6	6	2.95
Lohandi dhan	92	128	29.3	41.1	1.3	9	9	8

3. RESULTS AND DISCUSSION:

Radiation-induced mutation can result in micro as well as macro mutations. Macro mutations are easily visible to the naked eyes and micro mutations mostly occur at the molecular level. The present study focused on macro mutations which are required to improve the overall agro-morphological traits of the rice to evolve new superior varieties in future.

3.1. Mutation frequency: Mutation frequencies obtained in Dawar dhan, Gudma dhan and Lohandi dhan are mentioned in Table 4. The highest numbers of mutants were isolated from Dawar dhan, followed by Gudmadhan and Lohandi Dhan.

Table 4: Mutation frequencies obtained from the landraces

Landrace	Albino mutants	Normal mutants	Total mutants	Total plants in M2	Mutation frequency (%)
Dawar dhan	13	22	35	11,105	0.31
Gudma dhan	18	15	33	10,987	0.30
Lohandi dhan	12	9	21	11,256	0.18

3.2. Selection of mutants: Based on the observations and desirable traits, mutants were carefully selected from a population of around 11000 plants. The various observations recorded are presented in Table 5.

Table 5: Morphological observations of the putative mutants.

Genotype	Days to 50% flowering	Plant height (cm)	Panicle length (cm)	Flag leaf length (cm)	Flag leaf width (cm)	Total tillers	Effective tillers	Yield/ plant (g)
Gudma dhan mutants								
GM-1	81	110	18	22.5	1.5	12	9	4.54
GM-2	88	116	18	26.5	1.2	5	2	6.23
GM-3	79	103	11	31	1.3	16	10	10.00
GM-4	90	106	21	35	1.2	5	5	6.20
GM-5	78	97	9.5	29	1.3	7	7	6.53
GM-6	80	102	16	28	1.4	7	6	3.84
GM-7	81	102	11	38.5	1.1	8	5	4.58
GM-8	82	101	21	27	1.7	8	7	5.64
GM-9	77	139	28	48	1.7	15	13	5.38
GM-10	81	76	13	21.2	0.8	3	2	4.02
GM-11	76	107	18.5	27.3	1.3	10	7	3.20
GM-12	81	99	19	34.6	1.1	3	2	3.94
GM-13	86	82	11.5	31	1.7	3	3	4.98
GM-14	80	103	16.5	22.3	1.3	8	9	6.83
GM-15	81	102	18.5	28.5	1.1	6	7	5.74
Lohandi dhan								
LM-2	85	89	12	24.2	1.1	6	3	12.33

LM-3	91	110	15	22.8	0.9	10	6	11.87
LM-4	91	76	13	20.5	1.1	2	1	9.51
LM-5	90	95	18	16	0.6	5	4	8.30
LM-6	89	117	17	27.3	1.5	5	2	7.67
LM-7	89	114	18.3	19.1	0.9	13	14	6.37
LM-8	90	99	17.3	15.4	1.6	2	6	7.00
LM-9	89	77.1	11.4	18.5	1.3	1	1	6.20
Dawar dhan								
DM-1	91	123	27.1	31.6	1.1	4	4	10.83
DM-2	110	80	14.7	30	0.9	3	3	10.2
DM-3	97	86	15	18.6	1.1	7	4	13.71
DM-4	95	74	11	8.3	1.2	3	3	11.20
DM-5	115	91	17.5	24.3	1.1	6	6	12.33
DM-6	110	94	15	20.1	1.1	6	6	10.00
DM-7	112	89	13	19	1.3	3	3	12.13
DM-8	108	89	13	19	1.3	3	3	12.67
DM-9	107	89	15.1	26.3	1.1	8	7	13.73
DM-10	97	95	15.5	19.1	1.2	7	7	12.33
DM-11	91	82	12.5	19.3	0.9	3	3	10.93
DM-12	94	82	13.5	17.4	0.5	6	4	8.67
DM-13	113	86	13.5	25	1.2	10	4	9.33
DM-14	110	93	11.5	18.3	1.1	7	5	11.47
DM-15	111	95	17.2	26.4	1.5	6	3	13.60
DM-16	98	99	17.3	15.4	1.6	6	2	9.83

