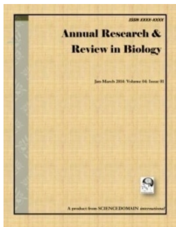

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# Integration of Mutation and Conventional Breeding Approaches to Develop New Superior Drought-tolerant Plants in Rice (*Oryza sativa*)

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***Authors' contributions***

*This work was carried out in collaboration between all authors. Authors MTH and HM designed the study, authors MTH and AAE managed the research and wrote the protocol, authors SSJ, MA and MM performed the statistical analysis and authors AAE, MM and MTH wrote the first draft of the manuscript. All authors read and approved the final manuscript.*

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## ABSTRACT

Present study was carried out to investigate the possibility of developing new superior drought-tolerant plants in rice by mutation breeding. Major regions of rice cultivation especially in Iran and Asia are affected continuously by drought stress especially at the end of flowering stage. To identify resistant plants, water stress was imposed on mutant rice plants in the field in flowering stage. In total, 23 tolerant mutant plants under drought stress were obtained after three generations of selection on mutants. Only 17 $M_4$ lines had high yield and were selected as resistant. Also, morphological parameter evaluations of 29 mutant lines grown under natural conditions indicated that 14 lines were early flowering by 7 days or more and the height of 8 mutant lines had been decreased by 30 cm or more than control. Also, 11 lines had yields of more than 5000 Kg/ hectare. On obtained results of phenotypic evaluations of mutant plants under drought stress and normal conditions, 11 lines were selected for descent test and yield experiments in several regions and years.

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*Keywords: Drought stress; gamma irradiation; mutant; rice; tolerant.*

## ABBREVIATIONS

*TM: Tarom Mutant; VE: Very Early Flowering; HD: Heading date; H.P: Height of plant; N.T: Number of fertile tiller; P.L: Panicle length; L.L: Length of flag leaf; W.L: Width of flag leaf; F.S: mean of fertile seeds number in any spike; S.S: mean of sterile seeds number in any spike; T.S: mean of number of total seeds in any spike; N.T×F.S: number of fertile seeds in any plant; N.T×S.S: number of sterility seeds in any plant N.T×T.S: number of total seeds in any plant; Y: Yield of any line in plot.*

## 1. INTRODUCTION

Rice is one of the most important cereals which are cultivated in about one-third of the world's total cereal cultivation area. Major regions of rice cultivation especially in Iran and Asia are encountered with drought stress problem at the end of flowering stage annually. Therefore, developing of early flowering and efficient crops by water utilization seems necessary. In spite of considerable efforts on improvement of resistance to drought stress in high yielding rice varieties, little progression has been achieved so far. Because of low variability available in gene pool of Iranian cultivars especially drought tolerance, application of mutation breeding (for example. using gamma irradiation) is evident to induce more variation. Ionizing radiations have been successful in inducing genetic variability in Iranian rice germplasm. Genetic variability induced in rice through gamma rays for selecting new genotypes with improved grain rice quality and high yield potentials have been documented [1,2]. Various attempts have been made in this direction by different scientists to determine the most effective mutagenic treatment for the induction of desirable traits in rice [3,4]. Induced mutations have played a significant role for the improvement of rice by developing a large number of semi-dwarf and high yielding varieties in many countries [5,6]. Bughio et al. developed a high yielding rice mutant variety; Mehak (fragrance) from a fine aromatic variety of Basmati-370, through gamma rays (150 Gy) that is significantly better than its mother in respect of yield and yield contributing traits [7]. Cheema and Atta used three Basmati rice varieties to examine varietal differences in radiosensitivity to gamma radiation. They found that $LD_{50}$ values for seed fertility were 238, 232 and 223Gy for Basmati 37, Basmati Pak and Super Basmati, respectively [8]. IAEA/FAO reported that 443 rice varieties have been developed through induced mutations during the period of 1966-2004 [9]. In a wide-scale backcross project for tolerance improving to drought stress in field experiments in Philippines, Lafitte et al. evaluated 160 donor cultivars from 25 countries for response to drought on plant height, heading date and seed yield and produced 325 $BC_2F_2$ bulk populations using backcrossing of donor plants to one of three elite recurrent parents which were evaluated under drought stress in lowland and upland nursery [10]. Tahmasebi Sarvestani et al. conducted a field experiment during 2001-2003 to evaluate the effect of water stress on the yield and yield components of four rice cultivars named, Tarom, Kazar, Fajr and Nemat that are commonly grown in Mazandaran province, Iran. Results of their research indicated that water shortage during vegetative, flowering and grain filling stages reduced grain yield by 21, 50 and 21 percents on average in comparison to control, respectively [11]. It is necessary to mention that last stage of flowering is coincident with very sensitive stage to drought stress in rice. The objective of this study was to investigate the possibility of developing mutant rice plants with superior qualitative and quantitative traits under drought stress using gamma irradiation and conventional breeding strategies.

