Original Research Article

Enhancing agro morphological traits of three unique traditional ricelandraces 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 raymutagenesis was performed on three traditional rice varieties(Gudmadhan, Dawardhan, and Lohandidhan) to identifypotentially beneficial mutants with desirable agro-morphological agricultural characteristics. The major constraints to address were their tall staturesand weak culms, fewer panicles and tillers, and late maturity. Using a fixed dose of 300 Gy, 35 mutants were identified in Dawardhan, 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%, 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% (Dawardhan), 32.63% (Gudmadhan), and 7.0% (Lohandidhan). These findings demonstrate the utility of mutation breeding through Gamma radiation inalleviating 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 (Mahajan et al., 2017)[1]. 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) [2]. Various traits, likeabiotic and biotic resistance,pigmentation, and medicinal properties have been found inthese accessions. Traditional knowledge about these rice varieties was gathered from local farmers and villagers who have 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.Ricchhariaover several years of his servicewere 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) [3]. Climate change has increased the incidences of biotic and abiotic stresses. Development of new cultivars or hybrid varieties through conventional breeding methods require a long time but 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 generally have a broader genetic base, so can play a very important role in today's scenario, particularly adapt to and can adapt to varying climatic conditionsHour et al., 2020 [4]. Unfortunately the traditional rice varieties have several undesirable traits like late maturing, uneven panicle maturity, tall stature, lodging susceptibilityand low yield. To improve these traits, radiation-induced mutation breeding was exercisedas demonstrated by Tiwari et al., 2018 [5].In the present study, 3 traditionalrice landraces of Chhattisgarh, Dawardhan, Gudmadhan and Lohandidhanwhich are popular for their medicinal properties were exposed to 300 Gy of Gamma ray. These landraces arewell 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 generationwould be the most effective for identifying the potentially improved lines(Zhu et al., 2017) [6].

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, Dawardhan 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: Lohandidhan was the tallest with an average height of 158 cm, followed by Dawardhan with a height of 156 cm and Gudmadhan had a height of 154 cm. Required amounts of seeds of the landraceswere 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 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 distanceswere maintained at 20 cm x 20 cm. Around10,987 plants were raised for Gudmadhan, 11,256 for Lohandidhanand 11,105 plants of Dawardhan. 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 M_2 generation.

2.3. Screening of mutants: Rigorous screening was carried out in the M_2generation to identify desirable mutantsconsidering 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 startedwith one with a prefix P and the numbering progressed with screening and selection.

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

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

DawardhanM_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.

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

Table 1: Details of medicinal properties of landraces according toDr. R.H. Ricchharia

Sr. No.	Name of rice Landrace	Medicinal properties
1.	**Gudmadhan**	• 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.	**Lohandidhan**	Used to enhance theimmunity and good nutritional value
3.	**Dawardhan**	Safe pregnancy and easy removal of placenta in cows, good nutritional values
4.	**Layacha**	Rice is eaten by females for expectingdelivery of a healthy infant.
5.	**Maharaji**	Rice is eaten by 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: Passportinformation of landraces used in the experiment.

Entry	**Genotype**	**CGR No.**	**Location**			
			Village	**Block**	**District**	**Province**
1.	Dawardhan	CGR-725	Pidapal	Kanker	Bastar	C.G.
2.	Gudmadhan	G-1042	Faraspal	Gidam	Dantewada	C.G.
3.	Lohandidhan	CGR-249	Chilhari	Manpur	Shahadol	M.P.

Table 3: Agro-morphological details for Dawardhan, Gudmadhan and Lohandidhan

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)**
Dawardhan	110	156	28.6	31.5	1.1	9	9	9.5
Gudmadhan	91	124	31.3	29.2	1.6	6	6	2.95
Lohandidhan	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 Dawardhan, Gudmadhanand Lohandi are dhanmentioned in Table 4. The highest number of mutants were isolated in Dawardhan, followed by Gudmadhan and Lohandi Dhan.

