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MUTAGENESIS FOR THE DEVELOPMENT OF SUBMERGENCE TOLERANCE RICE GENOTYPES in *Indica* BY GAMMA IRRADIATION-INDUCED MUTATION USING ^{60}CO SOURCE ISOTOPE WITH MARKER ASSAY

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ABSTRACT

The high selection pressure applied to rice breeding since it was domesticated thousands of years ago has initiated a narrowing its genetic variability. Obtaining new rice varieties therefore becomes a major challenge for breeders and the development of techniques to increase genetic variability has attracted the attention of various research groups. Understanding mutations and their application has paved the way for the interpretation of immersion tolerant rice. Creating variability through mutations has therefore become the most important tool for rice improvement. Gamma-rays were able to create genetic variables for abiotic stress tolerance, such as submergence tolerance as well as plant height, number of tillers, shoot and root weight, total biomass, and panicle length. Among the three parents of BRRI dhan52, Guti swarna and Mamun swarna, the highest average yield plant-1 (46.56 g) was produced by Mamun swarna (cont.) and the lowest (18.16 g) by BRRI dhan52. On the other hand, the group of all mutants, Mamun swarna-250 (M3) was more productive (39.30 g) and the maturation was 142.66 days from Guti swarna-300 (M3) (20.20 g) and maturity duration was 151.66 days. The first three principal components studied, including the Eigen value, explained 80.4% of the total variation in 11 rice genotypes for 9 quantitative traits. The gene diversity value of allele across 11 genotypes was 0.469. The value of polymorphism information content (PIC) was 0.359 (SC34 / RM23679) and the allele frequency (%) was 62. However, this assessment may be effective in developing reliable mutants for significant submergence stresses in rice.

Keywords: Mutation, Extraction, Submergence, Rice, Radiation, DNA.

1. INTRODUCTION

A mutation is an inheritable change in the genetic material and the organism that carries the mutation known as mutants. One has to keep in mind that most of the important agronomic traits are of complex inheritance and therefore more difficult to improve. In this case, mutant or variants alleles can be identified and easily introgressed by performing Genome Wide Association Studies (GWAS) on populations with mutant genotypes. Mutations can be used as a tool for functional study of genes and for generation of genetic variability (Lo et al., 2016). However, the rate of spontaneous mutations is lower in higher plants, ranging from 10−5 to 10−8 (Jiang and Ramachandran, 2010). Thus, mutagenesis is an important strategy to increase the mutation frequency by 0.1% (Maluszynski et al., 2000; Da Luz et al., 2016) enables the study of

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functional genomics and the development of new genotypes. Considering the importance of developing mutants in rice to understand gene functionality and generate genetic variability, this review reports on recent advances in gene identification and taps the genetic base obtained through mutations in the rice genome. Indeed, the degree of mutation depends on the tissue, dose, and duration of exposure (Parry et al., 2009). However, seeds are easy to handle and do not require a special structure, and are therefore the most widely used ingredients (Da Luz et al., 2016; Oladosu et al., 2016). When seeds are mutagenized, it is important to standardize the mutagenic absorption of seeds by soaking the seeds in distilled water, as it also activates seed metabolism and aids in mutagen action. In plant breeding, the induction and identification of seed mutations is a simple process. Once mutant populations have been recognized and mutations have been identified, mutants form the next generation of seeds from plants where phenotype analysis will be performed to investigate mutation effects (Henry et al., 2014).

A mutagen is an agent that causes cellular genetic material, usually irreversible and hereditary mutations in DNA. In genetics, a mutagen is three types such as physical agents (heat and radiation-gamma rays), chemical agents (base analogues) and biological agents (viruses, bacteria and transposons) that are genetic components of an organism, usually DNA. There are three main types of ionizing radiation, such as alpha particles, which contain two protons and two neutrons that carry a positive charge. Beta particles, which are basically electrons and beta particles carry negative charges. Gamma rays and X-rays, which are pure electromagnetic energy, or photons and gamma rays neutral. Ionizing radiation is a type of energy emitted by an atom that travels in the form of electromagnetic waves (gamma or X-rays) or particles (neutrons, beta or alpha). Ionizing radiation can remove electrons from an atom, meaning it can ionize an atom, ^{60}Co. Due to their high penetration power, the effects of gamma radiation can occur throughout an organism, but they are less ionizing than alpha particles. Gamma radiation is the most preferred physical mutation by plant breeders. Several mutant varieties have been successfully introduced in this method for commercial production. The combined use of mutation breeding and in vitro tissue culture methods contributes significantly to the development of new crops.

Rice is a main food in half of the world population and hence, is referred to as “Global Grain” (Prasad et al., 2018; Kumar et al., 2020; Ray et al., 2022) and “Rice is life” (FAO, 2004; Ray, 2014) but its production chronologically decline because of submergence and other climate change. Rice is a popular subject of mutagenesis because it is the world's leading food grain and it’s diploid in nature. Most of the crops can be improved by conventional breeding methods using the natural variability of germplasm. However, for plant type changes, mutation breeding is effectively employed as an alternative source. Mutations have been successfully used in the breeding of various food grain varieties, ornamental and export crops (Mohamad et al., 2005). Before starting any breeding program, knowledge about the relative biological efficacy and efficacy of different mutations in mutation breeding is effective. Various efforts in this direction have been made by various scientists to determine the most effective mutagenic treatment for bringing the desired traits in rice. Over the last few years, research institutes have undertaken several new projects aimed at creating mutant populations of gamma-induced rice.

Gamma radiation can be used as a mutagenic agent to induce genetic variability in plant species. The purpose of mutation induction is to produce genetic and phenotypic variables that can select

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plants with traits not found in nature. However, adequate doses of radiation must be established before starting a mutagenesis-based breeding program. The median lethal dose (LD50) and median growth reduction (GR50) are parameters used to establish adequate radiation doses to induce mutations in plant breeding programs. LD means "lethal dose". LD50 is 50% of the test organism (one half) that is killed by a specific dose (radiation) is called LD50 (Acquaah, 2007). The LD50 dose is initially determined, which is used as the optimal dose for mutation induction. Many researchers agree that the highest probability of creating mutations useful for breeding programs occurs in doses where 50% of irradiated individuals die (1-4). Similarly, other researchers have noted that in addition to LD50 there is another dose with a high probability of producing effective mutations where a 50% increase in growth (GR50) occurs (5, 6). Significantly, both parameters (LD50 and GR50) are based on the assumption that low-dose radiation has minimal effects on the genome, which rarely causes phenotypic changes; Where high levels can have multiple effects on the genome that produce a series of catastrophic or negative changes (7,8). Therefore, the first step in a mutagenesis-based breeding process is to determine LD50 and GR50. The half-life of gamma rays is 5.3 years.