DM-17	88	86	13.5	25	1.2	4	3	8.00
DM-18	100	84	13.5	25	1.2	4	3	9.33
DM-19	91	85	21	11	1.4	9	7	12.25
DM-20	94	86	12.5	18	1	8	7	11.52
DM-21	91	91	21.5	18.5	1.2	7	6	8.95
DM-22	85	95	18.4	22.5	1.3	9	8	8.54

*GM = Gudma mutant, DM = Dawar mutant, LM = Lohandi mutant,

3.2.1. Decrease in height: One of the most important characteristics of high-yielding paddy varieties is dwarfness. This reduces the risk of lodging and early shattering of grains (ref). In this study, induced mutation with Gamma rays reduced the height of landraces. In Dawar dhan, 20 mutants, in Gudma dhan 15 mutants and in Lohandi dhan 8 mutants were identified with significantly reduced height as compared to their respective parents. The average height of Dawar dhan's mutants was 89.4 cm ranging from 74 to 123 cm. The selected mutants exhibited a decrease of 42.69% in height as compared to the parent. In the case of Gudma dhan, the mutants measured 33.11% less with an average of 103 cm, ranging from 76 to 139 cm, while Lohandi dhan's mutants exhibited a decrease of 38.52% in height, with a mean value of 97.13 cm and a range of 76 to 110 cm (Fig 1.).

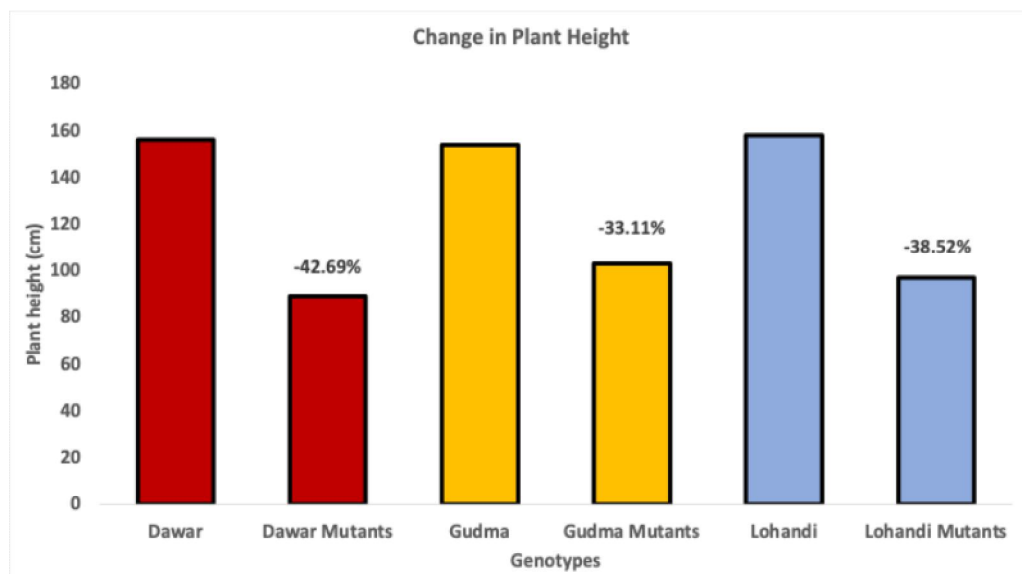


Fig. 1: Bar graphs representing a decrease in average height in selected mutants as compared to their parents

3.2.2. Decrease in days to 50% flowering: One of the major aims in mutation breeding was to decrease the maturity duration. The average number of days to 50% flowering in selected mutants were 101.6, 81.4 and 89.17 respectively. The number of days to 50% flowering in parents of Dawar dhan, Gudma dhan, and Lohandi dhan, were 110, 91 and 92 respectively. A decrease of 7.63%, 10.55% and 3.08% was recorded in the Dawar dhan, Gudma dhan and Lohandi dhan mutants respectively.

