Genetic diversity in mutated and non-mutated rice varieties

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ABSTRACT. We studied the genetic variability due to mutation induced by γ-rays (10, 15, and 20 Kr) on various traits of twelve rice genotypes. Mutated and non-mutated seeds were sown in the field between July 2013 and 2014 using a split plot design. Yield and yield-related trait data was recorded, which showed significant ($P < 0.05$) genotypic and irradiation effects. Gamma radiation exerted non-significant effects on the panicle length of all plants, indicating the uniformity of performance of this character at different radiation levels. The plant height, grains per main panicle, panicle length, 1000-grain weight, grain weight per main panicle, and fertility percentage was minimum in Basmati-198, Basmati-Pak, Shaheen-2000, Super Basmati, Basmati-385, and Super Basmati, respectively, when exposed to radiation at 20 Kr. However, Basmati-370 attained maximum flag leaf area at this level of radiation. Broad sense heritability ranged from 72.0 to 97.7%, indicating the

possibility of selection during earlier generations. Phenotypic correlation was positive and significant between grain weight per main panicle with panicle length and flag leaf area. Number of grains per main panicle and genotypic correlation were positive among grain weight per main panicle, panicle length, fertility percentage, 1000-grain weight, and plant height, indicating the effectiveness of these traits in the selection for yield. Phylogenetic analyses revealed a pair-wise similarity ranging from 0.51-0.76 before mutation and 0.39-0.89 after mutation. This study proved that γ-rays at 20 Kr would be most effective in creating genetic variability in the existing germplasm.

Key words: Genetic diversity; Genetic variation; Mutagenesis; Mutation breeding; *Oryza sativa*

INTRODUCTION

Rice is an important cereal crop in Pakistan, an excellent source of foreign exchange earnings, and is the staple food of approximately half the world population. According to The Food and Agriculture Organization of the United Nations, 70.0% more food must be produced in 2050 in order to feed a population of over nine billion people. Pakistan produces Basmati rice, which occupies a unique position in the world economy. Basmati rice accounts for 40.0%, while coarse rice accounts for 60% of the national rice production. Rice crop contributes to 0.7% of the gross domestic product (GDP) of Pakistan and 3.1% of the value addition in agriculture. The aim of plant breeding is to improve crop production, as well as to improve the quality by changing the hereditary characteristics of plants. Semi-dwarf growth and rapid growth are important characteristics of rice breeding. There is an urgent need to adopt some innovative technologies to break the yield gap in rice, and to assist conventional breeding programs for the development of good varieties of rice. Greater knowledge of the genetic architecture of various genotypes is therefore necessary to formulate an efficient breeding methodology. Ionizing radiations are an efficient source for the generation and improvement of genetic variability in rice crop. Historically, the use of mutagenesis in breeding has involved forward genetic screens, the selection of individual mutants with improved traits, and their incorporation into breeding programs. Inducible mutation is a suitable source of variations for crop improvement.

Exposure of plant material to ionic radiations inhibits meiosis. Among the different ionic radiations, gamma rays are frequently utilized in the crop improvement program, owing to their shorter wavelength, excessive energy per photon, and ability to penetrate deep within the tissues. However, the ability of these rays to induce genetic changes varies at an inter- and intra-specific level. The dimmers altering the strand finally alter normal base pairing, which hinders normal replication and transcription. Germination and survival decreases with the increase in radiation dosage of gamma rays and fast neutrons (Din et al., 2004). Gamma rays have been used to develop early maturing semi-dwarf varieties of rice. Increased intensity of gamma rays up to 35 Kr remarkably reduces the number of tillers per plant, plant height, and length of main panicle. Ibrahim (2013) discovered that maximum variations in fertility percentage and filled grains per panicle could be achieved by deputing 25 Kr gamma rays to Egyptian rice cultivar Sakha 105.

The mutation-based variety with higher yield potential YuaFengZao, was developed from IR8 using gamma radiations. The Nuclear Institute of Agriculture and Biology in Pakistan has

developed 24 mutation-based varieties of various crops. As many as 3000 mutant varieties of crop plants (Shu and Lagoda, 2007) have been developed worldwide since the discovery of the mutation-inducing ability of X-rays in *Drosophila melanogaster* and barley. China has produced the largest number of mutation-based varieties of rice, ahead of India (11.5%), the USSR and Russia (9.3%), the Netherlands (7.8%), USA (5.7%), and Japan 5.3%) (Ahloowalia et al., 2004). Of the 434 mutant varieties of rice, 225 (56.0%) have been induced by gamma rays, and a majority (67.0%) were developed as direct mutants (Maluszynski et al., 2001).

Repeated utilization of limited basmati varieties for the evolution of new varieties has narrowed the genetic base of recently evolved varieties in Pakistan. The development of broad gene-based varieties could efficiently slow down the evolution of strains and biotypes of parasites. Basmati 370 is the principal component of 4 major existing varieties of rice in Pakistan (Khan, 1996; Rabbani et al., 2008). Owing to the narrow genetic base, all aromatic varieties are susceptible to various biotic and abiotic stresses (Junaid et al., 2000). Genetic diversity can be estimated on the basis of morphological data; however, accuracy can be ensured by the use of DNA markers such as RAPD (Rabbani et al., 2008) and SSR markers (Shah et al., 2013). SSR markers have been used in a number of mutation analyses (Li et al., 2010), and correlation and genetic diversity studies (Zhao et al., 2010), owing to their easy availability throughout the genome.