Rice field in flooding is a serious problem in the lowland and deep water rice areas of the world in the river basins of South and Southeast Asia. Flash flood adversely affect in at least 16% of the world's rice fields (~ 22 m ha) (Singh et al., 2017: Ray et al., 2017). Its estimated annual economic loss is more than 600 million US dollars. Submerged stress regularly affects 15 million hectares or more of rain-fed lowland rice fields in South and Southeast Asia (Neeraja et al., 2007, Ray et al., 2014). Rice is the only cereal crops that adapts to the water logging conditions(Singh et al., 2017 ; Ray et al.,2013) because of its well-developed aerenchyma tissue which facilitates the diffusion of oxygen through a continuous air space from the shoots to the roots and prevents the development of anoxia in the roots. However, complete submergence due to frequent floods can adversely affect the growth and yield of the plant. Rice is the staple food of choice in Bangladesh but the state is facing a chronic rice shortage due to floods and / or climate change (Ray et al., 2016). More than 2.0 million-hectare area was affected by flash floods of various grades and reduced the average yield in Bangladesh by 5% (Iftekharuddaula et al., 2016; Ray et al., 2014; 2018). Many research studies have been recognized quantitative trait loci (QTLs) for submergence tolerance obtained from several populations (Nandi et al., 1997; Siangliw et al., 2003; Toojinda et al., 2003; Xu and Mackill 1996; Septiningsih et al., 2012; Gonzaga et al., 2016; Iftekharuddaula et al., 2016a; Ray et al., 2022). The *Sub1* QTL was about 70% on chromosome 9 of the phenotypic variation for survival under submergence has fine mapped on chromosome 9 and the cluster of genes underlying the QTL cloned (Xu et al., 1996; 2000; 2006; Ray et al., 2022). In this situation, mutation breeding with marker assay is the main objective of developing varieties for submerged, rain-fed environments with enhancement of submergence tolerance to rice. This technique allows sudden changes in the inherited DNA of a living cell or an organism without genetic recombination or natural breeding.

2. MATERIALS AND METHODS

Different Methods of Mutation

Gamma-ray (γ-ray) has been widely used to create mutants in rice, ca. 92% of rice mutants obtained with physical agents were generated with γ-ray (FAO / IAEA, 2019). Small deletions (1–16 pb) had the most frequent exposure γ-ray in the rice genome, but large deletions (9.4–129.7 kb) and large fragment inversions (1284.8 - 3208.5 kb) were also detected (Morita et al.,

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2009). Some mutation detection methods are used in rice such as TILLING (Targeting Induced Local Lesions IN Genomes), TILLING-NGS, and TILLING-HRM. Exome-capture, Eco-TILLING, MutMap, CRISPR-S, PCR-based, Amplicon labeling Based and some types of mutation detection same as SNPs, (single nucleotide polymorphisms), SNPs-Indel short indel. (Table-9). Indel and single base substitutions with higher frequencies of heterozygous were also identified when compared with homozygous mutations (Li et al., 2016c). Also, treatment of dried seeds resulted in heritable mutations at frequencies ranging from 7.5 × 10−6 to 9.8 × 10−6 (Li et al., 2016c). The report suggests that γ-rays produce genetic variability across a wide range of rice genotypes, making it an effective tool for rice improvement (Kole et al., 2008; Harding, 2012). Gamma-ray create genetic variability for abiotic stress tolerance such as submergence , cold , salinity in the ST-87 and ST-301 lines (Song et al., 2012), as well as plant height, number of tillers, shoot and root weight, total biomass and length of panicle (Joshi et al., 2016) .

Table 1: Different types of methods of mutation in rice

Name of methods	Mutagen name	Mutation types	pros	Reference
TILLING	EMS, γ-Ray	SNPs	Detect induced and naturally occurring homozygous and heterozygous SNPs; Suitable for polyploids	Taheri et al., 2017
TILLING-NGS	MNU,SA	SNPs	Mutation detection in pools deeper than eight individuals.	Kumar et al., 2017; reviewed in Taheri et al., 2017
TILLING-HRM	γ-Ray	SNPs, Indels	No require enzymatic digestion; High sensitivity; Time and cost saving.	Li et al., 2018b; reviewed in Taheri et al., 2017
Exome capture	EMS	SNPs, Indels	Large-scale mutation discovery; High-throughput; Cost-effective; Applicable in polyploids.	Henry et al., 2014; reviewed in Taheri et al., 2017
Eco-TILLING	Natural mutations	SNPs	Provides the approximate location within a few base pairs of the induced mutation; Detect induced and naturally occurring homozygous and heterozygous SNPs;	reviewed in Barkley and Wang, 2008
MutMap	EMS	SNPs	Minimizes the number of crosses in crop species and required mutant F2 progeny.	Abe et al., 2012; Takagi et al., 2013; Taheri et al., 2017
CRISPR-S	CRISPR/Cas9	-	Enable a PCR-free, phenotype-based identification of genome-edited T0 plants, and a subsequent selection of transgene-free T1 plants.	Lu et al., 2017
PCR-based	CRISPR/Cas9	short indels (± 1pb)	Accurately identify indel sizes down to ± 1 bp	Biswas et al., 2019
Amplicon labelingbased	CRISPR/Cas9	short indels (± 1pb)	Accurately identify indel sizes down to ± 1 bp	Biswas et al., 2019

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Activity of γ-Ray					
Physical agent	method	size	Dose	Development in Rice	Mutation
γ-Ray	Single nucleotide substitution, inversion and deletion.	7.5×10−6 to 9.8×10−6 (Li et al., 2016c)	50 - 350 Gy	Plant development and metabolism (Hirano et al., 2010; Han et al., 2012; Smillie et al., 2012; Li et al., 2017a; Mbaraka et al., 2017; Li et al., 2018c)	Higher DNA damage, affecting many traits.

Target mutations

Physical and chemical mutagens cause random mutations, providing a limiting mutation frequency in the desired / target location. On the other hand, genome editing systems (Meganucleases - MN; Zinc Finger Nucleases - ZFN; Transcription Activator-Like Effect Nucleus - TALENS; Clustered Regular Intercepted Short Palindromic Repeat-CRISPR) may be targeted (Table 9). Targeted genome editing is mediated by different nucleases that introduce DNA double-strand breaks (DSBs). Cellular DNA can promote single or double cut by stimulating the repair process (Zhu et al., 2017). Cells represent two different repair mechanisms, homologous recombination (HR) and nonhomologous end-joining (NHEJ) (Bortesy and Fisher, 2015). Depending on the repair system used, different changes will be obtained by promoting the creation of genetic variability from the nucleases-induced DBS.