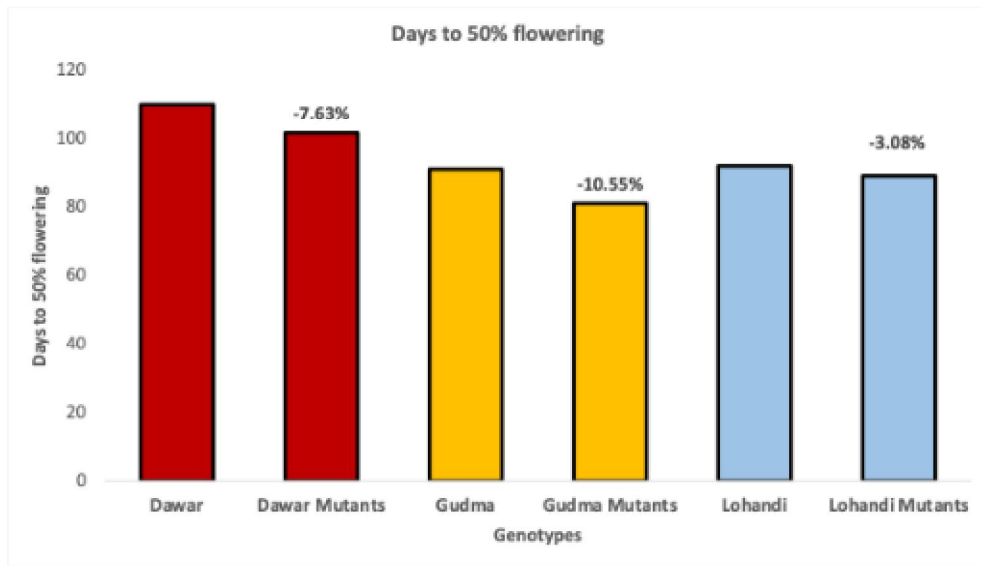


Fig. 2: Bar graphs representing a decrease in the days to 50% flowering in selected mutants as compared to their parents

3.2.3. Increase in yield per plant: Tillers harbour panicle at the end of their vegetative growth contributed positively to increasing the grain yield. Mutants were screened for yield/plant. It was observed that Dawar dhan, Gudma dhan and Lohandi dhan mutants exhibited an increase in yield by 38.11%, 32.63% and 7.0% respectively. The average yield of Dawar dhan mutants was 5.65 g while of that of Dawar dhan parent was 3.5 g. In the case of Gudma dhan mutants, the average yield per plant was 4.38 g and that of Gudma dhan parent was 2.95 g. Lohandi dhan parent had a yield of 3.0 g while its mutants exhibited 3.25 g of average yield per plant.

3.3. Correlation analysis: Selection in M_2 generation was done as most of the mutations were recessive. Variations in M_2 generation may be due to genetic changes or they could be the influence of environment. The influence of environment or gene segregation can only be ruled out after 3-4 generations of progeny testing. Correlation analysis in early generations can help in determining promising mutants which align with the desired traits.

3.3.1. Gudma dhan and its mutants: Observations like days to 50% flowering, flag leaf length, and flag leaf width exhibited a positive correlation with yield per plant. The Fig 4. represents the scatter diagram and Fig 5 represents the coefficients of correlation among different pairs of observations.