MATERIAL AND METHODS

Field studies

Well filled, dry, healthy, dormant (200 g) seeds of Basmati-370, Basmati-Pak, Basmati-198, Basmati-385, Super Basmati, Basmati-2000, Shaheen-2000, Basmati-515, IRRI-6, KS-282, Niab-IRRI-9, and KSK-133, with an average moisture content of 13.0% were treated in the gamma chamber at 10, 15, and 20 kg Roentgen for 30 min to 2 hours, depending on the dose of gamma radiation. The wave length of gamma rays ranged from 0.15 to 0.005 Å. The gamma irradiation was performed at the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad, Pakistan.

Treated seeds (100 M_0) of each genotype were cultivated in fully saturated loamy soil on May, 2011, in order to obtain M_1 seeds. A thin layer of silt and farmyard manure mixture (3:1) was used to cover the seeds. The beds were moistened with showers of canal water twice a day. Wheat straw was used to save seedlings from heat shocks and conserve moisture, until the seedlings attained a height of 5 cm. Thirty-day-old seedlings were transplanted to the field for space planting. All material was propagated for M_1 in 2012 in the Experimental area of the Department of Plant Breeding and Genetics, UCA & ES, The Islamia University of Bahawalpur. This evaluation was conducted in the M_2 generation, grown in 2013 and 2014 at the same experimental area: M_2 seeds of each genotype subjected to each type of irradiation were divided into two parts; half was sown each year. The seeds were cultivated in a split plot design, with the original genotype cultivated in the main plots, and the gamma-irradiated seeds grown in the sub-plots (four replications). The sub-plot size was maintained by ensuring a row to row and plant to plant distance of 45 cm and 30 cm, respectively. Standard agronomic and crop management practices were employed to maintain a healthy crop stand. Plant height, flag leaf area, panicle length, number of grains per panicle, grain weight per main panicle, fertility percentage, and seed index data was collected and analyzed. Data collected for each trait over two years was pooled before being analyzed. The genotypic and phenotypic correlation coefficients of twelve varieties were analyzed according to the formula given

by Kwon and Torrie (1964). Data obtained over two years was pooled, as the yearly effect was not significant. Statistical analyses were performed using Fisher's analysis of variance (ANOVA), and the mean values were compared using the least difference test at a probability level of 5% (Steel et al., 1997) using Statistix 8.1 (Analytical Software, Tallahassee, FL, USA).

Genetic diversity studies

Marker analysis

During 2014, leaves were collected from young seedlings 15 days after transplantation, and DNA was extracted using a previously-described method (Liu et al., 2011). Eighty seven SSR primers equally distributed on twelve chromosomes (McCouch et al., 2002) were analyzed. The SSR analysis was done as described by Tong et al. (2011) and Wu et al. (2013). The amplified fragments were electrophoresed on a 1.2% agarose gel at 80 V in 1X TBE buffer (Yang et al., 2012). The gels were stained with ethidium bromide, and visualized in a gel documentation system.

Marker data analysis

Amplified products of each SSR primer were scored as present or absent (1, 0). The PowerMarker program (Liu and Muse, 2005) was used to calculate the number of alleles per locus for SSR analysis. Genetic similarity estimates were obtained for all pairs of cultivars according to the method described by Nei and Li (1979). The resulting similarity data was used to prepare a dendrogram, based on un-weighted pair group method with arithmetic average (UPGMA), to determine the genetic relationship among varieties. The phylogenetic tree was visualized using the TreeView software (Page, 2001).

RESULTS AND DISCUSSION

ANOVA revealed highly significant (P < 0.01) genotypic irradiation and the effects of its interaction on all traits of rice excluding the fertility percentage; only significant differences (P < 0.05) were observed among the fertility percentages of all genotypes (Table 1).

Table 1. Mean square values for various traits of rice varieties used in this experiment.

So. Var.	d.f	PH	FLA	PL	NGP	GWMP	FP	SI
Replication	2	54.2[NS]	14.44[NS]	1.58[NS]	793.02[NS]	0.16386[NS]	9.819[NS]	7.842[NS]
Varieties	11	1366.3**	189.14**	13.65**	3797.13**	2.93412**	395.106*	23.004**
Error$_1$	22	30.6	9.34	0.82	335.93	0.09187	26.933	6.419
Radiation	3	58098**	1136.67**	2656.67**	5498.89**	0.37003**	108.640*	220.021**
V * R	33	152.7**	44.71**	32.26**	815.00**	0.29122**	233.795*	64.677**
Error$_2$	72	36.9	11.17	1.36	375.65	0.11929	37.196	11.506
Total	133							

[NS]Non significant. *,**Significant at 5%, 1%. PH = plant height; FLA = flag leaf area; PL = panicle length; NGP = number of grains per panicle; GWMP = grain weight per main panicle; FP = fertility percentage; SI = seed index.

The interaction between genotypes and irradiation was significant; therefore, the means of both factors were compared separately. Mutated plants displayed obvious phenotypic changes, in the color, shape, and size, which distinguished them from non-irradiated plants, as previously

reported by Haris et al. (2013). The post-hoc test revealed that all genotypes showed significant differences between their control and irradiated plants; however, within a single or among different doses of radiation, a majority of the genotypes showed non-significant differences (Tables 2-8).

Table 2. Comparison of mean values for Plant height of rice varieties at different radiation doses.