Table 4: Mutation frequencies obtained from the landraces

Landrace	**Albino mutants**	**Normal mutants**	**Total mutants**	**Total plants in M_2**	**Mutation frequency (%)**
Dawardhan	13	22	35	11,105	0.31
Gudmadhan	18	15	33	10,987	0.30
Lohandidhan	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)
Gudmadhan 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
Lohandidhan mutants								
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
Dawardhan mutants								
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 shatteringof grains (Kumar V and Ladha, 2011) [7]. In this study, induced mutation with Gamma rays reduced the height of landraces. In Dawardhan, 20 mutants, in Gudmadhan 15 mutants and inLohandidhan 8 mutants were identified with significantly reduced height as compared to their respective parents. The average height of Dawardhan'smutants 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, whileLohandidhan'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.).

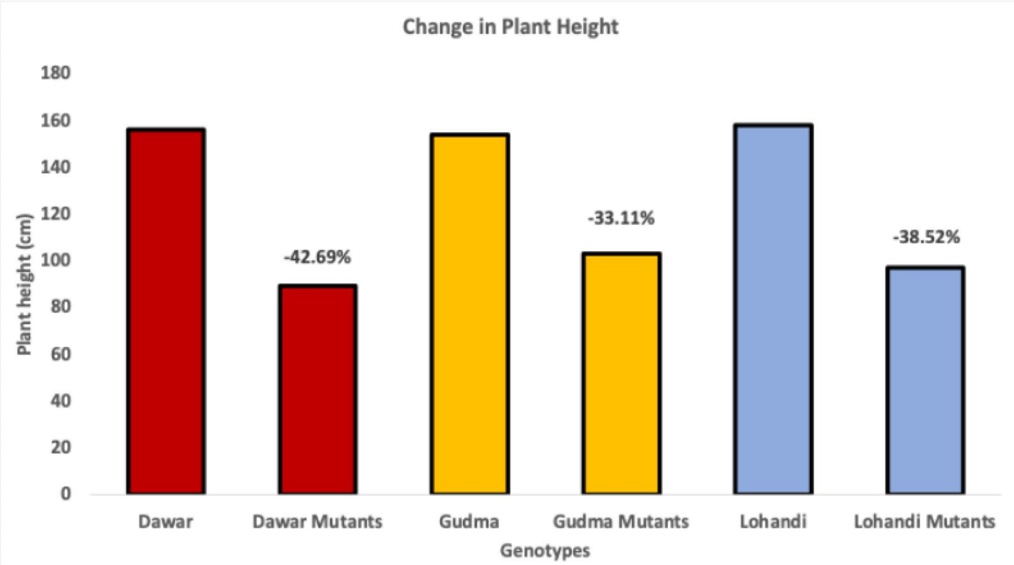

Fig. 1: Bar graphs representing a decrease in average height in selected mutants as compared to their parents

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

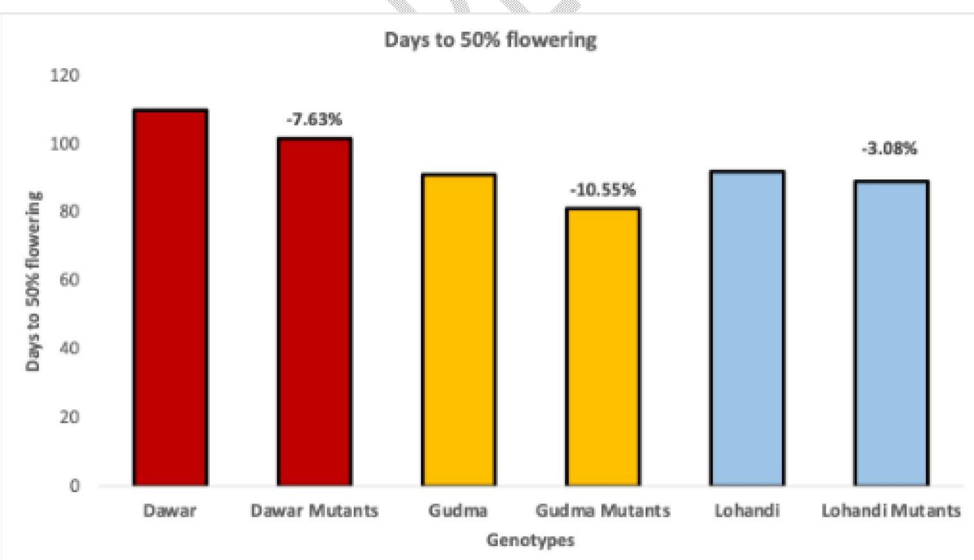

Fig. 2: Bar graphs representing a decrease in the days to 50% flowering in selected mutants as compared to their parents

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

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. Gudmadhan and its mutants: Observations like days to 50% flowering, flag leaf length, and flag leaf width exhibited a positive correlation with grain yieldperplant. Fig 4. represents the scatter diagram and Fig 5 represents thecoefficents ofcorrelation among different pairs of observations.