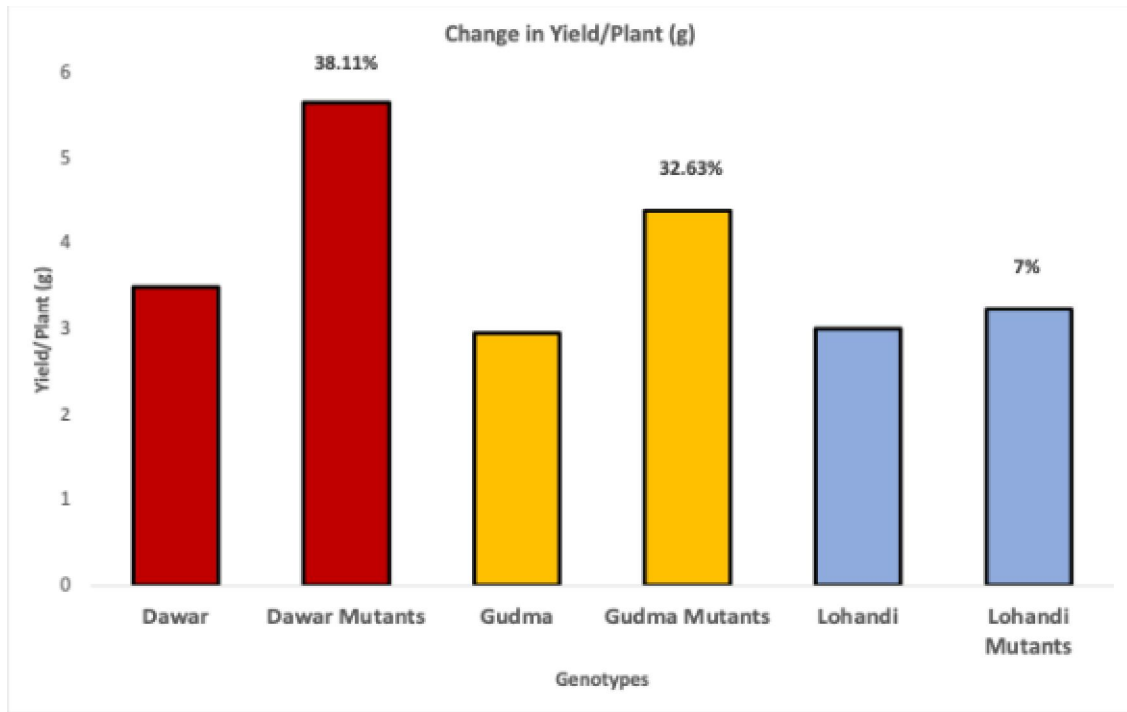


Fig. 3: Bar graphs representing an increase in the yield/ plant in selected mutants as compared to their parents

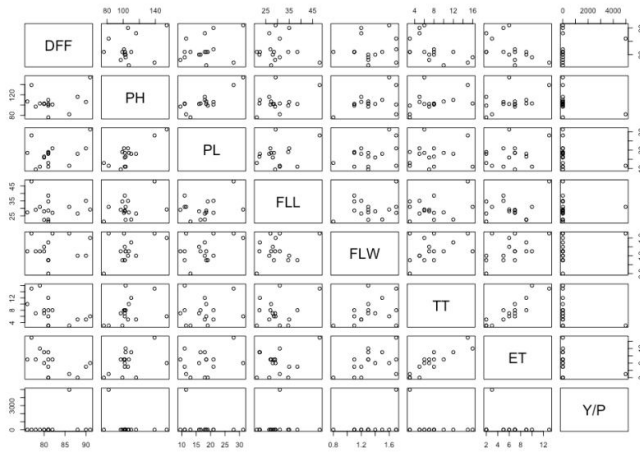


Fig. 4: Scatter plots representing the variation between different observations in Gudma Dhan and its mutants

