Original Research Article

Enhancing agro morphological traits of three unique traditional landraces of Chhattisgarh through Gamma ray-induced mutation breeding.

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 raymutagenesis was performed on three traditional rice varieties(Gudma Dhan, Dawar Dhan, and Lohandi Dhan) to identifypotentially beneficial mutants with valuable agricultural characteristics as well as the other morphological mutants, which can be easily recognized. The major constraints to address were their tall and weak stature, fewer panicles and tillers, and late maturity. Using a fixed dose of 300 Gy, 35 mutants were identified in Dawar dhan, 33 in Gudmadhan, and 21 in Lohandidhan. The selected mutants showed an average reduction in plant height that ranged from 42.69% to 33.11%, in the selected land races. 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% (Gudmadhan), and 7% (Lohandidhan). These findings demonstrate the utility of mutation breeding through gamma radiation inalleviating the key constraints inherent to traditional rice landraces.

Keywords: *Mutation breeding, rice breeding, landraces, gamma rays, mutation frequency*

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. The state of Chhattisgarh, known as the "Rice Bowl of India," is particularly renowned 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, including abiotic and biotic resistance, pigmentation, and medicinal properties have characterized these accessions. Traditional knowledge about these rice varieties has been gathered from local farmers and villagers who have cultivated and consumed these for generations. The preservation and study of these diverse rice varieties, along with the documentation of 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 were classified according to their traits. The medicinal rice landraces were documented along with their pharmacological traits (Table 1). Mutation breeding is a very powerful and effective tool which can help in the development of varieties in a short duration(Stadler, 1928) [1]. Climate change has increased the incidences of biotic and abiotic stresses. Development of new cultivars or hybrid varieties through conventional breeding methods requires a long amount of time. Such varieties have narrow genetic bases which makes them more susceptible to changing climatic conditions. Landraces can play a very important role in today's scenario. Landraces have a broader genetic base and can adapt to varying climatic conditionsHour et al., 2020 [2]. Traditional rice varieties have several undesirable traits including late maturing, uneven panicle maturity, tall stature, and low yield. To improve these traits, radiation-induced mutation breeding was employedas demonstrated by Tiwari et al., 2018 [3].In the present study, 3 traditional landraces of Chhattisgarh, Dawar dhan, Gudmadhan and Lohandi Dhan which are known for their medicinal properties were exposed to 300 Gy of Gamma rays. These landraces are known to have high iron and zinc content as well. Due to these properties, these landraces were included in the radiation-induced mutation breeding programme to improve the overall architecture and plant type. The selection of mutants was carried out using changes in morphological traits. Mutations are mostly recessive, hence selections in M_2 generation is the most effective for identifying the potentially improved variety(Zhu et al., 2017) [4].

2. MATERIAL AND METHODS:

2.1. Mutation Breeding: Three traditional medicinal landraces from Chhattisgarh,Gudma Dhan, Lohandi Dhan and Dawar Dhan (Table 2), were subjected to mutation breeding. Gudmadhan has short and bold grain type, Dawar dhan has medium bold grains while Lohandidhan had long and slender grain type. Gamma rays were used to generate 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 isolated for 7 days for radioactivity decay. The irradiated seeds were sown in the Research cum Instructional farm of the Department of Genetics and Plant Breeding in Indira Gandhi Krishi Vishwavidyalaya (IGKV), Raipur in the Summer (Rabi) season of 2020-21 as M1 generation.

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 Gudmadhan had a height of 154 cm. The seeds were procured from R.H. Richharia medium-term germplasm storage, IGKV (Raipur). All the required cultural and agronomic properties were adopted to cultivate the M1 generation. Mother panicle from each plant was harvested and stored separately was sowing in the subsequent season. The seeds harvested in the M1 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 distance was maintained at 20 cm x 20 cm. Around10,987 plants were sown for Gudma Dhan, 11,256 for Lohandi and 11,105 plants of Dawar. Along with the seeds from the M1 generation, untreated seeds of landraces were planted alongside to act as control or checks. All the required agronomic practices were adopted for the cultivation of the M_2 generation.