Rice materials

BRRI dhan52, Guti swarna and Mamun swarna were irradiated with 4 doses of 200, 250,300 and 350 Gy {Gy = Gray (1Grey = 10krad)} rays, with the target of developing submergence tolerant mutant line at gamma rays from ^{60}Co source of Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh, Bangladesh (Table 1). Irradiated mutagen seeds, different M1 were sown separately on according to dosage. One thousand seeds were used for each dose. After receipt the irradiated seeds are placed on a petri dish separately according to the dose and variety. Sprouted seeds are then sown in earthen tray. Finally, single seedlings are planted in rows 15 cm apart at a distance of 15 cm, depending on the dose and variety. Germination counts were recorded when the plants germinated completely in petri dishes during the surviving time of harvest. Finally, M2 seeds were collected and kept separately according to dose and variety for M3 generation in the next season. Surviving plants make their selfed to get M3 seeds and get M4 again. All mutants were grown in the plant progeny row to select the true breeding line of desirable character such as submergence tolerance, short duration, high grain yield, fine and medium grain and resistance / major disease / insect tolerance.

Genotypic analysis

Genome DNA was extracted from the leaves of each genotype (2-3 cm piece) using Cetyl Trimethyl Ammonium Bromide (CTAB) mini-prep method (IRRI. 1997) at Biotechnology Lab, Bangladesh Institute of Nuclear Agriculture (BINA), Mymensingh. The cetyltrimethyl ammonium bromide (CTAB) method described by Zheng et al. (1995) is easier and faster than other methods and requires no liquid nitrogen (Ray et al., 2016). The quality of isolated DNA in the protocol was sufficient for PCR analysis. PCR was performed using Chen et al. method. (1997) and the products were visualized in 1.5% agarose gel. After initial denaturation for 2 min

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at 94 ° C, each cycle consisted of 30 sec denaturation at 94 ° C, 30 sec annealing at 55 ° C, and 2 min extension with final extension for 5 min at 72 ° C at the end of 34 cycle. PCR products were mixed with bromophenol blue gel loading dye and electrophoresis was performed by 6% polyacrylamide gel (PAGE) for all SSR markers using mini vertical polyacrylamide gel for high throughput manual genotyping. The gels were stained with 0.5 mg / ml of ethidium bromide and photographed using the Molecular Imager Gel Documentation Unit. SSR profiling was done to tag submergence tolerant QTL using sub1 flanking markers (Figure 6).

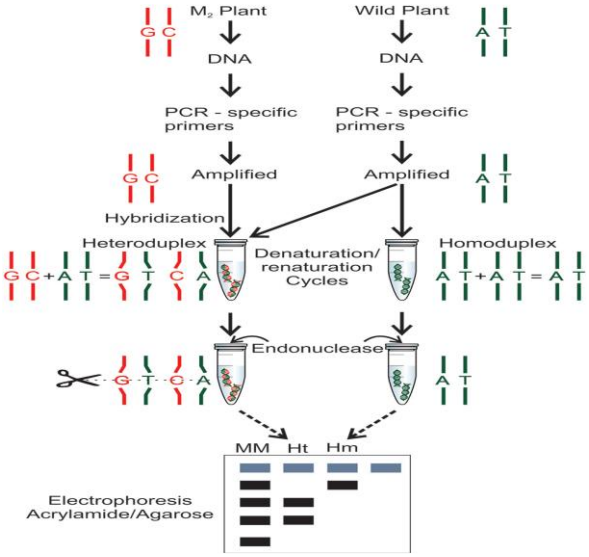

Figure 6: Mutation detection by TILLING technique (Viana,et al.,2019, Till et al., 2003).

Data analysis

The molecular weight of each SSR marker allele was measured using Alpha-Ease 5.5 software. SSR marker alleles were analysed using programmable power marker version 3.25 (Liu and Muse, 2005). This software was used to perform brief statistical analysis of the number of alleles in each locus, major allele frequency, gene variation, polymorphism data content (PIC) values. The genetic distance coefficient of Nei's and a dendrogram representing the genetic relationship between genotypes were created by looking at the same program and tree view (MEGA software) based on the unweighted pair group method with arithmetic averages (UPGMA). Maturity days, plant height, number of tillers hill^{-1}, and effective tiller hill^{-1}, panicle length, number of filled grain panicles^{-1}, and number of unfilled grain panicles^{-1} 1000-seed weight and grain yield for each population was recorded. Data were analyzed to calculate the yield for mutant (M3) and yield contributing characters using Analysis of variance (ANOVA), mean performance, correlation coefficient, Principle component analysis (PCA), dendogram by using the software igetintopc.com_Minitab_18.1. Data calculated on significance difference (P <0.001) which is *** = significant at 0.01% level of probability, ** = significant at 1% level of probability (P <0.01), * = at 5% level of significant probability (P <0.05).

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3. RESULTS

Germination and Survival %

Germination in M1 generation decreased with increasing gray of Gamma dose. In addition, with the increases in gray of gamma dose the decrease in germination (%) and survival (%) in M1 generation was observed from the non-irradiated control (0 Gy). Conversely, of all non-irradiated control seeds, Mamun swarna (0 Gy) has the highest germination (%) which is 96% and BRRI dhan52 (0 Gy) has the highest survival% which is 79.00%. (Table-1).

Table 2. Effect of different doses of gamma rays from ^{60}Co source on germination and plant survival of these rice cultivars in Boro season.

Variety	Dose (Gy)	Seed irradiated (no.)	Seed Germinated at M_1 (no.)	Germination (%)	Plants survived to maturity (no.)	Survival (%)
BRRI dhan52	Cont.(0)	1000	940	94.00	790	79.00
Guti Swarna	Cont.(0)	1000	950	95.00	725	72.50
	200 Gy	1000	750	75.00	690	69.00
	250 Gy	1000	710	71.00	355	35.50
	300 Gy	1000	530	53.00	175	17.50
	350 Gy	1000	225	22.50	155	15.50
Mamun Swarna	Cont.(0)	1000	960	96.00	721	72.18
	200 Gy	1000	815	81.50	719	71.90
	250 Gy	1000	875	87.50	480	48.00
	300 Gy	1000	560	56.00	260	26.00
	350 Gy	1000	205	20.50	160	16.00

Seedling performance

Seedling height has also increased and the height of different rice seedlings has been significantly affected due to complete submergence (Table 8). Mamun swarna (350) - (M2) has a maximum plant height of 45.00 cm and Guti swarna (cont.) (Figure 7 & 8) has a minimum plant height of 34.50 cm. At all submerged levels, the maximum final seedling height was BRRI dhan52 to 51.02 cm and the minimum final seedling height was 39.61 cm from Guti swarna (cont.). The final seedling height was increased as the submerged level increased. The highest elongation% (21.59%) was recorded from BRRI dhan52 and the lowest (7.66%) was recorded from Guti swarna (350) - (M2). The weight and strength of seedlings of different rice genotypes were affected by submerged stress. In all genotypes Mamun swarna (350) - (M2) weight 1.97 g and Guti swarna (250) - (M2) minimum 0.57 g. Plant height from Mamun swarna (350) - (M2) were 4.37 cm and minimum 1.44 cm from Guti swarna (250) - (M2).