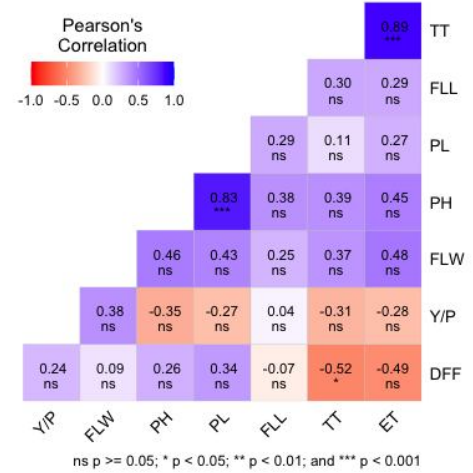


Fig. 5: Correlation coefficients of morphological observations in Gudma Dhan and its mutants

3.3.2. Dawar dhan and its mutants: Both the Fig 6 and 7 represent scatter diagrams and correlation coefficients among different pairs of observations. The results exhibited a positive correlation between flag leaf length and flag leaf width with yield per plant.

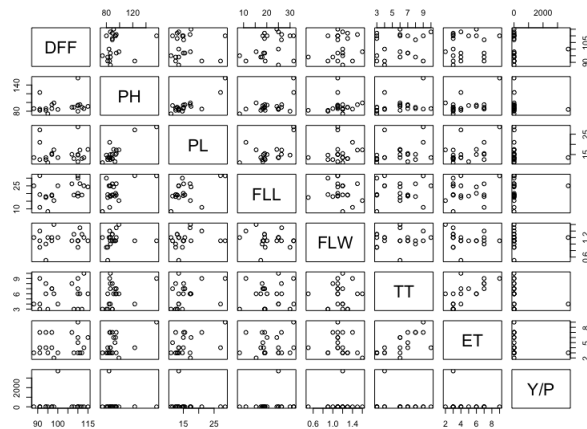


Fig. 6: Scatter plots representing the variation between different observations in Dawar Dhan and its mutants

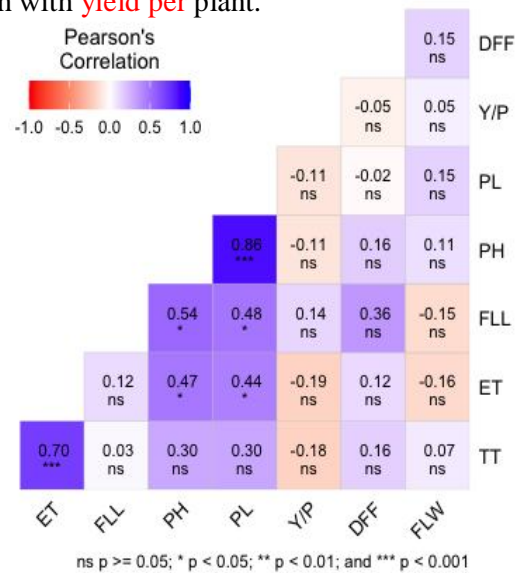


Fig. 7: Correlation coefficients of morphological observations in Gudma Dhan and its mutants

3.3.3. Lohandi dhan and its mutants: Both the Fig 8 and 9 represent scatter diagrams and correlation coefficients among different pairs of observations. The results exhibited a positive correlation between flag leaf length, flag leaf width, effective tillers, total number of tillers, plant height and panicle length with yield per plant.

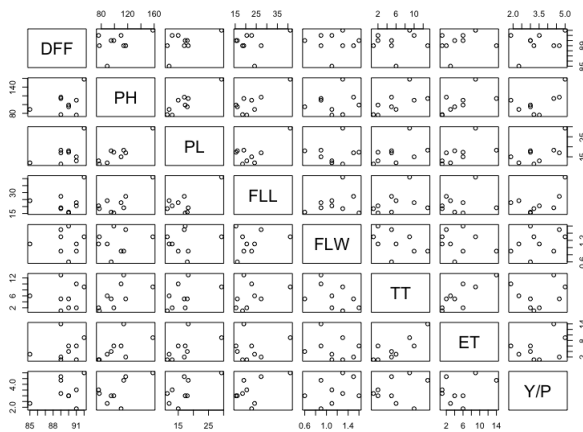


Fig. 8: Scatter plots representing the variation between different observations in Lohandi Dhan and its mutants