2.3. Screening of mutants: Rigorous screening was carried out in the M_2 generation for mutants byfollowing the understated 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 were selected.
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 indicates the name of the parent variety

2). The second line represents the generation of mutants

3). The third line indicates the mutant number. The number starts with one with a prefix P and the numbering progresses with discovery and screening.

4). Lastly, the tag denotes any special characters the mutant is displaying like early flowering, early maturity etc.

For Example: A mutant of Dawar dhan discovered in 2^{nd} generation, 5^{th} in line will tagged as:

Dawar

M_2, P5

Early flowering

2.5. Mutation frequency: The mutagenic frequency of induced mutations was estimated using the progenies in the M_2 population with the following formula as given by Gual, 1964 :

$$Mutation\ frequency = \frac{Number\ of\ mutated\ progenies}{Total\ progenies\ in\ M2\ population}$$

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 breeding.

2.6. Statistical analysis: All the data analysis was carried out using MS Excel and Rstudio.

Table 1: Details of medicinal properties of landraces collectedby Dr. R.H. Ricchharia

Sr. No.	Name of rice Landrace	Medicinal properties
1.	**Gudma Dhan**	• 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, good nutritional value
3.	**Dawar Dhan**	Safe pregnancy and easy removal of placenta in cows, good nutritional values
4.	**Layacha**	Rice is fed to expecting females for delivery of a healthy infant.
5.	**Maharaji**	Rice is fed to 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 data of landraces used in the experiment.

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

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

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 breeding can result in micro as well as macro mutations. Macro mutations are easily visible to the naked eye and micro mutations mostly occur at the molecular level. The present study focuses on macro mutations which are required to improve the overall agro-morphological traits of the landraces to make them more cultivable.

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

Table 4: Mutation frequency

Landrace	**Albino mutants**	**Normal mutants**	**Total mutants**	**Total plants**	**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

3.1. 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,

3.1.2. 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. In this study, induced mutation with gamma rays reduced the height of landraces. In Dawar dhan, 20 mutants, in Gudmadhan 15 mutants and Lohandidhan 8 mutants were identified with significantly reduced height as compared to their respective parents. The average height of Dawar 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 Gudmadhan, the mutants measured 33.11% less with an average of 103 cm, ranging from 76 to 139 cm. While Lohandidhan'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.).