## 2. MATERIALS AND METHODS

### 2.1 Plant Material

An Iranian rice landrace "Tarom Mahalli" was used in this research that is a high yield genotype and sensitive to drought stress.

### 2.2 Irradiation of Seeds and Determination of Irradiation Optimum Dose

As plant genotypes and cultivars indicate different responses to gamma irradiation, optimum dose is different in various genotypes and cultivars. Optimum dose is a dose that causes high frequency of favorable mutations with minimum damage to the plant. In order to determine optimum dose, Survival $LD_{50}$ of seedlings was calculated in different doses, three weeks after sowing the seeds. To obtain the optimum dose, seeds were irradiated with different doses of (0, 150, 180, 200, 230, 250 and 300 Gy) of gamma rays from $Co_{60}$ source in gamma cell (dose rate, 0.2 Gy/sec and special activity, 2000 Ci). Then, $M_1$ generation plant populations were developed with doses (220, 230, 250 and 300 Gy) and sent to Rice Research Institute of Iran-Rasht for sowing in the nursery. Five mutant populations (220, 230, 250, 300 Gy and bulk) were established from these seeds in the field. Bulk population is a mixture of four mutant populations that was investigated in field.

### 2.3 Sowing Mutant Seeds and Establishment of Different Mutant Generations

On Koornneef, et al. study [12], frequency of induced mutations is often $5\times10^{-4}$ per one locus in any genome after gamma irradiation. On the other hand, rice is considered a diploid plant; therefore, the frequency of mutation in diploid rice is expected to be $2 \times 10^{-3}$ per any $M_1$ plant. To obtain the 95 percent probability of discovering a special mutation in population of $M_1$ the generation plants need to have 3000 $M_1$ plants [12]. Three weeks after sowing the seeds in the nursery, seedlings were transferred to the main field. Inter and intra distance of the rows was 25×25 cm. $M_1$ generation plants were raised up to flowering (heading) stage in the field. Then, the seeds of individual $M_1$ generation plants were collected. Seeds of a spike from any plant of $M_1$ generation were sowed in the field and resulted plants evaluated in $M_2$ generation. $M_3$ generation seedlings of the selective plants were transplanted in separate lines in the field. Also, $M_4$ generation seedlings were sowed in separate plots.

### 2.4 Drought Stress Treatment of Rice Mutant Plants

Two big drainage channels of 1m (width × depth) length were made along with the experimental field length and width. It was used from flood irrigation system for watering the plants in the experimental plots. All plots were surface (flood) irrigated once a week, except when the water stress was imposed. Changes in moisture and stability of soil were measured periodically in the plots under stress using tensiometer (Fig. 1). Prior to impose the drought stress, field soil was drained up to the field capacity. Field capacity was determined by gravimetric method and tensiometer. In gravimetric method, the moisture content of the soil is calculated by the following formula (Soil moisture calculation) (International Crops Research Institute for the Semi-Arid Tropics [ICRISAT]) [13]:

$$\text{Moisture content on weight basis} = ((\text{Wet Weight} - \text{Dry Weight}) / \text{Dry Weight}) \times 100$$

**Fig. 1. Schematic pictures of tensiometer and mutant rice plants two weeks after drought stress in experimental field**

By this method, the field capacity of plots was determined as 40 and 41 %. After the soil moisture approached to a field capacity, water stress was imposed to $M_2$ generation plants about 10 days before flowering by two weeks in the field (Fig. 1) [14]. It is necessary to mention that flowering date of landrace Tarom mahalli is 95 days after seed sowing. In $M_3$ and $M_4$ generations, water stress was imposed to the plants for one month. In tensiometery method, when water is available in the field, tensiometer shows number 0. Tensiometer represents the number over 2 when soil moisture approaches to the field capacity. In this situation, almost all of the plants express the rolling signs in their leaves. This method can be applied as a scale for evaluation of leaf rolling of rice plants under drought stress.

## 2.5 Phenotypic Analysis of Mutant Rice Plants under Drought Stress

Phenotypic analysis of mutant rice plants under drought stress was performed in flowering stage on standard evaluation systems of International Rice Research Institute [IRRI] [15].