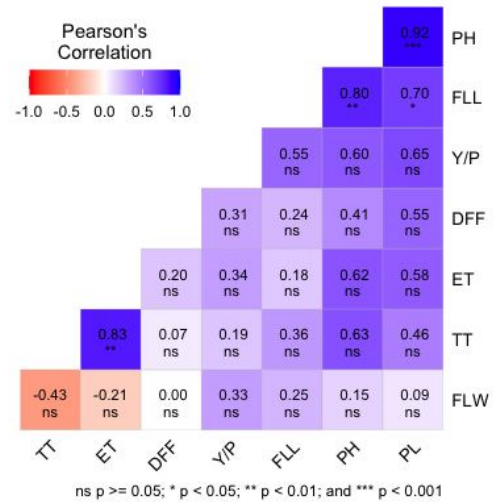


Fig. 9: Correlation coefficients of morphological observations in Lohandi Dhan and its mutants

The selected mutants derived from three landraces have one or more better morphological traits as compared to their respective parents. Reducing the height of the landraces is very important in order to prevent lodging of plants, Hu, 1973 [5]. Lodging leads to a decrease in yield as paddy can germinate or gets soaked in water which deteriorates its quality or germination ability, Lang et al., 2012 [6]. In this study, dwarf mutants from all three landraces were isolated. The mutation frequency of spontaneous mutation is 10^{-6} which can be increased significantly through induced mutations as studied by Jana & Roy, 1973 [7].

However, in the present study, both albino mutants and normal mutants were found. Gene

encoding chlorophyll production is a hot spot for mutation and easily gets mutated. These mutants do not survive for long **time** and disintegrate quickly (**ref**). Mutants with normal chlorophyll production were also **isolated which were** used for selection of desirable traits as experimented by Sao et al., 2021 [8]. The average height of mutants was decreased as compared to their parent **plants**, yield was increased and maturity duration was decreased. Some mutants also exhibited a higher number of tillers. The selection of mutants was based on any potential improvement which could be used for the development of a better cultivar **who is the author?** [9]. The correlation studies revealed scattered variations amongst the traits. As these observations were made on single-plant basis without any replication, the results can be used for directing the selection in a proper direction [10]. The coefficient of correlations with positive values can be further evaluated in replicated trials **for authentication**. **Development of rice varieties having high medicinal value** is crucial as farmers prefer to cultivate high-yielding varieties with profitable returns [11]. The selected mutants **may be** advanced **further for selection** and release as varieties (Fig. 10 **Where is the Fig. 10? irritating**). Mutation breeding offers a powerful tool for achieving this, as it modifies only a few traits while preserving desirable characteristics [12,13].