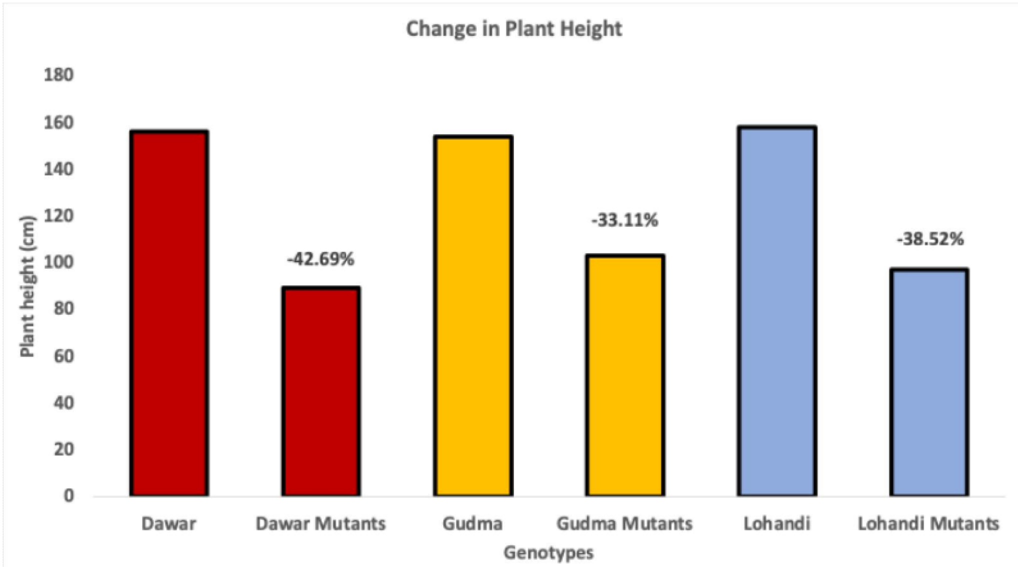

Fig. 1: Bar graphs representing a decrease in average height in selected mutants as compared to their parents

3.1.2. 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 amd 89.17 respectively. The number of days to 50% flowering in parents of Dawar, Gudma and Lohandi were 110, 91 and 92. A decreaseof 7.63%, 10.55% and 3.08% was recorded in the Dawar, Gudma and Lohandi mutants respectively.

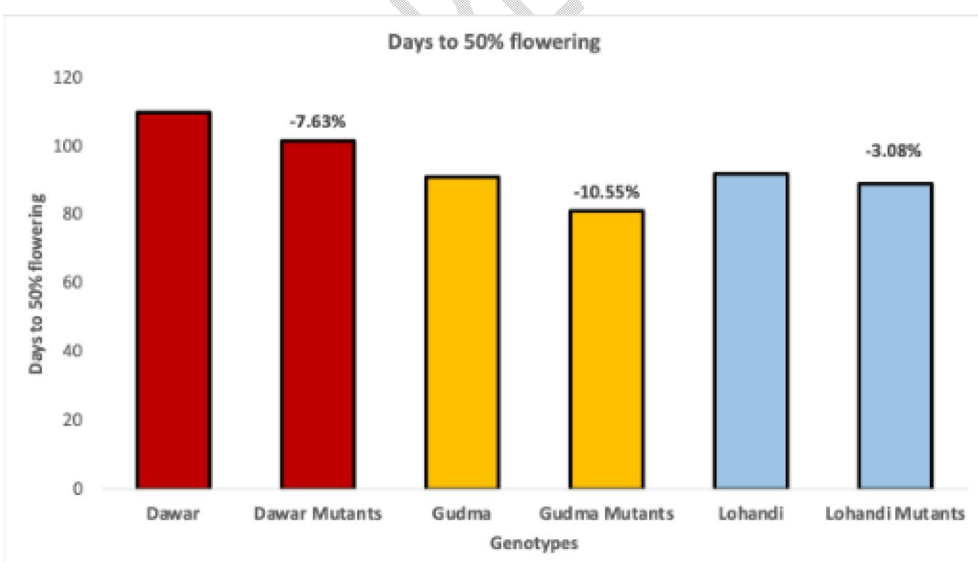

Fig. 2: Bar graphs representing a decrease in the days to 50% flowering in selected mutants as compared to their parents

3.1.3. Increase in Yield/Plant: Tillers harbour panicle at the end of their vegetative growth and contribute positively to increasing the grain yield. Mutants were screened for yield/plant. It was observed that Dawar, Gudma and Lohandi mutants exhibited an increase in yield by 38.11%, 32.63% and 7% respectively. The average yield of Dawar mutants was 5.65 grams while of that of Dawar parent was 3.5 grams. In the case of Gudma mutants, the average yield/plant was 4.38 grams and that of Gudma parent was 2.95 grams. Lohandi parent had a yield of 3 grams while its mutants exhibited 3.25 grams of average yield/plant.

3.2. Correlation analysis: Selection in M_2 generation is done as most of the mutations are recessive. Variations in M_2 generation may be due to genetic changes or they could be a result 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.

3.2.1. Gudma Dhan and its mutants: Observations like Days to 50% flowering, Flag leaf length, and Flag leaf width exhibited a positive correlation with yield/plant. Fig 4. represents the scatter diagram and Fig 5 represents the coefficients of correlation between different observations.