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Table 3: Average performance of the genotypes regarding difference score at seedling stage

Cross combination	Seedling age	No. of leaf at submergence	Seedling height(cm)	Seedling weight(gm)	Seedling strength(cm)	Final Seedling height(cm)	Elongation (%)
BRRI dhan52 (cont.)	30 Days	6	40.00	1.26	3.15	51.02	21.59
Guti Swarna(Cont.)	30 Days	5	34.50	1.43	4.14	39.61	12.90
Guti Swarna(200)-(M2)	30 Days	6	37.00	1.05	2.83	43.09	14.13
Guti Swarna (250)-(M2)	30 Days	4	39.50	0.57	1.44	45.26	12.72
Guti Swarna (300)- (M2)	30 Days	4	36.50	0.91	2.49	40.75	11.65
Guti Swarna (350)-(M2)	30 Days	5	44.00	1.50	3.40	47.65	7.66
Mamun Swarna(Cont.)	30 Days	5	41.00	1.32	3.21	45.09	9.07
Mamun Swarna(200)-(M2)	30 Days	6	41.50	0.97	2.39	46.25	10.27
Mamun Swarna (250)- (M2)	30 Days	6	40.00	1.36	3.40	44.76	10.63
Mamun Swarna(300)-(M2)	30 Days	5	42.00	1.28	3.04	45.65	7.99
Mamun Swarna(350)-(M2)	30 Days	5	45.00	1.97	4.37	50.67	11.19

Figure7. Seedling height for mutant of Mamun swarna rice genotypes in M2 generation

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Figure 8. Seedling height for mutant of Guti swarna rice genotypes in M2 generation

Genetic variation for vegetative characters

The analysis of variance for all the characters (viz., days to maturity, number of effective tillers per plant, plant height, , number of filled grains per panicle, number of unfilled grains per panicle, 1000-seed weight, yield per plant) showed highly significant ($p < 0.01$) variation among the genotypes studied but except panicle length showed significant ($p < 0.05$) in Table 2. These parameters related to plant vegetative growth were analyzed for variation assessment (Table 3). The varietal impact on plant height was significant for all genotypes ($P < 0.001$) which was significant at 0.01% level probability. Plant height varied from 101.30 to 109.13 cm. The tallest plant (109.13 cm) was from Mamun swarna-250 (M3), while the shortest plant (101.30 cm) was from Guti Swarna-250 (M3). On the other hand, other genotypes such as BRRI dhan52, Guti swarna-200 (M3), Guti swarna-300 (M3) , Guti swarna-350 (M3), Mamun swarna-200 (M3), Mamun swarna-300 (M3) and Mamun swarna-350 (M3) was almost identical in height. Maturity days varied significantly among genotypes ($P < 0.001$) and ranged from 142.00 to 155.33 days. The earliest mature (142 days) genotype was Mamun swarna (cont.) while the genotype BRRI dhan52 matured from all genotypes (155.33 days). On the other hand, other such as Guti swarna (cont.), Guti swarna-200 (M3), Guti swarna-250 (M3), Guti swarna-300 (M3), Guti swarna-350 (M3), Mamun swarna-200 (M3), Mamun swarna -250 (M3), Mamun Swarna-300 (M3) and Mamun Swarna-350 (M3) had similar average day to maturity.

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Table 4: Analysis of variance (ANOVA) of mutant (M3) for yield and yields contributing characters of rice genotypes in Boro season

Source of variation	df	Mean sum of squares								
		dm	ph	tt	et	pl	fg	ug	sw	yld
Replication	2	0.63	0.76	1.55	0.75	4.34	4.73	2.82	0.11	0.629
Genotype	10	53.29***	16.63***	22.11***	23.09***	11.32*	1467.93***	1201.36***	8.61***	241.49***
Error	20	1.16	0.40	0.4548	0.55	3.51	0.96	1.08	0.14	0.584

*** = Significant at 0.01% level of probability, * = Significant at 5 % level of probability, Here, dm= Days to maturity, ph= Plant height (cm), tt = Total tillers/plant, et= Effective tillers/plant, pl= Panicle length (cm), fg= Filled grains/panicle, ug= Unfilled grains/panicle, sw=Thousand seed weight (g), Yld= Yield/plant (g).

Yield and its components

These parameters of rice yield were analyzed for genetic variability (Table 3). The total tiller per plant varied significantly between genotypes with values ranging from 22.00 to 15.00. The highest total tiller (22.00) was found in Guti Swarna-350 (M3) and Mamun Swarna (cont.), while the lowest value (15.00) was observed in Mamun Swarna-300 (M3). The number of effective tillers per plant varied from 14.33 to 21.33. The highest number of effective tillers per plant has been found (21.33) in Mamun swarna (cont.) which is statistically similar to Mamun swarna (cont.), Mamun swarna-250 (M3), Mamun swarna-350 (M3), Guti swarna. (Cont.), Guti swarna-250 (M3), swarna swarna-350 (M3) and BRRI dhan52. The lowest effective tiller per plant (14.33) has been observed in Mamun swarna-300 (M3) which is statistically similar to Mamun swarna-200 (M3), Guti Swarna-300 (M3), Guti Swarna-200 (M3). The difference in the number of effective tillers per plant produced among the genotypes was significant ($P < 0.001$). Panicle length ranged from 20.13 to 26.30 cm and maximum (26.30 cm) were observed in Mamun swarna-250 (M3) followed by Mamun swarna (Cont.), Mamun swarna-200 (M3), Mamun swarna-300 (M3), Mamun swarna-350 (M3), Guti swarna (cont.), Guti swarna-200 (M3), Guti swarna-200 (M3) and BRRI dhan52 when the shortest panicle length (20.13 cm) was recorded in Guti swarna-250(M3) which was statistically similar to Guti Swarna-300 (M3), Guti Swarna-350 (M3). Differences in panicle length produced between genotypes were significant ($p < 0.05$). The maximum number of grains per panicle (161) was Mamun swarna-300 (M3) and the lowest (82.33) was Guti swaran-350 (M3). Significant differences were observed for filled grains per panicle among all varieties with values from 161 to 82.33. Mamun swarna (cont.) has the highest number of unfilled grains per panicle (97.00) which is statistically similar to Guti swarna-200 (M3), Guti swarna-250 (M3), Guti Swarna-300 (M3), Guti. Swarna-350 (M3) but different from the rest of the genotype. Mamun swarna-350 (M3) has the lowest number of unfilled granules per panicle (27.00). The thousands seed weight varied significantly among genotypes with weights ranging from 21.76 to 16.30 g. The maximum value of BRRI dhan52 was the thousand