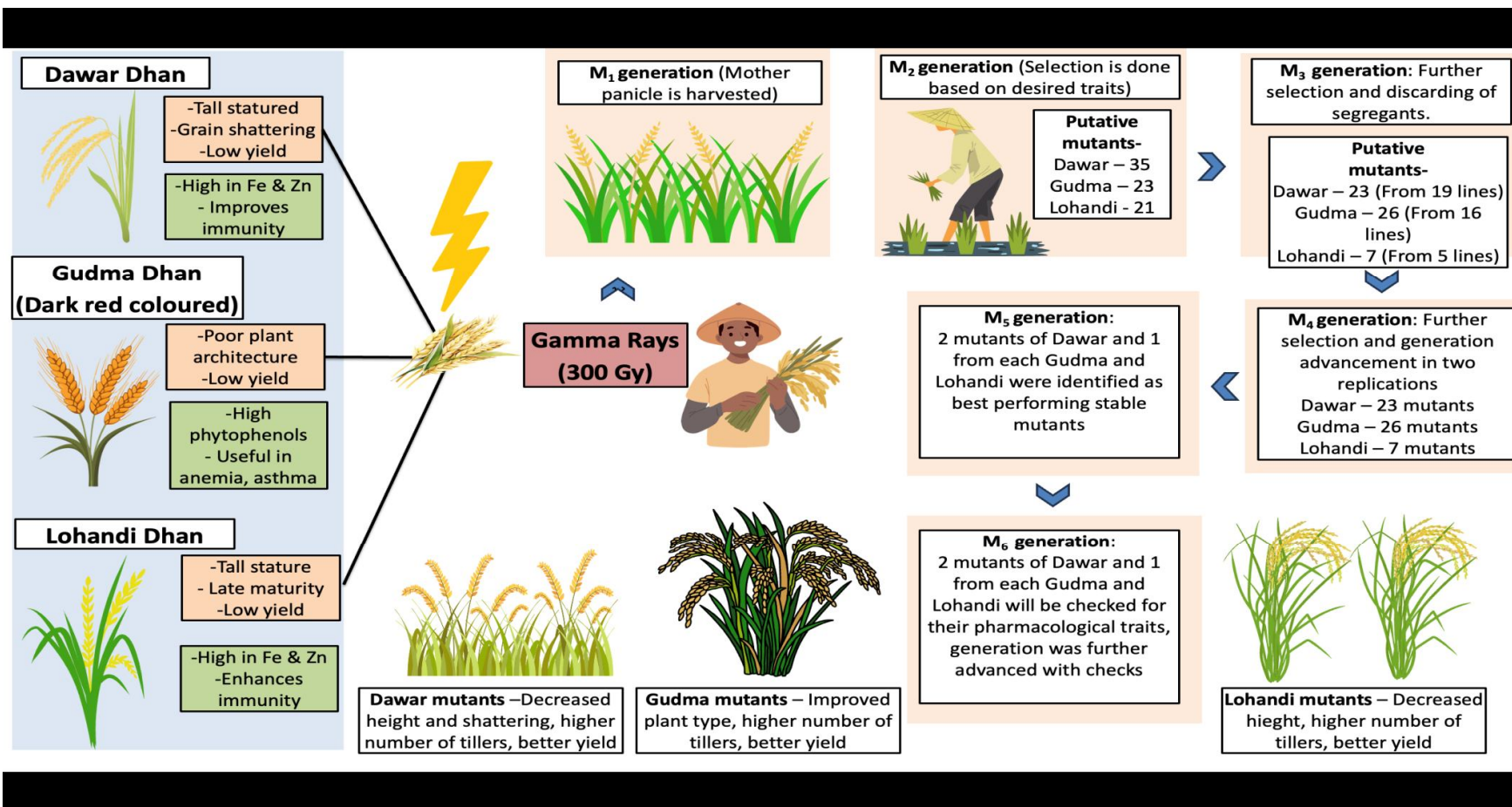
As demonstrated by Thada et al. 2024 [1], untargeted metabolomics can be used to analyze the biochemical composition of mutants and their parents. **(Why you failed to analyze biochemical compositions of your selected mutants?)** Their findings revealed that mutation breeding effectively **improve** antioxidant properties and aroma while addressing undesirable traits. Therefore, mutation breeding can be a valuable technique for enhancing landraces suitable for large-scale cultivation [14,15].

4. CONCLUSION:

The selection of mutants in the M₂ generation **is the most** tedious process and is entirely based on the skills of breeders and their observations. Mutants can be often confused with

environmental influences. Improvement of landraces as better cultivars is the need of the hour as they can better acclimatise, require fewer inputs and are less susceptible to biotic and abiotic stresses. Regardless of how, 35 mutants derived from Dawar dhan, 33 from Gudma dhan, and 21 from Lohandi dhan need further evaluation through progeny trials to eliminate undesirable segregants and unwanted phenotypic variations, eventually to evolve outstanding new rice varieties for cultivation.

UNDER PEER REVIEW



The picture of Lohandi dhan seems picture of unknown cereal

Image 1: Selection of mutants in the M₂ generation

Image 2 - Pictures of some selected mutants of Gudma Dhan



Image -3 Pictures of some selected mutants of Dawar Dhan



Image -4 Pictures of some selected mutants of Lohandi Dhan



All the mutants are lodging susceptible.