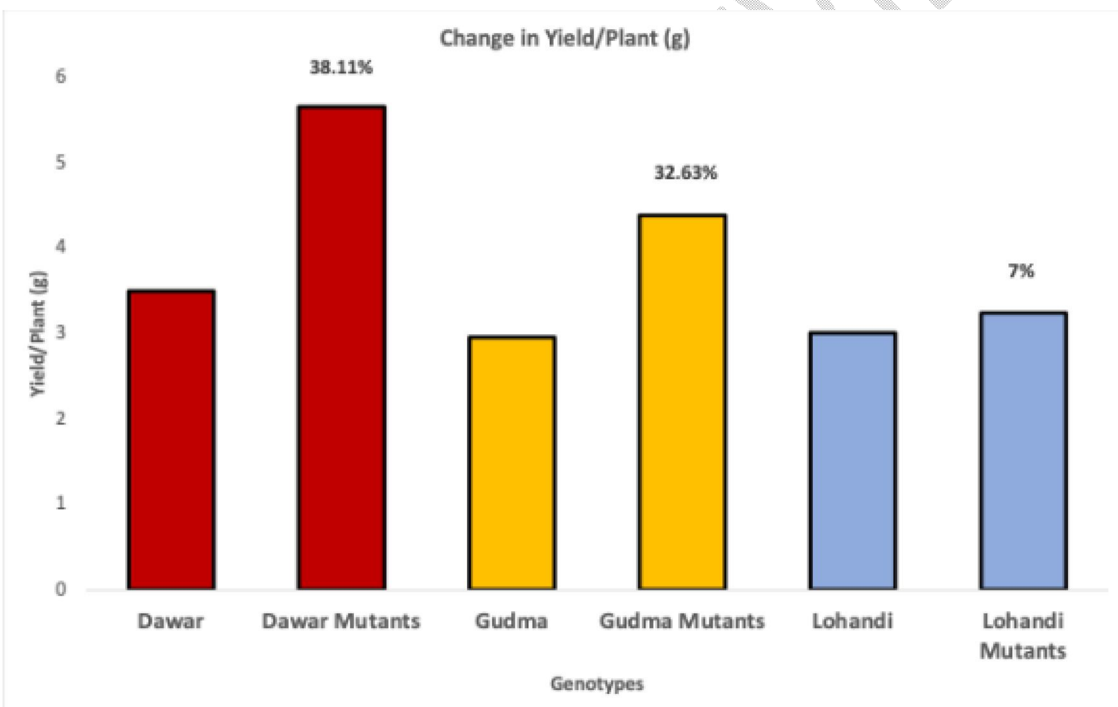

Fig. 3: Bar graphs representing an increase in the yield/ plant in selected mutants as compared to their parents

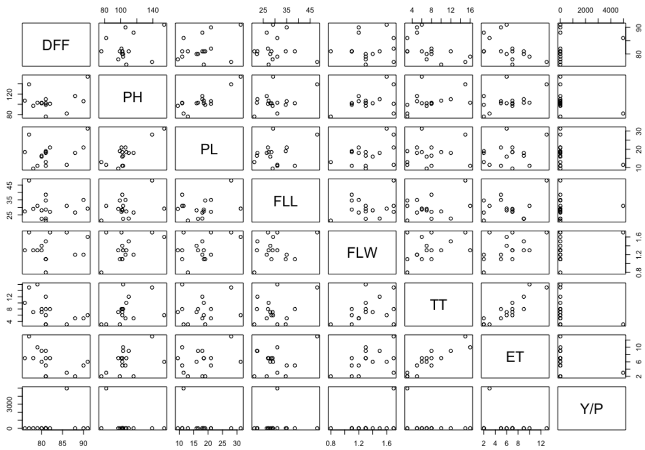

Fig. 4: Scatter plots representing the variation between different observations in Gudma Dhan an d its mutants