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seed weight (21.76 g) and the thousand seed weight (16.30 g) was recorded in Guti swarna (cont.). The yield of plant^{-1} varied significantly among genotypes (P <0.001) from 46.56 to 18.16 g. The highest average value among the three parents of BRRI dhan52, Guti swarna and Mamun swarna was produced by yield plant^{-1} (46.56 g) from Mamun swarna (cont.) and the lowest (18.16 g) from BRRI dhan52. Of all the mutant groups, on the other hand, Mamun swarna-250 (M3) (39.30 g) was more productive than Guti swarna-300 (M3) (20.20 g) (Figure 3). It was found that each mutant population had a lower yielding plant^{-1} from their parents due to segregation from the M3 generation (Figure 5).

Table 5: Mean performance of parents and Mutants (M3) for morphological traits, grain yield plant^{-1} and yield contributing traits in Boro season.

Genotypes	gen	dm	ph	tt	et	pl	fg	ug	sw	yld
BRRI dhan52	1	155.33 a	104.36 bc	20.00 bc	19.00 bc	25.53 ab	97.33 f	62.00 d	21.76 a	18.16 f
Guti swarna (Cont.)	2	150.00 bc	102.93cde	21.66 ab	20.66 abc	24.93 ab	111.00 cd	64.00 d	16.30 f	38.36 bc
Guti swarna-200(M3)	3	148.00 cd	102.76 cde	18.33 c	15.66 d	24.80 ab	109.00 d	80.33 b	17.53 de	24.50 e
Guti swarna-250(M3)	4	151.00 bc	101.30 e	19.00 c	19.06 bc	20.13 b	112.00 c	75.00 c	17.30 def	25.83 de
Guti swarna-300(M3)	5	151.66 b	101.83de	15.33 d	15.00 d	23.93 ab	102.66 e	78.00 bc	18.00 d	20.20 f
Guti swarna-350(M3)	6	149.00 bc	101.63de	22.00 a	21.00 ab	22.26 ab	82.33 g	76.00 c	18.10 d	27.16 d
Mamun swarna (Cont.)	7	142.00 f	105.96 b	22.00 a	21.33 a	26.13 a	134.00 b	97.00 a	16.46 ef	46.56 a
Mamun swarna-200(M3)	8	144.00 ef	104.63 bc	15.50 d	14.46 d	25.90 a	133.00 b	46.00 e	19.33 c	25.33 de
Mamun swarna-250(M3)	9	142.66 f	109.13 a	19.20 c	18.66 c	26.30 a	132.00 b	48.00 e	19.26 c	39.30 b
Mamun swarna-300(M3)	10	146.00 de	101.60 de	15.00 d	14.33 d	25.96 a	161.00 a	48.66 e	17.76 d	25.50 de
Mamun swarna-350(M3)	11	144.33 ef	103.2 cd	21.33 ab	20.66 abc	26.11 a	134.66 b	27.00 f	20.63 b	36.40 c

Similar letter indicates there is no significant difference at 5% level of probability whereas different letter indicates significant difference at 5% level of probability

Here, dm= Days to maturity, ph= Plant height (cm), tt = Total tillers/plant, et= Effective tillers/plant, pl= Panicle length (cm), fg= Filled grains/panicle, ug= Unfilled grains/panicle, sw=Thousand seed weight (g), Yld= Yield/plant (g).

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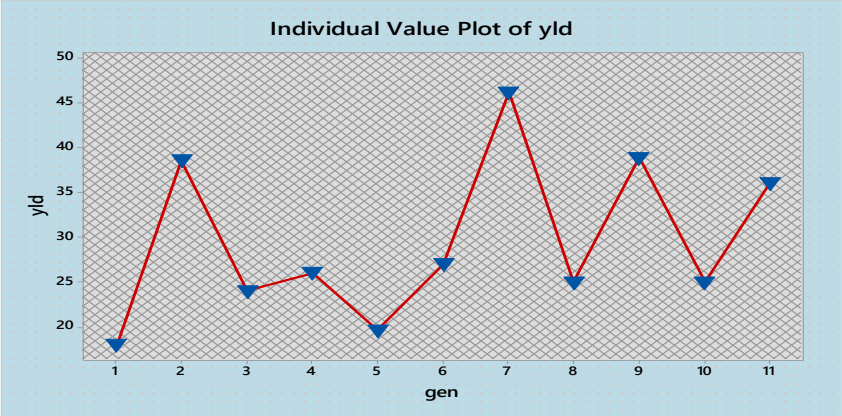

Figure 3: Individual value plot of yield with 11 rice genotypes for different mutant genotypes.

Here, 1= BRRI dhan52, 2= Guti swarna, 3= Guti swarna-200(M3), 4= Guti swarna-250(M3), 5= Guti swarna-300(M3), 6= Guti swarna-350(M3), 7= Mamun swarna, 8= Mamun swarna-200(M3), 9= Mamun swarna-250(M3), 10= Mamun swarna-300(M3), 11= Mamun swarna-350(M3).

Figure 5. Phenotypic performance for mutant of Guti swarna rice genotypes

Correlations between traits

The morphological traits of rice, yield and its components, correlation co-efficient were presented in Table 4. The correlations between the traits of mutants and parental populations were also analyzed ($p < 0.05$, 0.01 and 0.001). The relationship between traits showed that all traits had a positive correlation with yield, except the days to maturity and thousand grain weight. In addition, non-significant but negative relationship has been observed with thousands grain weight and total tiller, effective tiller, filled grain / panicle also.