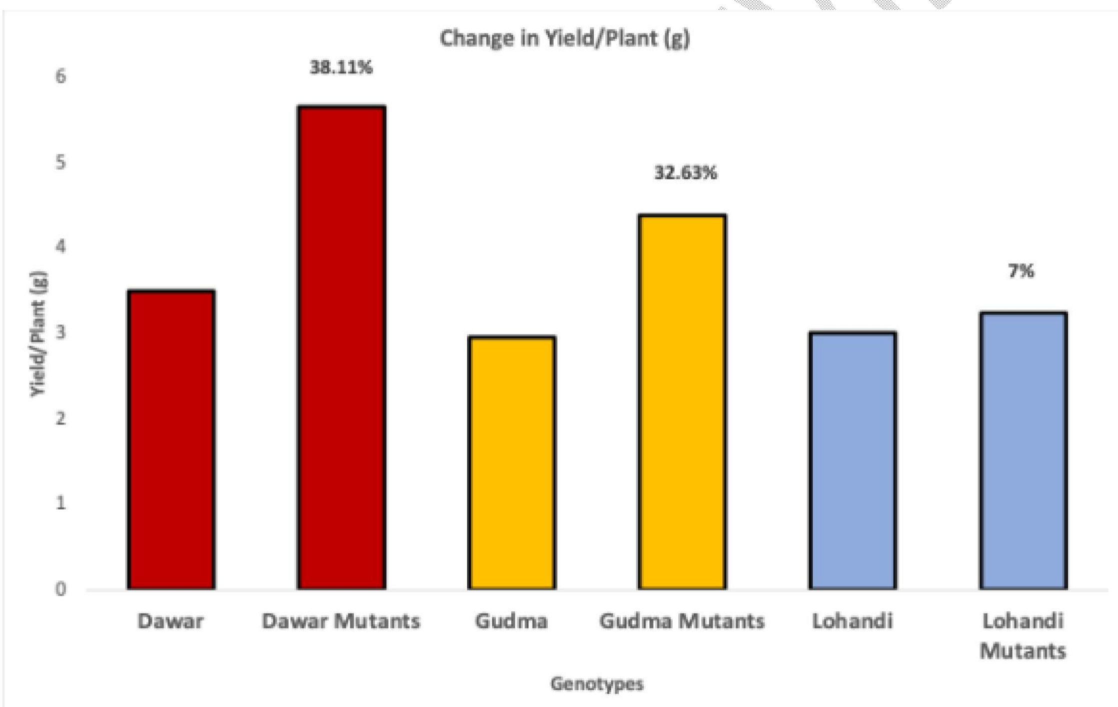

Fig. 3: Bar graphs representing an increase in the yield/ plant in selected mutants as compared to their parents

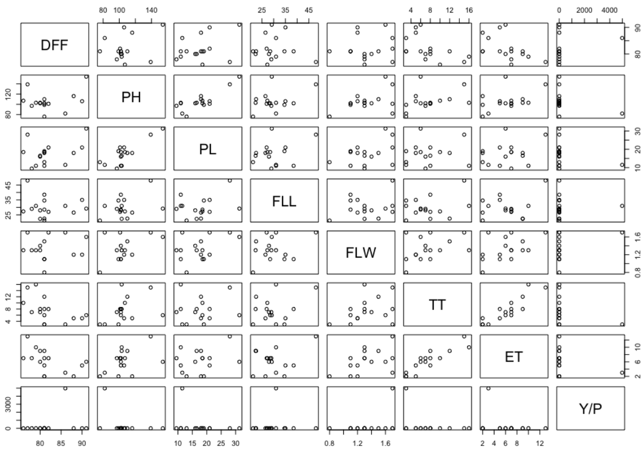

Fig. 4: Scatter plots representing the variation between different observations in Gudma Dhan an d its mutants