Varieties	Control	10 Kr	15 Kr	20 Kr	Mean value
Basmati-370	158.31[aA]	59.53[aC]	65.30[aB]	60.80[aC]	85.98
Basmati-Pak	141.60[bA]	52.00[bB]	49.97[cB]	51.07[cB]	73.66
Basmati-198	127.95[cA]	38.07[eB]	37.73[fgB]	37.40[efB]	60.28
Basmati-385	120.77[cdA]	32.47[gfB]	33.93[gB]	30.63[gB]	54.45
Super Basmati	115.80[dA]	39.00[eC]	43.10[efB]	43.10[eB]	60.25
Basmati-2000	126.09[cA]	48.00[bcB]	47.20[deB]	51.33[bcB]	68.15
Shaheen 2000	115.92[dA]	47.13[cB]	43.80[efB]	47.30[cdB]	63.53
Basmati-515	128.62[cA]	46.47[cB]	45.88[eB]	46.[B]	66.87
IRRI-6	105.80[eA]	39.73[eB]	35.60[gBC]	34.45[fC]	53.89
KS-282	113.65[deA]	34.27[fB]	33.60[gB]	33.07[fB]	53.64
NIAB-IRRI-9	126.25[cA]	30.27[gB]	30.13[hB]	28.30[gB]	53.73
KSK-133	105.80[efA]	35.13[efB]	34.30[gB]	34.53[fB]	52.44
Mean value	123.88	41.83	41.71	41.54	

LSD value = 9.7580. Mean values sharing common letters for a given trait do not differ significantly at 5% probability level. Capital and small letters shows similarity and differences with in a same variety (rows) and among varieties (Columns).

Table 3. Comparison of mean values for flag leaf area of rice varieties at different radiation doses.

Varieties	Control	10 kR	15 kR	20 kR	Mean value
Basmati-370	35.40[aA]	24.68[bcB]	23.60[aB]	18.27[bC]	22.18
Basmati-Pak	31.22[bA]	22.91[cB]	19.15[bC]	18.86[bC]	20.31
Basmati-198	32.78[abA]	7.20[fC]	9.04[eB]	7.55[fC]	7.93
Basmati-385	29.28[bcA]	8.28[fB]	8.99[fB]	5.06[fC]	7.45
Super Basmati	29.65[bcA]	22.44[cB]	19.35[bC]	21.79[aBC]	21.19
Basmati-2000	29.37[bcA]	28.31[aA]	19.18[bB]	19.32[abB]	22.27
Shaheen 2000	25.46[cdA]	23.82[bcAB]	19.76[bB]	17.41[bcB]	20.34
Basmati-515	23.25[dA]	22.47[cA]	21.13[abAB]	19.82[bB]	21.14
IRRI-6	22.41[deA]	22.25[dcA]	20.19[abAB]	18.34[bB]	20.26
KS-282	28.7[bcA]	17.13[dB]	17.31[cdB]	17.62[bcB]	17.36
NIAB-IRRI 9	28.57[bcA]	15.58[deB]	14.85[cdB]	14.24[cdB]	14.89
KSK-133	24.92[dA]	12.46[eC]	14.00[cdB]	13.0[cdBC]	13.15
Mean value	28.33	18.96	17.21	15.94	

LSD = 5.37437580. Mean values sharing the common letters for a given trait do not differ significantly at 5% probability level. Capital and small letters shows similarity and differences with in a same variety (rows) and among varieties (Columns).

Plant height

Plant height is an important agro-morphological attribute of crop plants that is generally indicative of the relative growth and vigor of crop plants. The genotypes showed a wide range of variability in plant height, varying from 28.30 cm (NIAB-IRRI-9 @ 20 Kr) to 158.31 cm (Basmati-370 @ 0 radiation) (Table 2). The plant height was significantly reduced in irradiated plants; for example, NIAB-IRI-9 irradiated at 20 Kr showed a 97.95 cm reduction in plant height compared to its control. The post-hoc test revealed significant differences between the mean of control and irradiated plant heights; however, the three irradiated means of plant height showed only non-significant differences. These results are supported by the results of Wattoo et al. (2013), who reported a gradual decrease in plant height with the increase in gamma radiation intensity (15-25 kR); the plant height of mutated plants was significantly lower than that of control plants.

Table 4. Comparison of mean values for Panicle length of rice varieties at different radiation doses.

Varieties	Control	10 kR	15 kR	20 kR	Mean value
Basmati-370	29.87[aA]	10.26[abB]	11.03[aB]	10.73[abB]	10.67
Basmati-Pak	27.57[bA]	9.80[bB]	10.30[bB]	9.53[bB]	9.877
Basmati-198	28.26[abA]	10.20[abB]	10.10[bB]	10.30[abB]	10.2
Basmati-385	26.53[bA]	11.33[aB]	10.16[bB]	11.16[aB]	10.88
Super Basmati	27.60[bA]	9.60[bB]	10.33[bB]	10.20[bB]	10.04
Basmati-2000	27.82[bA]	10.60[abB]	10.60[bB]	10.76[abB]	10.65
Shaheen 2000	27.31[bA]	11.50[aB]	10.83[bB]	9.80[cB]	10.71
Basmati-515	27.08[bA]	11.13[aB]	11.40[aB]	10.53[abB]	11.02
IRRI-6	23.49[cA]	9.66[bB]	8.56[cB]	8.86[bcB]	9.03
KS-282	27.58[bA]	9.06[bB]	8.83[cB]	8.63[bcB]	8.84
NIAB-IRRI 9	27.78[bA]	8.10[cB]	8.83[cB]	7.73[cB]	8.22
KSK-133	23.66[cA]	8.40[cB]	8.10[cB]	8.06[cB]	8.18
Mean value	27.04	9.97	9.92	9.69	10.67

LSD = 1.8138. Mean values sharing the common letters for a given trait do not differ significantly at 5% probability level. Capital and small letters shows similarity and differences with in a same variety (rows) and among varieties (Columns).