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Table.6: Pearson correlation coefficients among different pairs of yield and yield contributing characters for different genotype of rice in Boro season

	dm	ph	tt	et	pl	fg	ug	sw
ph	-0.52*							
tt	-0.013	0.157						
et	-0.015	0.165	0.96***					
pl	-0.334	0.46**	-0.063	-0.097				
fg	-0.682***	0.271	-0.356*	-0.307	0.431*			
ug	0.239	-0.148	0.185	0.150	-0.30	-0.464**		
sw	0.147	0.255	-0.051	-0.033	0.272	-0.051	-0.605***	
yld	-0.694***	0.51**	0.57**	0.593***	0.257	0.378*	0.017	-0.355*

*** = Significant at 0.01% level of probability, ** = Significant at 1% level of probability, * = Significant at 5% level of probability

Here, dm= Days to maturity, ph= Plant height (cm), tt = Total tillers/plant, et= Effective tillers/plant, pl= Panicle length (cm), fg= Filled grains/panicle, ug= Unfilled grains/panicle, sw=Thousand seed weight (g), Yld= Yield/plant (g).

Cluster analysis of morphological traits, yield and its components

The observational cluster analysis (dm, ph, tt, et, pl, fg, ug, sw, yld) was assigned to calculate Euclidean distances among 11 genotypes and a UPGMA dendrogram was created using the values indicated in Table 5 and Figure 2. In this dendrogram, 11 rice genotypes were divided into 5 primary clusters based on 9 morphological traits. Of the five clusters, Cluster II had the highest number of genotypes (4), Cluster I had 2 genotypes, and Cluster III had 1 genotype. Cluster IV had 3 genotypes whereas cluster V has only one genotype.

Table 7: Grouping of 11 genotypes according to cluster analysis.

Cluster number	Number of observations	Within cluster sum of squares	Average distance from centroid	Maximum distance from centroid
Cluster1	2	307.18	12.39	12.39
Cluster2	4	466.67	9.75	16.34
Cluster3	1	0.00	0.00	0.00
Cluster4	3	451.66	12.14	14.58
Cluster5	1	0.000	0.0000	0.00

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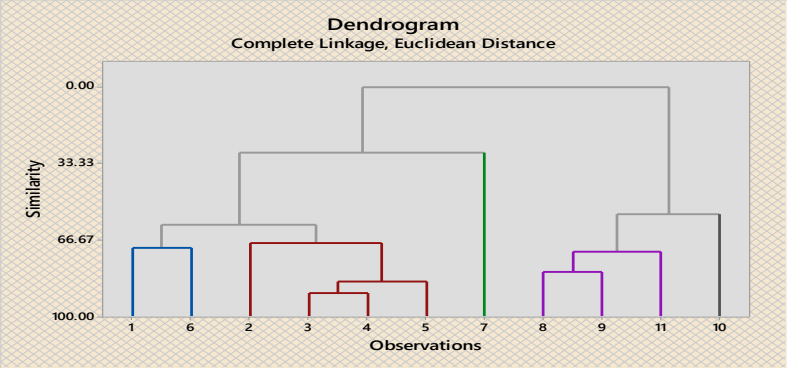

Figure 2. Clustering pattern showing the genetic relationship among rice genotypes

Here, 1= BRRI dhan52, 2= Guti swarna, 3= Guti swarna-200(M3), 4= Guti swarna-250(M3), 5= Guti swarna-300(M3), 6= Guti swarna-350(M3), 7= Mamun swarna,8= Mamun swarna-200(M3), 9= Mamun swarna-250(M3), 10= Mamun swarna-300(M3) , 11= Mamun swarna-350(M3).

Principal Component Analysis (Trait Association)

The nine yields and yield-related traits with their other contrition characters for PCA and total genetic divergence, were presented in Table 6. According to the suggestion of Brejda et al. (2000), data with an Eigen value greater than 1 in each component were considered, as it determines the minimum 10% of variation. Superior Eigen values were considered to be the best attributes in principle components. A sree plot shows graphically important PCs that explained major variability obtained by drawing a graph between Eigen values and principle component numbers (Figure 1). The results of the study show that the three components indicated Eigen value of greater than one. The first three principle components studied, including the Eigen value, explained 80.4% of the total variation in 11 rice genotypes for 9 quantitative traits. These first three main components PC1, PC2 and PC3 explained 33.4%, 29.3% and 17.7% of data variability, respectively (Table 6). The first major component (PC1) is responsible for more than 33.4% of the total variance. The results showed that grain yield per plant has the most positive loading (0.476), followed by plant height (0.423), filled grain per panicle (0.383), panicle length (0.345), effective tillers per plant (0.159), total tillers per plant (0.149) and thousand grain weight (0.028). Only two characters showed negative loading in the first principle component (PC1) which is the day to maturity (-0.487), unfilled grains per panicle (-0.209). The second principle component (PC2) accounted for more than 29.3% of total variance whereby filled grain per panicle (0.323), thousand seed weight (0.238), panicle length (0.209), plant height (0.002), days to maturity (-0.070). In contrast, total tiller per plant (-0.543), effective tiller per plant (-0.536), unfilled grains per panicle (-0.343), grain yield per plant (-0.301) show negative loading. (Table 6). The third principle component (PC3) were more than 17.7% of the total variance and thousands seeds weight (0.698), days to maturity (0.264), effective tiller per plant (0.263), total tiller per plant (0.261), panicle length (0.261) , Plant height (0.169) showed positive loading. In

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addition, they show negative loading unfilled grains per panicle (-0.387), filled grain per panicle (-0.263), grain yield per plant (-0.171).

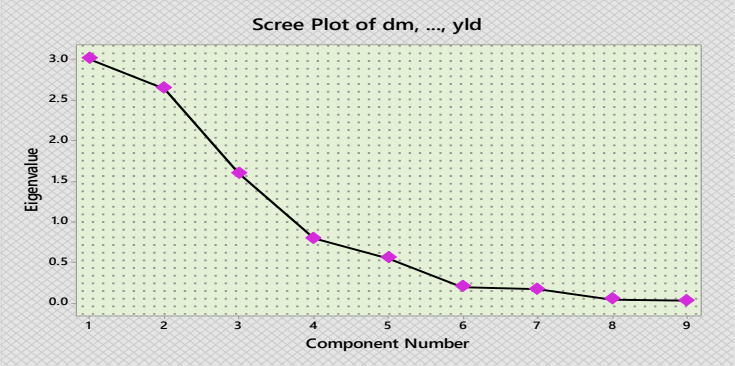

Figure 1: Sree plot of principal component analysis of 11 rice genotypes for yield related traits.