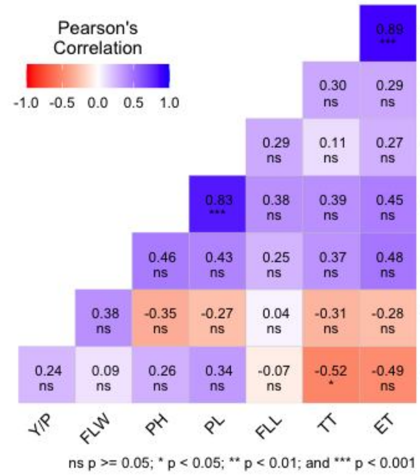

Fig. 5: Correlation coefficients of morphological observations in Gudma Dhan and its mutants

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

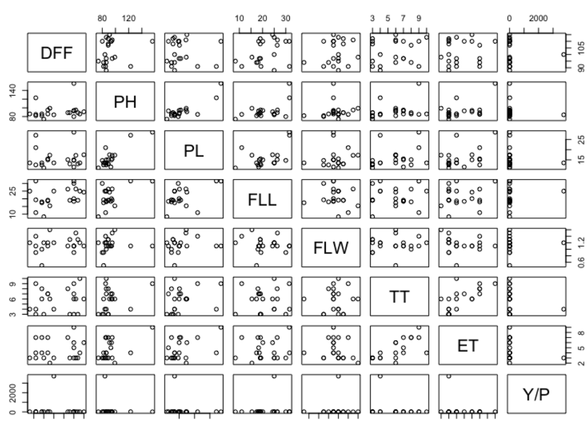

Fig. 6: Scatter plots representing the variation between different observations in Dawar Dhan and its mutants

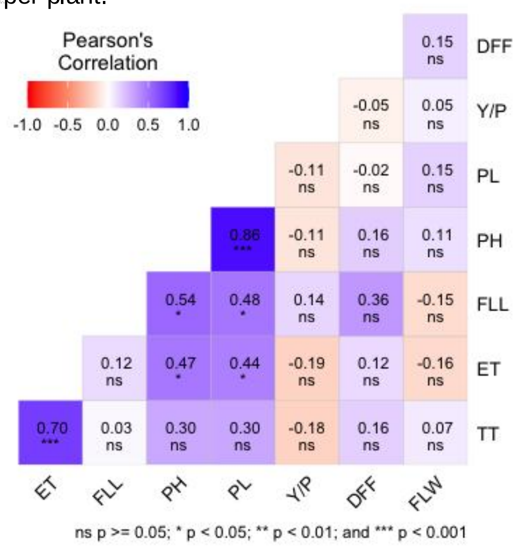

Fig. 7: Correlation coefficients of morphological observations in Gudma Dhan and its mutants

3.3.3. Lohandidhan and its mutants: Both theFig 8 and 9 represent scatter diagrams and correlation coefficients among different pairs ofobservations. The results exhibited a positive correlation between flag leaf length, flag leaf width, effective tillers, total number of tillers, plant height and anicle length with grain yield per plant.

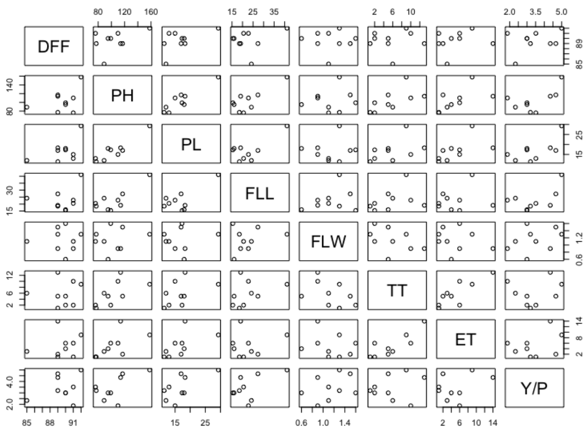

Fig. 8: Scatter plots representing the variation between different observations in Lohandi Dhan and its mutants