Table 5. Comparison of mean values for Number of grains per panicle of rice varieties at different radiation doses.

Varieties	Control	10 kR	15 kR	20 Kr	Mean value
Basmati-370	162.27[aA]	126.63[cdIJ]	128.63[dI]	134.63[abH]	138.04
Basmati-Pak	110.67[fA]	69.30[hiIJ]	75.30[iHI]	78.83[gH]	88.26667
Basmati-198	125.73[eA]	111.73[dD]	90.47[ghIJ]	110.57[dDE]	108.9233
Basmati-385	160.60[abA]	118.27[deJK]	158.27[aAB]	129.93[bH]	149.6
Super Basmati	124.80[eE]	106.60[eEF]	119.17[eB]	74.17[ghOP]	106.0467
Basmati-2000	163.60[aA]	142.87[bE]	130.00[cdH]	114.27[cdKL]	135.9567
Shaheen 2000	156.63[Ba]	119.07[deK]	122.80[deJK]	141.23[aEF]	140.22
Basmati-515	143.33[Ca]	119.23[deG]	129.33[dDE]	127.23[bcE]	133.2967
IRRI-6	143.43[cA]	131.60[cD]	117.53[eGH]	130.17[bDE]	135.0667
KS-282	156.63[Ba]	147.93[abC]	130.03[cdGH]	135.57[abF]	144.8633
NIAB-IRRI 9	136.93[Da]	130.67[cdBC]	111.10[efH]	126.93[cDE]	124.9867
KSK-133	159.83[abA]	152.67[aC]	152.47[abC]	100.73[eOP]	154.99
Mean value	111.85	146.1	125.41	123.70	

LSD = 31.415. Mean values sharing the common letters for a given trait do not differ significantly at 5% probability level. Capital and small letters shows similarity and differences with in a same variety (rows) and among varieties (Columns).

Table 6. Comparison of mean values for Grains Weight per main Panicle (g) of rice varieties at different radiation doses.

Varieties	Control	10 KR	15 KR	20 KR	Mean value
Basmati-370	1.91[bcA]	1.51[cdB]	1.73[bcAB]	1.71[bAB]	1.71
Basmati-Pak	1.65[cA]	1.41[cdAB]	1.21[cB]	1.23[bcAB]	1.37
Basmati-198	2.15[bcA]	1.53[cBC]	1.78[bcB]	1.61[bcBC]	1.76
Basmati-385	1.94[bcA]	0.97[cdCD]	0.98[cdCD]	1.22[bcBC]	1.27
Super Basmati	2.22[bcA]	1.70[bcAB]	1.98[bAB]	1.18[bcCD]	1.77
Basmati2000	2.57[abA]	2.09[bcBC]	2.08[abBC]	2.40[abAB]	2.28
Shaheen2000	2.10[bcA]	1.57[bcBC]	1.66[bcBC]	1.80[abAB]	1.78
Basmati-515	2.53[dA]	2.05[bcBC]	2.13[abB]	2.04[abBC]	2.23
IRRI-6	2.81[abA]	2.52[abB]	2.49[abAB]	2.40[abB]	2.55
KS-282	3.29[aA]	3.14[aAB]	2.79[aABC]	2.54[aBC]	2.94
NIAB-IRRI-9	2.72[abA]	1.86[bcCD]	2.31[abB]	1.80[abCD]	2.17
KSK-133	3.0[abA]	2.17[bcCD]	2.32[abBC]	2.45[abBC]	2.49
Mean value	2.41	1.87	1.79	1.69	

LSD value = 0.5503. Mean values sharing the common letters for a given trait do not differ significantly at 5% probability level. Capital and small letters shows similarity and differences with in a same variety (rows) and among varieties (Columns).

Table 7. Comparison of mean values for Fertility Percentage of rice varieties at different radiation doses.

Varieties	Control	10 kR	15 kR	20 kR	Mean value
Basmati-370	79.83bcA	73.73bcBC	78.53bcAB	61.24dDE	73.59
Basmati-Pak	86.33bA	71.29cdCD	82.11bcB	62.66cdDE	79.91
Basmati-198	93.33aA	76.67bcDE	82.33bC	75.33bDE	81.77
Basmati-385	78.53cA	69.26cdBC	71.45cdBC	68.46bcC	69.73
Super Basmati	83.43bcA	74.56bcC	76.07cBC	48.10efH	66.24
Basmati-2000	81.3bcA	74.27bcBC	80.76bcAB	73.0bcBC	78.78
Shaheen 2000	87.10abA	82.07bD	85.30abAB	75.53bCD	80.96
Basmati-515	93.20Aa	88.10aB	91.36aAB	74.96bcDE	90.88
IRRI-6	92.96bA	84.53abBC	89.83abAB	83.80aC	87.1
KS-282	88.13abA	73.74bcD	82.38bB	70.76bcDE	75.63
NIABIRRI9	91.93abA	85.16abD	87.33abAB	68.39cEF	80.29
KSK-133	80.86bcA	76.82bcAB	76.56cdAB	61.34Dde	71.57
Mean value	80.94	79.39	78.03	76.69	73.59

LSD value = 9.6534. Mean values sharing the common letters for a given trait do not differ significantly at 5% probability level. Capital and small letters shows similarity and differences with in a same variety (rows) and among varieties (Columns).