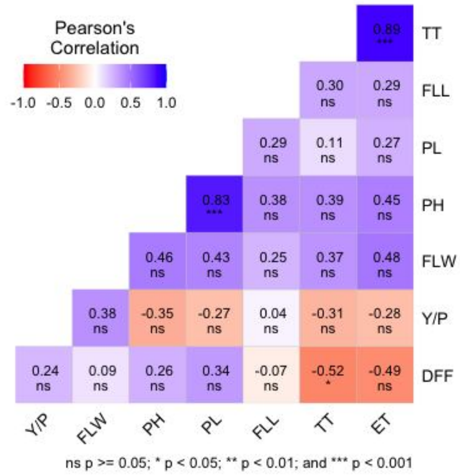

Fig. 5: Correlation coefficients of morphological observations in Gudma Dhan and its mutants

Dawar Dhan and its mutants: Fig 6 and 7 represent scatter diagram and correlation coefficients between different observations. The results exhibited a positive correlation between Flag leaf length and Flag leaf width with Yield/Plant.

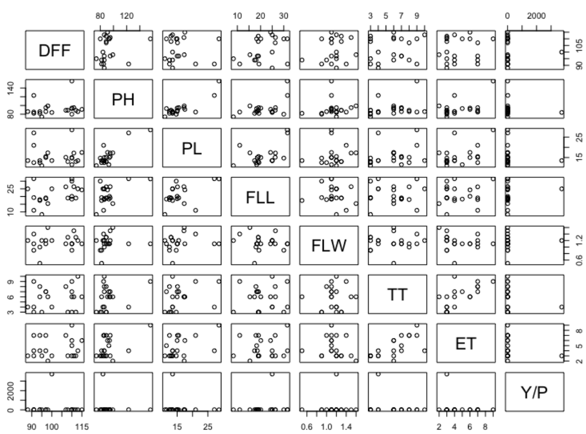

Fig. 6: Scatter plots representing the variation between different observations in Dawar Dhan and its mutants

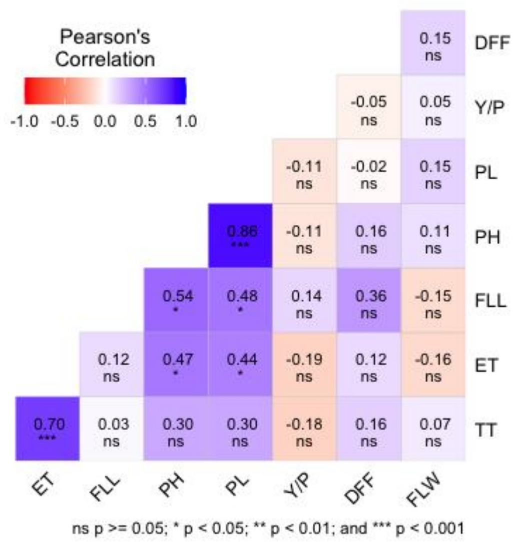

Fig. 7: Correlation coefficients of morphological observations in Gudma Dhan and its mutants

Lohandi Dhan and its mutants: Fig 8 and 9 represent scatter diagram and correlation coefficients between different 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.

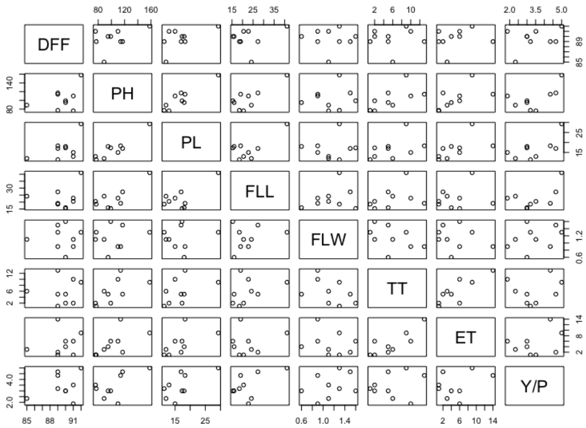

Fig. 8: Scatter plots representing the variation between different observations in Lohandi Dhan and its mutants