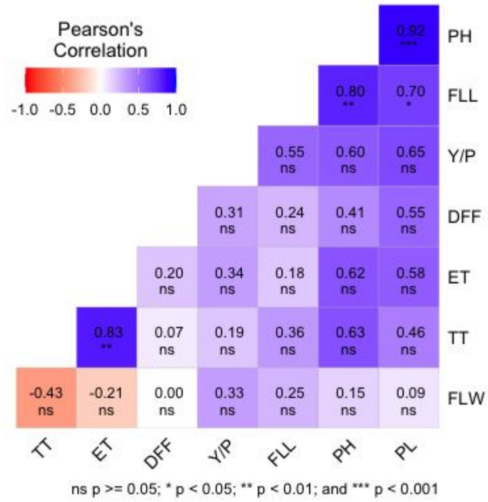

Fig. 9: Correlation coefficients of morphological observations in Lohandi Dhan and its mutants

The selected mutants derived from three landraces have one or more better morphological traits as compared to their respective parents. Reducing the height of thelandraces is very important in order to prevent lodging of plants,Hu, 1973 [8]. 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 [9]. In this study, dwarf mutants from all three landraces were isolated. The mutation frequency of spontaneous mutations is 10^{-6} which can be increased significantly through induced mutations as studied byJana & Roy, 1973 [10]. However, 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 [11]. The average height of mutants wasdecreased 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 used for the development of a better cultivar (Mahantashivayogayya et al., 2016) [12]. 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 directions as studied by Aravind et al, 2019[13]. The coefficient of correlations with positive values can be further evaluated in replicated trials for authentication. Development of rice varieties having high medicinal valueis crucial as farmers prefer to cultivate high-yielding varieties with profitable returns(Tu et al., 2018) [14]. The selected mutants may be advanced for further forselection 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 characteristicsBabu et al., 2012 and Tu et al., 2018) [15,16].

As demonstrated by Thada et al. 2024 [2], untargeted metabolomics can be used to analyze the biochemical composition of mutants and their parents. (The study of metabolomics is beyond the scope of this paper as the manuscript focuses on selection of putative mutants based on morphology) Their findings revealed that mutation breeding effectivelyimprove antioxidant properties and aroma while addressing undesirable traits. Therefore, mutation breeding can be a valuable technique for enhancing landraces suitable for large-scale cultivation(Liakat et al., 2011 and Domingo 2007)[16,17].

4. CONCLUSION:

The selection of mutants in the M_2 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. 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.Regardless of how, 35 mutants derived from Dawardhan, 33 from Gudmadhan, and 21 from Lohandidhan need further evaluation through progeny trials to eliminate undesirable segregants and unwanted phenotypic variations, eventually to evolve outstanding new rice varieties for cultivation.