### 2.5.1 Evaluation of leaf rolling of mutant rice plants

The first standard phenotypic evaluation system of rice plants was on leaf rolling scale 0-9: 0, leaves healthy; 1, leaves start to fold (shallow); 3, leaves folding (deep V-shape); 5, leaves fully cupped (U-shape); 7, leaf margins touching (O-shape); 9, leaves are tightly rolled (V-shape).

### 2.5.2 Evaluation of spikelet fertility of mutant rice plants

The second standard phenotypic evaluation system of rice plants was on spikelet fertility scale 1-9: 1, more than 80%; 3, 61 - 80%; 5, 41 - 60%; 7, 11 - 40%; 9, less than 11%.

## 2.6 Statistical Analysis of Data from Mutant Rice Populations under Drought Stress

Traits such as number of tillers, number of fertile tillers, number of total seeds, number of full seeds, percentage of fertility and thousand seeds weight were studied in the populations.

Mean, standard Deviation, minimum and maximum of value were also calculated for each trait. Test of homogeneity of variances was done to ensure the assumption required for parametric analysis. Analysis of variance (ANOVA) and multiple comparisons were performed between treatments by Tukey HSD method for each trait. The whole data analysis was accomplished by version 16.0 of SPSS (Software Package for Statistics and Simulation) software.

### 2.7 Evaluation of Morphological Characters of Selected Mutant Lines Grown Under Natural Conditions

Simultaneous with phenotypic analysis of selective mutant rice lines under drought stress in third year of evaluation, yield of 29 tolerant mutant lines of $M_4$ generation were evaluated in separate plots grown under natural conditions. This experiment was done in a farm of Rice Research Institute of Iran-Rasht. 29 plots were designed on number of studied lines. There were 16 plants in any plot. Also, size of any plot and interval of plots were about 1m$^2$ and 0.5 m, respectively. Traits such as heading date, height of plant, number of fertile tiller, panicle length, length of flag leaf, width of flag leaf, mean of number of empty seeds in any spike, mean of number of empty seeds in any spike, mean of number of total seeds in any spike, number of full seeds in any plant, number of empty seeds in any plant, number of total seeds in any plant and yield of any line in plot were estimated.

## 3. RESULTS

### 3.1 Determination of Optimum Irradiation Dose and Phenotypic Analysis of Mutant Plants under Drought Stress

Three weeks after sowing of seeds, optimum dose and $LD_{50}$ for survival of seedlings in landrace "Tarom mahalli" was determined to be 230 Gy (Fig. 2).

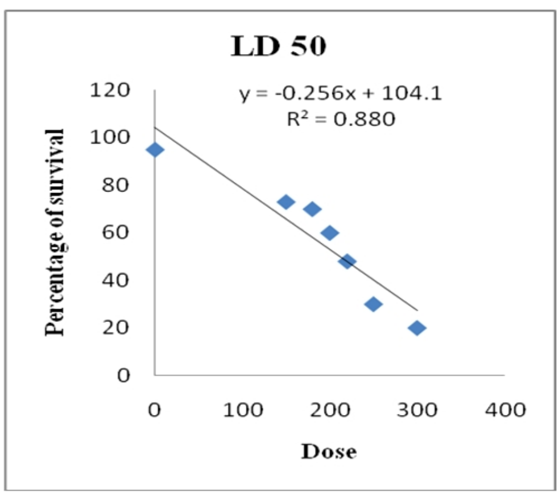


**Fig. 2. Survival $LD_{50}$ of seedlings in genotype Tarom Mahalli**

Two weeks after drought stress, we selected 64 tolerant mutant plants to drought stress on leaf rolling scale and 81 early flowering mutant plants on flowering date from $M_2$ generation population. Furthermore, three mutant plants ($TM_2$ - 230 - 3($VE_7$), $TM_2$ - 230 - 4($VE_8$) and $TM_2$ - 230 - 6($VE_{10}$)) were tolerant and very early flowering (Table 1 and Figs 3, 4).


**Fig. 3. Schematic pictures of tolerant and sensitive mutant rice plants after two weeks drought stress**


**Fig. 4. Schematic picture of very early flowering mutant rice plant**

64 tolerant plants were analyzed on spikelet fertility scale. Only three mutant plants ($TM_2$ - 230 - 3($VE_7$), $TM_2$ - 230 - 4($VE_8$) and $TM_2$ - 230 – 5) were identified as resistant on two scales (Table 1). So, two mutant plants ($TM_2$ - 230 - 3($VE_7$) and $TM_2$ - 230 - 4($VE_8$)) not only were resistant on two scale but also very early flowering.