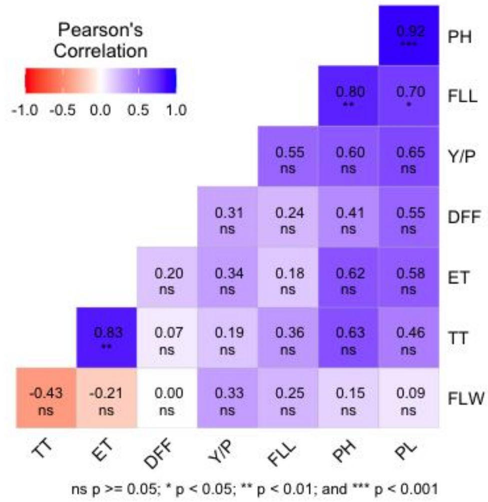

Fig. 9: Correlation coefficients of morphological observations in Lohandi Dhan and its mutants

The selected mutants in all three landraces have one or other better morphological traits as compared to their respective parents. Reducing the height of 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 naturally induced mutations is 10^{-6} which can be increased significantly through induced mutations as studied byJana & Roy, 1973 [7]. In the present study, 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 and disintegrate quickly. Mutants with normal chlorophyll production were also isolated in this study which were used for selection of desirable traits as experimented by Sao et al., 2021 [8]. The average height of mutants wasdecreased as compared to their parent plant, 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 used for the development of a better cultivar [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 better understanding. improving medicinal rice varieties is crucial as farmers prefer to cultivate high-yielding varieties with profitable returns[11]. The selected mutants are advanced for further selection and release as varieties (Fig.

10). 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. Their findings revealed that mutation breeding effectively maintained 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 M_2 generation is one of the most tedious process and is entirely based on the skills of breeders and their observations. Mutants can be often confused with environmental influences. The selected mutants in this study need to be further evaluated through progeny trials to eliminate segregation and phenotypic variations. Improvement of landraces as better cultivars is the need of the hour as they can better acclimatise, require less inputs and are less susceptible to biotic and abiotic stresses.