Table 8. Comparison of mean values for 1000-grains weight (g) of rice varieties at different radiation doses.

Varieties	Control	10 kR	15 Kr	20 kR	Mean value
Basmati-370	20.91bAB	21.08aA	13.19cBC	8.84deCD	14.37
Basmati-Pak	24.90abA	20.59abAB	9.18cdD	12.15dCD	13.97
Basmati-198	19.09bcA	13.86bcBC	19.34abA	16.35cAB	16.53
Basmati-385	16.48cB	19.17abBC	20.52abA	8.98deCD	16.22
SuperBasmati	18.47bcA	17.51abAB	11.83cdBC	7.22eCD	12.19
Basmati-2000	18.49bcAB	18.63abA	16.44bcAB	13.04cdB	16.03
Shaheen 2000	14.14cdCD	14.96bcCD	15.51bcCD	27.99aA	19.49
Basmati-515	17.32bcAB	11.79cBC	18.25bA	16.10cdAB	15.38
IRRI-6	20.20bcA	17.43abAB	19.90abAB	20.01bcA	19.11
KS-282	20.20bcAB	13.15bcC	23.22aA	9.73deCD	15.37
NIAB-IRRI 9	20.76bA	16.58bAB	12.72cdBC	11.45deC	13.587
KSK-133	26.43aA	13.01bcCD	13.26cCD	14.20cdCD	13.49
Mean value	19.78	16.48	16.11	13.84	

LSD value = 5.2400. Capital and small letters shows similarity and differences with in a same variety (rows) and among varieties (Columns).

Genotypic and phenotypic coefficients of variability for plant height are presented in Table 9. The genotypic coefficient of variation measures the variability of any trait. The extent of environmental influence on a trait indicates the magnitude of differences between the genotypic and phenotypic coefficients of variation. Large differences reflect high environmental influence, while small differences reveal high genetic influence (Idris and Muhammad, 2013). Phenotypic coefficients of variability (34.5%) were slightly higher than the genotypic coefficients (34.15%; Table 9), which indicated that the variation is due to genetic content, along with considerable influence by the environment. Similar findings were reported by Idris and Muhammad (2013) and Rao et al. (2014), who reported a high phenotypic and low genotypic coefficient of variation for plant height. Very high broad sense heritability 97.7% was recorded for plant height by Mehla et al. (2000), allowing for selection during the early attempts at improving plant height.

Table 9. Heritability, Genotypic, and Phenotypic coefficient of variation for various traits of rice.

Sr. No	Traits	H²%	G.C.V%	P.C.V%
1	Plant height	97.7	34.15	34.5
2	Flag leaf area	95.0	38.47	39.46
3	Panicle length	93.8	14.5	15.05
4	No. of grains per main panicle	91.1	27.01	28.2
5	Grains weight per main panicle	96.8	47.8	48.63
6	Fertility %age	93.1	14.07	14.57
7	1000-grains wt.	72.0	14.09	16.6

h^2 = heritability, G.C.V% = genotypic coefficient of variation, P.C.V% = phenotypic coefficient of variation.

Flag leaf area

Flag leaf area is an essential character reflecting the yield and yield-contributing traits. All varieties showed a wide range of variability in the flag leaf area [5.069 cm² (Basmati-385 @ 20 Kr) to 35.407 cm² (Basmati-370 @ Control)]. Basmati-385 showed the greatest reduction in flag leaf area (of 24.22 cm²) in response to gamma radiation at 20 Kr, compared to its control. Alternately, gamma radiation had the least effect on the flag leaf area of Basmati-2000 (1.06 cm² when exposed to 10 Kr; Table 3).

This data indicated that a higher dose of gamma radiation led to a reduction in flag leaf area, the mutation in various segment of chromosome. Similar results were obtained by (Shereen et al., 2009) who noted that the leaf area continuously increased from 3-47% in plants exposed to 150 Gy gamma radiation, while the flag leaf area did not change despite an increase in radiation under saline conditions. Similar findings were reported by Burton (1952) who reported that access to the genotypic and phenotypic coefficient of variability, together with heritability estimates, could provide information about the extent of advance to be expected from selection.

Genotypic and phenotypic coefficients of variations of flag leaf area are presented in Table 9. Phenotypic coefficients of variability (39.46%) were slightly higher than the genotypic coefficient of variability (38.47%) of flag leaf area, indicating that the variation was a result of the genetic content, was well as the environment. Similar findings were reported by Dhanwani et al. (2013) who reported that maximum phenotypic and minimum genotypic coefficient of variation in flag leaf area indicated the presence of variation, however appreciable, as a result of environmental factors. The high broad sense heritability (95.0%) obtained for this trait reflected the ease of selection in earlier generations. However (Ananadarao et al. (2011) and Dhanwani et al. (2013), showed findings that were inconsistent with those of this study: they stated that a high broad sense heritability determining the selection in earlier generation would improve the flag leaf area.