Table 8: Principal Components Analysis (PCA) for nine yield and yield related traits in 11 rice genotypes from principle component analysis with Eigen vectors (loadings) of the first three principal components in Boro season

Variable	PC1	PC2	PC3
Eigenvalue	3.00	2.63	1.59
Proportion (%)	33.4	29.3	17.7
Cumulative (%)	33.4	62.7	80.4
Days to maturity	-0.487	-0.070	0.264
Plant height (cm)	0.423	0.002	0.169
Total tillers per plant	0.149	-0.543	0.261
Effective tillers per plant	0.159	-0.536	0.263
Panicle length (cm)	0.345	0.209	0.169
Filled grains per panicle	0.383	0.323	-0.263
Unfilled grains per panicle	-0.209	-0.343	-0.387
Thousand seed weight (g)	0.028	0.238	0.698
Grain yield per plant (g)	0.476	-0.301	-0.171

SSR genotyping for mutant populations:

SSR genotyping is important for the production and maintenance of a mutant stock on a large scale for quality control of mutants. Rice is a self-pollinating plant but occasional outcrossing by unwanted pollen can occur, especially when a mutant plant is partially or completely male-sterile. However, it is unrealistic to control pollen by bagging panicles in a greenhouse or field. We genotype the original Mamun Swarna mutants and BRRI dhan52 parent with 11 SSR marker of marker (RM8300, RM23901, RM231, RM518, RM587, RM234, RM5799, RM5708, RM4112, RM313, Sc34/RM23679) which together fingerprint pattern available. We regularly used submergence tolerance primer as SC34 / RM23679. To date, almost all morphological

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mutants have tested the same SSR pattern as submergence tolerant BRRI dhan52; however, for conditional mutants where submergence tolerant mutant for selection pressure is high for identification of variants (Figure 4). Marker diversity across sub1 region of submergence tolerant primer (SC34 / RM23679) on chromosome9 and the sequence and position of this primer, number of alleles, allele's size range, allele frequency (%) and polymorphism information Content (PIC) of the primer (SC34 / RM23679) was obtained for SSR primer with 11 rice genotypes. Gene diversity values of the alleles across the 11 genotypes were 0.469. The Polymorphism Information Content (PIC) value was 0.359 (SC34 / RM23679) and the allele frequency (%) was 62 (Table 7).

Table 9. The sequence and position of submergence tolerant primer and number of alleles, allele size range, allele frequency (%) and Polymorphism Information Content (PIC) of 11 rice genotypes for SSR primer.

Sl no.	SSR Marker	DNA Sequence	No. of base	Ann. Temp.	Expected product size(bp)	Position (bp)	Chromosome Number	Repeat Motif
1	SC34/ RM23679	F-AGTGCATGTTGAGCTTGTGG R-ACCTGGCAATGAGAACGAGT	20 20	55	189	868,836 - 869,025	9	(AGAA)10

	Marker	Position	No. of allele	Allele Frequency (%)	Gen diversity	PIC	Chro. No.
1	RM23679	0.5 Mb	2	62	0.469	0.359	9

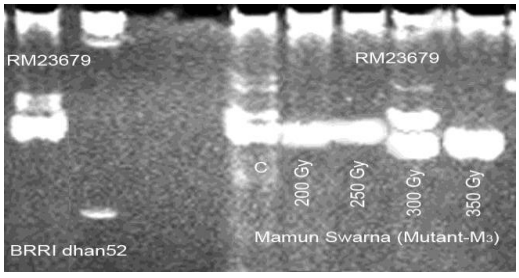

Figure 4. DNA profile of RM23679 for mutant of Mamun swarna rice genotypes

4. DISCUSSION

The trait assessed in the genotype of 11 rice showed significant differences among themselves. This indicates the existence of population variation. The differences displayed by the genotypes may be because they originated from different regions. In this regard, several reports have been published on phenotypic variations in rice genotypes (Mehetar et al., 1996). Similarly, Pandey et al. (2009) reported a very significant difference among 40 rice accessions using 12 quantitative characters. In the same vein, Padmaja Rao, (1991) discovered a 95% difference between five rice

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populations using 20 morphological characters. High-yielding genotypes were short. This feature was the result of a short internode. This can be attributed equally to the highly efficient assimilation partition at the expense of vegetable growth. Thus, instead of having tall plants, high yields came as compensation for the vegetative deficiency. This trait is also useful for protection against lodging. Although plant height is largely controlled by genetic makeup of the genotype, it is highly influenced by environmental factors. As mentioned indirectly earlier, the yield of rice is indirectly related to its height. This is due to the sinking competition for finite photosynthesis produced by limited sources. So what will be used to increase the yield will be unnecessarily used for somatic cell growth which in turn will increase the growth and height of the luxuriant plants. Therefore, taller varieties generally yield less than shorter varieties. Another serious disadvantage of tallness rice is lodging which significantly reduces the final yield and puts the plants at risk of some other natural attack. In this experiment, all high-yielding varieties were found to be of medium height. This means that for high-yielding varieties, medium height of plants is recommended during breeding. Flag leaf plays an important role in increasing the yield of rice as it is the only source of assimilation production for spikelets filled in the grain-filling stage (Raj and Tripathi, 2000). The larger the leaf area, the more solar barrier and photosynthate production that all other factors of production are not limited to. Therefore, the flag leaf area was found to be directly related to the yield components: number of panicles, panicle length, and number of grains per panicle, 100 grains weight and yield per hectare. Furthermore, flag leaf have been found to be metabolically active in supporting high grain yields. This work is supported by our research, Ashrafuzzaman et al. (2009) make it clear that yield components are positively correlated with flag leaf area. The number of tillers plays an important role in determining the yield of rice grain as it is directly related to the number of panicles that will be produced per unit area of land. Less tiller results in less panicles; Excess tillers reduce high tiller abortions, short panicles, poor grain filling, and grain yields (Pandey et al., 2009). When there is an adequate supply of nutrients, mitotic cell division will enhanced and the growth of tiller and the normal plant life of the plant will increase. In this work, tiller production was in the middle to lower levels. So there was no problem with tiller abortion during production period. The number of panicles per hill was moderate and this is correlated to the number of tillers produced. Thus the number of effective tillers depends on the number of tillers produced and is directly proportional to the panicle produced per unit area and ultimately depends on the variety (Hossain et al., 2008). Panicle number is an important character that directly affects yield. Therefore, if agricultural manipulation is used to increase the number of panicles produced per unit area, the yield may be increased. Panicle length in yield contributing characters. This is proportional to the number of potential spikelets to be filled at the grain-filling stage.