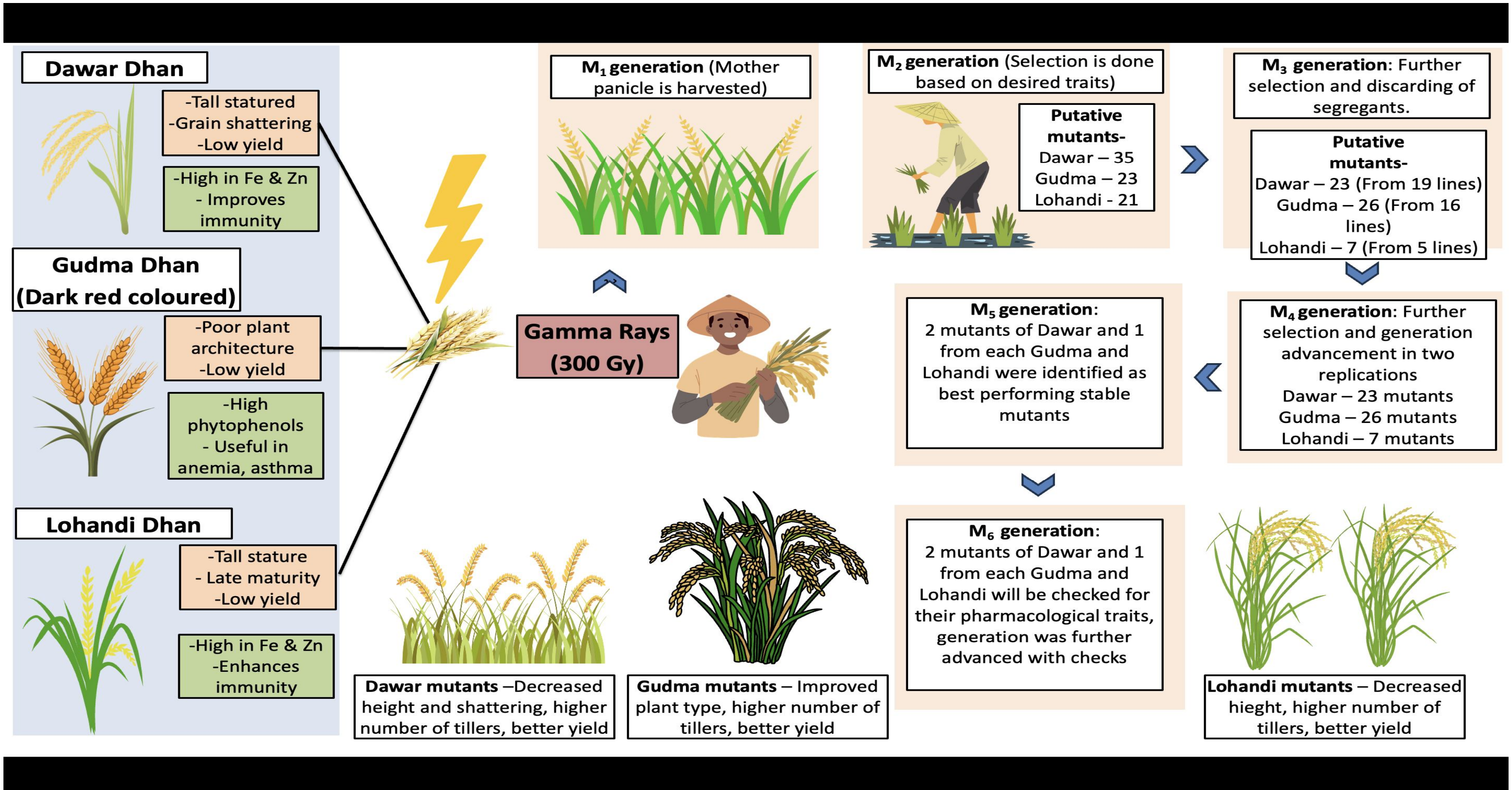

Image 1: Selection of mutants in the M2 generation

Image 2 - Pictures of some selected mutants of Gudma Dhan

Image -3 Pictures of some selected mutants of Dawar Dhan

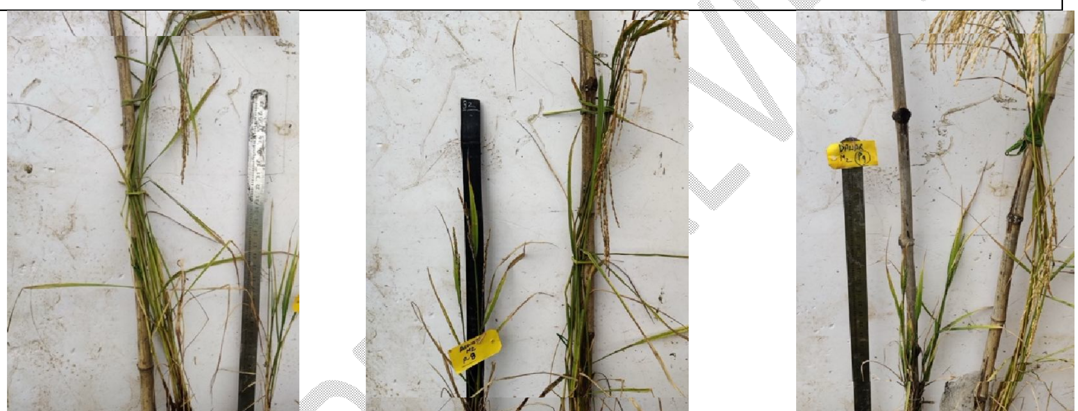

Image -4 Pictures of some selected mutants of Lohandi Dhan

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