Panicle length

The panicle length plays a significant role in raising the yield per unit area of rice crop. All varieties showed a wide range of variability in the panicle length, ranging from 7.733 cm in NIAB-IRRI-9 irradiated at 20 Kr to 29.87 cm in control Basmati-370. Radiation reduced the panicle length, thereby imparting a vigilant effect on KSK-133, reducing the PL up to 15.6 cm at 20 Kr (Table 4).

The genotypic and phenotypic coefficients of variation of panicle length are presented in Table 9. The phenotypic coefficients of variability (15.05%) were slightly higher than those of the genotype (14.5%), which indicated that the variation was due to genetic content, with countable influence by the environment. Similar results were reported by Watto et al. (2013) and Rao et al.

(2014), who reported that the panicle length showed high phenotypic and low genotypic coefficient of variation, indicating the presence of genetic variation in addition to noticeable impact by the environment, thereby allowing for its utilization in improving panicle length. High broad sense heritability (93.8%) was recorded, as in a previous study (Anandrao et al., 2011), which indicated that selection during an early generation could help improve this trait.

Number of grains per panicle

The number of grains per main panicle influences the grain weight, and is an important trait influencing the yield and yield-contributing characters. The varieties showed a wide range of variability in number of grains per main panicle. The mean value of number of grains per main panicle varied from 69.30 (Basmati Pak at 10 Kr) to 163.60 (Basmati- 2000, control). The maximum effect of radiation on the grains per panicle at 10 Kr was observed in Basmati Pak, revealing a difference of 41.37 grains per panicle compared to its control. Basmati-385 was least affected by radiation, as the mean value reduced by only 2.33 grains per panicle in response to radiation (Table 5). Similarly, Ibrahim and El-Degwy (2013) observed a range of genetic variability upon the application of different doses of gamma radiation in the number of grains per main panicle. Additionally, Haris et al. (2013) reported that 200 Gy of radiation could produce variation through induced mutation. Similar results were observed by El-Keredy (1990), who reported a gradual decrease in number of grains per main panicle with the increase in radiation dose from 15 kR to 25 kR. Wattoo et al. (2013) observed a decrease in the number of grains per panicle as a result of increase in gamma radiation from 15 kR to 25 kR; additionally, the mutant showed lesser number of grains per panicle compared to the non-radiated plant.

The genotypic and phenotypic coefficients of variability in number of grains per main panicle are presented in Table 9. The phenotypic coefficients of variation (28.2%) were slightly higher than the genotypic coefficients of variation (27.01%) in number of grains per main panicle, indicating that the variation is due to genetic content, as well as the environment. Similar results were reported by Rao et al. (2014), who revealed that high phenotypic coefficient of variation and low genotypic coefficient of variation in number of grains per main panicle was indicative of the presence of genetic variation, as well as environmental influence. The high broad sense heritability (91.2%) of the number of grains per panicle reflected the higher transmissibility described by Rao et al. (2014), who reported that the measurement of a maximal broad sense heritability during an earlier generation would affect the selection process.

Grain weight per main panicle

The grain weight per main panicle is the main characteristic of higher yield. We observed a range of variability for this trait. Mean values varied from 0.97 g (Basmati-385 @ 15 Kr) to 3.21 g (KS-282 @ control). The maximum mean value was observed in KS-282, followed by KS282 and KSK-133, whereas the minimum grains weight per main panicle was recorded in Basmati-385 at 10 Kr. Similar research was conducted by Ibrahim and El-Degwy (2013), who revealed that genetic variability increased with the increase in gamma radiation intensity of grains weight per main panicle (Table 6).

In this study, the various doses of gamma radiation imparted different effects on different rice varieties. Basmati-515, IRRI-6, KS-282, NIAB-IRRI-9, and KSK-133 displayed higher grain

weight per main panicle at different doses of radiation, and showed maximum genetic variability, which is desirable for the selection of crop breeds in breeding programs.

Genotypic and phenotypic coefficients of variation in grain weight per main panicle are presented in Table 9. Phenotypic coefficients of variability (48.63%) were higher than the genotypic coefficients (47.8%) for grains weight per main panicle, indicating that the variation is due to genetic content, with minimum influence from the environment. Similar results are reported by Bhutta et al. (2005), who reported a much higher phenotypic than genotypic coefficient of variation in grain weight per main panicle, indicating the occurrence of genetic variation with minimal interference from the environment. A high broad sense heritability (96.8%) of the grain weight per main panicle reflected the ease of selection during earlier generations. Similar findings were reported by Agrawal (2003), who stated that a high broad sense of heritability indicates selection during an earlier generation, speeding up the improvement in grain weight per main panicle.

Fertility percentage

Rice varieties used in this study showed a wide range of variability in fertility percentage [48.1 % (Super Basmati at 20 Kr) to 93.33 % (Basmati-198 at control)]. Similar research was conducted by Ibrahim and El-Degwy (2013), who observed a significant difference in fertility percentage in mutant rice cultivars. They also reported that a higher dose of gamma radiation reduced fertility percentage consistently, as observed in this study. In this study, the fertility percentage was affected by a gradual increase in radiation level. The fertility percentage was less influenced at 10 Kr, while maximum variability was observed at 20 kR. Super Basmati was more affected at 20 kR compared to the other varieties, which is an important achievement for further breeding programs (Table 7).

The genotypic and phenotypic coefficients of variation in fertility percentage are presented in Table 9. The phenotypic coefficients of variability (14.57%) were non-significantly higher than that of the genotype (14.07%) in fertility percentage, indicating that the variation is due to genetic content, with minimal involvement of the environment. Similar results were reported by Vanisree et al. (2013), who observed a slightly higher phenotypic than genotypic coefficient of variation in fertility percentage. The estimation of high broad sense heritability (93.1%) in fertility percentage reflected the ease of selection during an earlier generation (Vanisree et al., 2013).