The rice plant will naturally have a superior spikelet filled before the inferior plant. The superior spikelets are located on the primary branches at the top of the rice plant. So they fill up quickly and produce heavy and large grain weights. On the other hand, inferior spikelets are usually located on the secondary branch and are usually slow to fill. They also produce poorly filled spikelets or sterile (Yang and Zhang, 2006). Slow grain-filling rates and low grain weights of low quality spikelets have often been attributed to limitations in carbohydrate supply (Young and Zhang, 2006). According to the (Mostajeran and Rahimi-Eichi, 2009), the underlying factors responsible for the difference in grain filling between upper and lower spikelets remain unknown. This study shows that some varieties flower earlier than others. Those that flowered

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earlier mature early while those that flower late are late in their maturity. Early flowering indicates a short life cycle and is considered a positive characteristic for rice improvement. Early maturing varieties are advantageous in areas with short rainfall periods as they grow faster at plant stage and thus compete more with weeds. They reduce the cost of weed control and use less water (Khush, 1995). It is a known fact that frequent droughts hinder rice production in rainfed lowlands. So when drought occurs during the reproductive stage of rice production, pollination and fertilization as well as grain filling are severely damaged and panicles can be empty. In this situation, early maturing varieties will provide remedial measures instead of establishing irrigation facilities and developing drought-tolerant varieties (Haefele and Bouman, 2009). The increase in the number of filled grains can be attributed to the efficient transfer of carbohydrates from the source to the spikelet (sink) which in turn leads to increased grain yields (Xu and Zhu, 2007). There were different types of yields in this work between high and low. Yield differences between different rice varieties have been reported at any time in both field and glasshouse experiments when comparing different varieties of rice (Biswas, et al., 1998). The differences are genetically based, although the environment has a large contribution to make in revealing the underlying potential. In this work, there was a high number of genotypes of effective tillers, as well as higher grain yields per panicle (Dutta et al., 2001). The weight of 100 or 1000 grains weight contributes significantly to the final yield. This represents the weight of the individual seeds which cannot be measured directly due to the size of the individual seeds. The results of the present study showed that the 100 grains weight varied significantly among the tested varieties. This may be due to differences in their origin and genetic makeup. Similar reports have been published by BRRI Scientist (BRRI, 1997) and Ashrafuzzaman et al. (2009). The panicle length determines how many spikelets can be found in a panicle and hence the full spikelet and consequently of the final grain yield. The longer the panicle, the more spikelets and filled grains, if not limited by other environmental conditions. Found here, the length of the panicle is positively correlated with the final yield. The results of this study are believed to have been established by Chakraborty and Chakraborty, 2010 who found a significant positive relationship between the panicle length and the grain yield per hill.

The positive correlation coefficient between vegetative traits and yield parameters in this work was an indication that the measured quantitative traits are suitable for selection to obtain better strength or heterosis for yield prediction and breeding programs. A positive correlation between final yield and plant height and total number of grains per panicle and days to maturity indicates that a better exploration of these three traits may be used to develop the desired genotype (Ray et al., 2022). Individual panicle grain yield adds to the contribution of grain production in making the final yield. Therefore, high panicle grain yield can be successfully used as an important selection indicator for grain yield Meenakshi et al., 1999. In addition, when a panicle yield is related to the area per unit, a positive correlation coefficient will result (Mustafa and Elsheikh, 2007). In the present study, yield per panicle was best correlated with yield. Therefore, more attention is paid to this feature to determine the final yield in all evaluated genotypes.

The UPGMA dendrogram broadly divides the genotypes of rice into five main group's at1.43 dissimilarity coefficients. This refers to a high level of morphological diversity in rice genotypes. This study revealed the effectiveness of quantitative or morphological traits in grouping rice genotypes. It has been established that genetic divergence analysis of rice genotypes based on morphological traits can be used to classify and differentiate different genotypes of a population

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(Franco et al., 2001). This genetic divergence analysis also plays an important role in the selection of different genotypes for further improvement of rice varieties through breeding (Shahidullah et al., 2010). We managed to group 45 genotypes into 5 main groups using some quantitative traits to better manage diverse genotypes as well as better selection to achieve higher heterotic vigour strength among the resulting offspring after crossing. Similarly, Ahmadikhah et al. (2008) divided 58 rice varieties into four groups using 18 morphological traits, while Mazid et al. (2013) his 41 rice genotypes were clustered into 6 based on 13 morphological traits. Therefore, in order to achieve breeding success for higher strengths among the genotypes, Group I can be hybridized with Group V or Group VI in this work, and confirmed by Latif et al. (2011). These principal components have been found to contribute 78.26 percent to total variability (Kumar et al., 2020, Ray et al., 2022). Similar results were previously reported in rice by Gana et al. (2013) and Nachimuthu et al. (2014). Prasad etc. (2001) and Ali et al., (2010) reported that the number of alleles per locus ranged from 2 to 8, with an average of 3.8 using varieties published by BRRI. The comparative study is based on three previous estimates of rice microsatellite analysis, such as 0.26 to 0.65 with an average of 0.47 (Singh et al., 2015; Ray et al., 2022), 0.28-0.50 with an average of 0.45 {Umadevi, et al., 2014) and 0.239 to 0.765 with Average 0.508 (Hossain et al., 2012).

5. CONCLUSION AND FUTURE PROSPECTS

The history of mutation induction began long time ago. However, from the early days to now, many improvements have been made to increase the mutation frequency. Rice is a major grain species, and since it represents a small genome and exhibits synteny with other crops, many of the achievements of the structural and functional genomics of rice have been extended to other crop species. Rice was already a model from preliminary results of mutation induction, which includes more than 90 years of research. Much has been achieved in terms of agronomy through increasing the variability of rice. Based on the studies discussed here, it is possible to verify that mutagenesis is a key tool in the development of genetic resources, a new source of variability for the development of submergence tolerant genotypes, future submergence prone, and rainfed ecologies problems. All genotypes showed variation for vegetative and yield traits. Also, all the traits that are positively related to the final yield, excluding the day to maturity and the thousand seed weight. Finally, the evaluated genotypes were divided into five main clusters based on the traits assessed using the UPGMA dendrogram. The first three principal components studied, including the Eigen value, explained 80.4% of the total variation in 11 rice genotypes for 9 quantitative traits. These first three principal components PC1, PC2 and PC3 explained 33.4%, 29.3% and 17.7% of data variation, respectively. The highest average yield per plant among the three parents of BRRI dhan52, Guti swarna and Mamun swarna was produced by 46.56 g from Mamun swarna (cont.) and the lowest 18.16 g from BRRI dhan52. Of all the mutant groups, Mamun swarna-250 (M3) was higher yielding (39.30 g) than Guti swarna-300 (M3) (20.20 g). This assessment can be effective in developing reliable selection indicators for important agronomic traits of rice. Considering these aspects, mutagenesis had, has and will certainly have an effect on the genetics and breeding of rice.

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Conflicts of interest

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The authors declare that there is no conflicts of interest

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