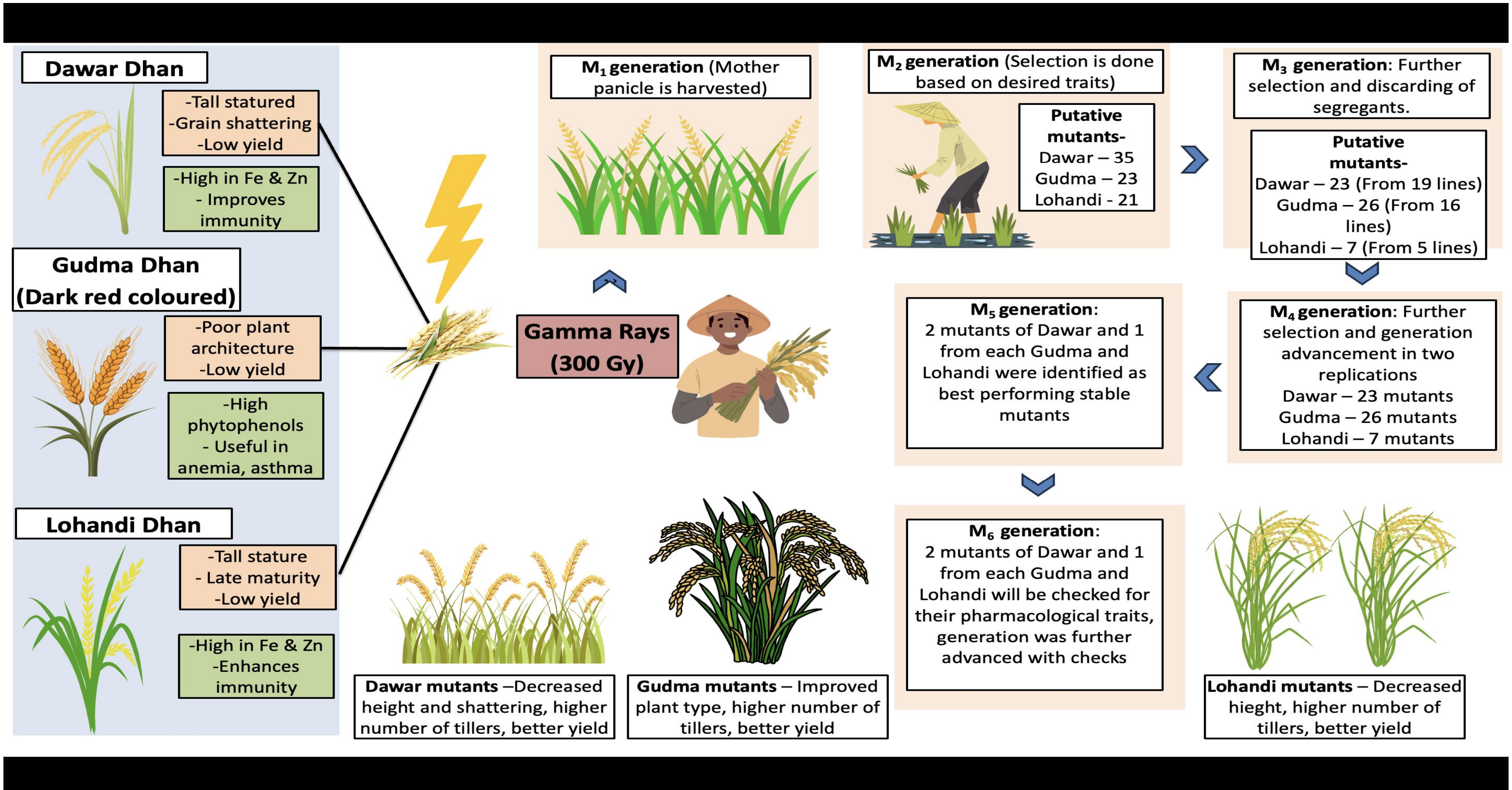

Fig. 10: Graphical abstract of mutation breeding program.

Image 1 - Pictures of some selected mutants of Gudma Dhan

Image 2-Pictures of some selected mutants of Dawar Dhan

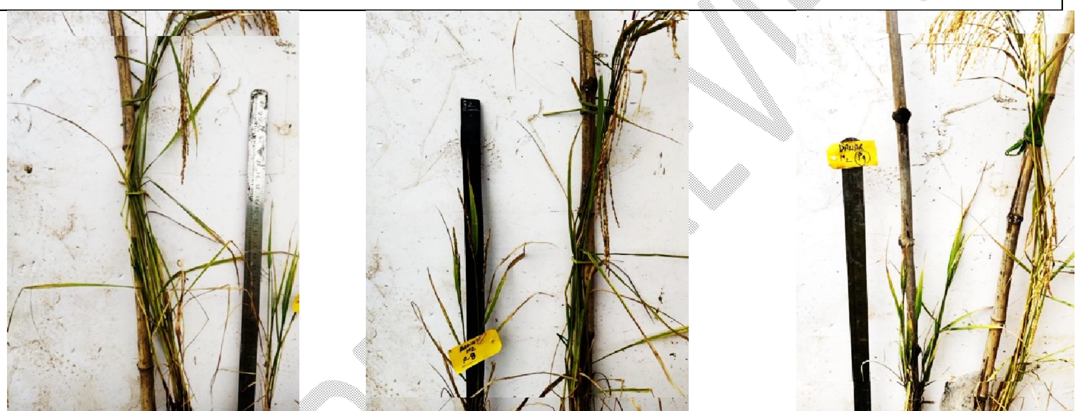

Image 3-Pictures of some selected mutants of Lohandi Dhan

Disclaimer (Artificial intelligence)

Option 1:

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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