1000-grain weight

The 1000 grain-weight is a major character influencing the yield and yield-contributing effect. The varieties showed a wide range of variability in 1000-grain weight at different radiation doses, varying from 7.22 g to 27.99 g (Table 8). The maximum mean 1000-grain weight value was observed in Shaheen-2000 at 20 Kr, which was 13.85 gm greater than its control. Similar results were reported by Bughio et al. (2007), who observed a remarkable mutagenic improvement in the 1000-grain weight of different rice varieties. Similar results were observed by Ibrahim and El-Degwy (2013), who reported a significant difference in the 1000-grain weight in various genotypes, when exposed to 15-25 kR radiation. A comparable result was obtained by Siddiqui and Singh (2010), who observed a higher mean value of 1000-grain weight at different radiation doses, which produced highly significant results among genotypes.

In this study, mutagenic treatments altered the 1000-grain weight in all varieties. Consequently, the mean 1000-grain weights of rice subjected to all types of treatment were virtually

the same; moreover, the range of variability of irradiated and control plants were similar. Therefore, the gamma-ray dosages utilized in this study were capable of inducing a marked variation in the 1000-grain weight. Nevertheless, the differences among the 3 mutagenic treatments were significant.

The extent of environmental influence on this trait is depicted by the magnitude of differences between the genotypic and phenotypic coefficients of variation. Large differences reflect a high environmental influence, while small differences indicated a high genetic influence (Idris and Mohamed, 2013). The genotypic and phenotypic coefficients of variation in 1000-grain weight are presented in Table 9. The phenotypic coefficients of variability (16.6%) are higher than the genotypic coefficients (14.09%) of grains weight per main panicle, indicating that the variation is due to genetic effects (Rao et al., 2014). A moderate broad sense heritability (72.0%) was recorded, similar to that in a previous study (Idris and Mohamed, 2013).

Correlation analyses

The crop yield is a very complex trait controlled by many genes and influenced by other traits. Therefore, other yield-related traits must be considered when selecting genotypes for yield. Correlation is an easy and efficient means to determine the association between different traits. In this study, phenotypic and genotypic correlation was evaluated. A majority of the traits depicted significant ($P < 0.5$) correlation among themselves, whereas the correlation among some traits was non-significant ($P > 0.5$). Maximum genotypic and phenotypic correlation was observed between the plant height and panicle length (r_g = 0.7465, r_p = 0.7294) followed by fertility percentage and 1000-grain weight (r_g = 0.7355, r_p = 0.5551). The flag leaf area and 1000-grain weight showed a non-significant correlation with a maximum number of traits, while the plant height and panicle length were significantly correlated with a maximum number of traits (Table 10). These results are supported by the findings of other researchers, who obtained similar results, when investigated the correlation among yield-related traits of rice (Shanthakumar et al., 1998; Ashfaq et al., 2012; Idris and Mohamed, 2013; Vanisree et al., 2013).

**Table 10.** Genotypic and phenotypic correlations for various traits of rice.

Traits	CR	Panicle length (cm)	No. of grains/ panicle	Grains weight/ panicle	Fertility percentage	Flag leaf area (cm²)	1000 grains weight (g)
Plant height (cm)	rg	0.7465*	-0.4241*	-0.4763*	-0.2657*	-0.6551*	-0.2643*
	rp	0.7294**	-0.4061*	-0.4623**	-0.2411[NS]	-0.6395**	-0.2333[NS]
Panicle length (cm)	rg		-0.2188*	-0.6191*	-0.1458*	0.3498*	-0.2942[NS]
	rp		-0.2136[NS]	-0.5986**	-0.1561[NS]	0.3200[NS]	-0.2648[NS]
No. of grains/ panicle	rg			0.5508[NS]	0.1122*	-0.2702*	0.3474*
	rp			0.5397**	0.1453[NS]	-0.2700[NS]	0.2479[NS]
Grains weight/ panicle	rg				0.4274*	0.0855[NS]	0.3414[NS]
	rp				0.4263**	0.834[NS]	0.2657[NS]
Fertility percentage	rg					-0.0176[NS]	0.7355*
	rp					0.0042[NS]	0.5551**
Flage leaf area (cm²)	rg						-0.1148[NS]
	rp						-0.1347[NS]

[NS]Non-significance. *$P \leq 0.05$%. CR = correlation, rg = genotypic correlation, rp = phenotypic correlation.

Genetic diversity studies

A phylogenetic analysis and similarity index of 12 coarse and fine varieties of rice (Basmati-370, Basmati Pak, Basmati-198, Basmati-515, Basmati-2000, Shaheen 2000, Basmati