References

1. Thada A, Sahu P, Sao R, Chauhan A, Checker R, Das B K, Sharma D, Sharma D, Ghanty T K. Biochemical foundation of the aroma and antioxidant activity of Indian traditional

- rice landrace maharaji and the effect of radiation-induced mutagenesis on its metabolome. *Food Chemistry*, 2024; 460:140668-14078.
- Stadler L. J. Mutations in Barley Induced by X-Rays and Radium. *Science*, 1928; 68(1756):186–187.
2. Hour A, Hsieh W, Chang S, Wu Y, Chin H, Lin Y. Genetic Diversity of Landraces and Improved Varieties of Rice (*Oryza sativa* L.) in Taiwan. *Rice*. 2020; 13(1): 82.
 3. Tiwari A K, Sharma D, Das B K, Kumar V. Improvement of Traditional Local Rice Varieties through Induced Mutations Using Gamma Radiations 2018;2249-9598.
 4. Zhu C, Bortesi L, Baysal C, Twyman R M, Fischer R, Capell T, Schillberg S, Christou P. Characteristics of Genome Editing Mutations in Cereal Crops. *Trends in Plant Science*, 2017; 22(1), 38–52.
 5. Hu C.-H. Evaluation of breeding semidwarf rice by induced mutation and hybridization. *Euphytica*, 1973;22(3): 562–574.
 6. Lang Y, Yang X, Wang M, Zhu, Q. Effects of Lodging at Different Filling Stages on Rice Yield and Grain Quality. *Rice Science*, 2012;19(4):315–319.
 7. Jana M K, Roy K. Induced quantitative mutation in rice. *Radiation Botany*, 1973;13(5):245–257.
 8. Sao R, Sahu, P K, Mondal S, Kumar V, Sharma D, Das B. K. Spectrum and frequency of macro and micro mutations induced through gamma rays in traditional rice landraces of Chhattisgarh. *Electronic Journal of Plant Breeding*, 2021; 12(3):693–706.
 9. Mahantashivayogayya K, Lakkundi BS, Kuchunur PH, Vishwanath J. Genetic variability studies on rice (*Oryza sativa* L.) mutants for yield and yield components in normal and saline stress soil. *Electronic Journal of Plant Breeding*, 2016; 7(4):1162-1168.
 10. Aravind K, Banumathy S, Vanniarajan C, Arunachalam P, Ilamaran M, Kalpana K. DUS characterization and genetic variability studies of rice mutants. *Electronic Journal of Plant Breeding*. 2019; 10: 451.

11. Viana VE, Pegoraro C, Busanello C, Costa de Oliveira, A. Mutagenesis in Rice: The Basis for Breeding a New Super Plant. *Frontiers in Plant Science*, 2019; 10.
12. Babu VR, Shreya K, Dangi KS, Usharani G, Nagesh P. Genetic variability studies for qualitative and quantitative traits in popular rice (*Oryza sativa* L.) hybrids of India. *International Journal of Scientific and Research Publications*, 2012; 2(6): 1-5.
13. Tu Anh, TT, Khanh TD, Dat TD, Xuan TD. Identification of phenotypic variation and genetic diversity in rice (*Oryza sativa* L.) mutants. *Agriculture*, 2018; 8(2):30.
14. Liakat Ali M, McClung AM, Jia MH, Kimball JA, McCouch SR, Eizenga GC. A rice diversity panel evaluated for genetic and agro-morphological diversity between subpopulations and its geographic distribution. *Crop Science*, 2011; 51(5):2021-2035.
15. Domingo C, Andrés F, Talón M. Rice cv. Bahia mutagenized population: a new resource for rice breeding in the Mediterranean basin. *Spanish Journal of Agricultural Research*, 2007; 5(3):341.