385, Super Basmati, KSK-282, KSK-133, IRRI-6, and NIAB-IRRI-9) before and after mutagenesis using 87 SSR markers (widely distributed on 12 chromosomes) were conducted. Genotypic banding patterns of 12 rice varieties with two SSR markers are shown in Figures 1 and 2.
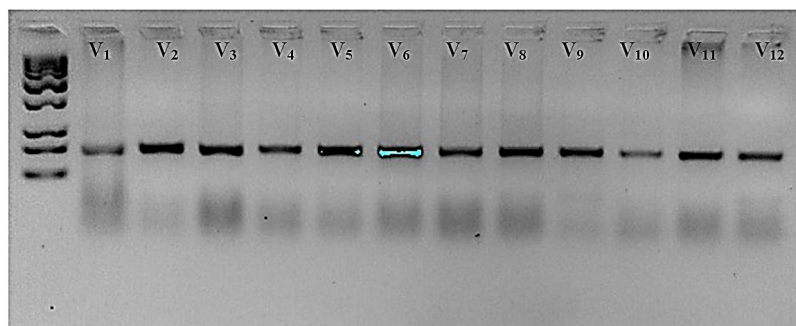

Figure 1. Gel electrophoresis of twelve rice varieties with SSR marker RM3202.
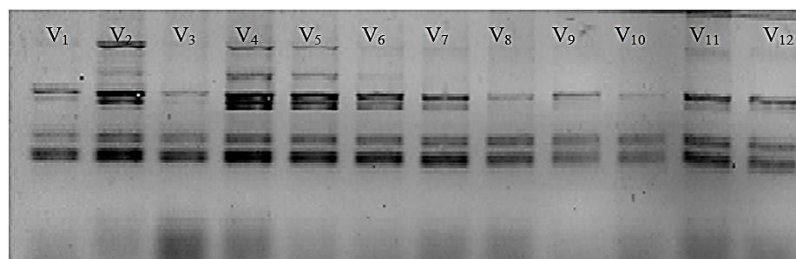

Figure 2. Gel electrophoresis of twelve rice varieties with SSR marker RM529.

Genetic analysis led to the grouping of rice varieties into two clades: fine (Basmati-370, Basmati Pak, Basmati-515, Basmati-2000, Shaheen 2000, Basmati 385, and Super Basmati) in clade-I, and coarse (KSK-282, KSK-133, IRRI-6, and NIAB-IRRI-9) in clade-II (Figure 3). A total of 153 polymorphic alleles were detected from 87 markers. A UPGMA-based dendrogram with two clusters was observed (Figure 3). Pairwise similarity ranged from 0.51 to 0.76 before (Figure 3) and 0.39 to 0.89 after mutagenesis (Figure 4).
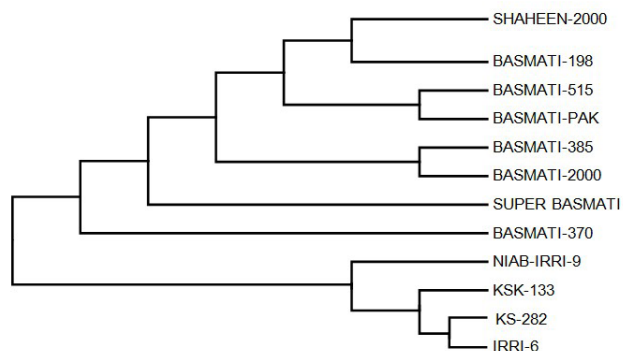
Figure 3. UPGMA cluster analysis showing the diversity and correlation among 12 rice varieties before mutagenesis.
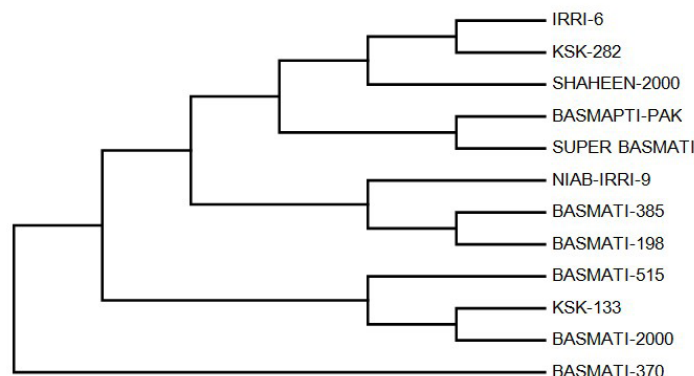
Figure 4. UPGMA cluster analysis showing the diversity and association between 12 rice varieties after mutagenesis.

Genetic distances between Basmati varieties were as low as 0.5-0.61 (Arif et al., 2005; Shah et al., 2013). A substantial genetic diversity was observed using SSR markers of various rice genotypes, and by clustering their results into 2, 3, 2, 3, and 4 groups. Similarly, the use of 87 SSR markers to determine the genetic diversity of mutated population revealed 187 polymorphic alleles in twelve varieties. Also, diversity in the genetic constitution was widened up to 0.39-0.89 after mutagenesis, and four clusters were developed by UPGMA. Our results are in accordance with those of Arif et al. (2005), who observed an increase in the diversity of mutated population compared to their respective controls. As a result of mutation (Figure 4), fine rice varieties Basmati-515 and Basmati 2000 were shuffled into the coarse variety cluster, while IRRI-6 and KSK-282 were incorporated into the fine variety group, indicating the effectiveness of gamma radiation for mutation. In conclusion, although the genetic base of Basmati varieties was diversified post-mutation, the varieties were less affected than non-Basmati varieties. Differences in the morphological uniqueness of Basmati and non-Basmati varieties remained unaltered even after mutagenesis. Twelve varieties with heading days 100-120 and plant height 60-110 cm showed a wide diversity among mutant and non-mutant lines. Genetic diversity generated by this mechanism can enrich the germplasm of rice crop, as the availability of a diverse gene pool for various economical traits can facilitate the crop improvement program.

Conflicts of interest

The authors declare no conflict of interest.

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