**Table 1. Characteristics of 17 tolerant mutant lines of $M_2$ generation in dose 230Gy**

| Plant name | Number of tillers | Number of fertile tillers | Thousand seed weight | Number of full seeds | Number of Total seeds | Percentage of fertility | Tolerance on leaf rolling | Tolerance on spikelet fertility |
|---|---|---|---|---|---|---|---|---|
| Control (without stress) | 12.7 | 11.2 | 23 | 1331.68 | 1652.27 | 100 | - | - |
| $TM_2$ - 230 - 2* | 9 | 9 | 25.6 | 616 | 822 | 46 | 0 | 5 |
| $TM_2$ - 230 - 3($VE_7$) | 25 | 21 | 26.3 | 1733 | 2415 | 100 | 0 - 1 | 1 |
| $TM_2$ - 230 - 4($VE_8$) | 30 | 25 | 28.5 | 2017 | 2575 | 100 | 0 - 1 | 1 |
| $TM_2$ - 230 - 5 | 30 | 22 | 20.6 | 1670 | 2399 | 100 | 0 - 1 | 1 |
| $TM_2$ - 230 - 6($VE_{10}$) | 22 | 7 | 27.1 | 453 | 630 | 34 | 0 | 7 |
| $TM_2$ - 230 - 8 | 9 | 7 | 28.3 | 559 | 686 | 42 | 0 - 1 | 5 |
| $TM_2$ - 230 - $VE_1$ | 10 | 7 | 26.3 | 407 | 518 | 31 | - | 7 |
| $TM_2$ - 230 - $VE_2$ | 19 | 13 | 28.1 | 837 | 1128 | 63 | - | 3 |
| $TM_2$ - 230 - $VE_3$ | 18 | 9 | 24.6 | 650 | 818 | 49 | - | 5 |
| $TM_2$ - 230 - $VE_4$ | 24 | 17 | 19.6 | 1227 | 1690 | 92 | - | 1 |
| $TM_2$ - 230 - $VE_5$ | 25 | 22 | 27.8 | 1799 | 2456 | 100 | - | 1 |
| $TM_2$ - 230 - $VE_6$ | 16 | 15 | 22.9 | 1426 | 1882 | 100 | - | 1 |
| $TM_2$ - 230 - $VE_7$ | 25 | 21 | 26.3 | 1733 | 2415 | 100 | 0 - 1 | 1 |
| $TM_2$ - 230 - $VE_8$ | 30 | 25 | 28.5 | 2017 | 2575 | 100 | 0 - 1 | 1 |
| $TM_2$ - 230 - $VE_9$ | 19 | 16 | 29.8 | 1314 | 1636 | 99 | - | 1 |
| $TM_2$ - 230 - $VE_{10}$ | 22 | 7 | 27.1 | 453 | 630 | 34 | 0 | 7 |
| $TM_2$ - 230 - $VE_{11}$ | 12 | 7 | 22.7 | 403 | 538 | 30 | - | 7 |

**TM is equal to Tarom Mutant. The $M_2$ generation plants that expressed tolerance to drought stress again in $M_3$ generation on leaf rolling scale, were characterized by bold names. VE is abbreviation to Very Early Flowering. Bold names indicate selected plants on flowering date and two scales of tolerance to drought stress.*

Drought stress was imposed on mutant plants of $M_3$ and $M_4$ generations for one month in the field. In the second year of evaluation ($M_3$ generation), 43 mutant plants expressed tolerance to drought stress on leaf rolling scale among 64 tolerant selective plants of $M_2$ generation during two weeks drought stress (Table 2). Among them, 25 lines were identified as tolerant to drought stress on spikelet fertility and had high yield. In the third year of evaluation ($M_4$ generation), from 25 selective lines, 23 lines were identified on leaf rolling scale as tolerant. Then, these 25 lines were screened on spikelet fertility scale. Only 17 $M_4$ lines had high yield and were selected as resistant.

### 3.2 Statistical Analysis of Data from Mutant Rice Populations under Drought Stress

These considerations indicated that highest of mean, standard deviation and value of traits "number of tillers, number of fertile tillers, number of total seeds and number of full seeds" were due to population (2) or 230 Gy (Table 3) [8].

Test of homogeneity of variances and F test of all studied traits in this research, were significant ($F_{4, 44} = 10.269$, $P < 0.01$; Table 4). The mean differences were significant only in comparisons between treatments (population 230 Gy) with other populations by Tukey HSD method in all studied traits ($P < 0.01$). Mutant plant populations were divided to completely distinct two groups on studied traits by above method. Also, the highest mean and standard deviation (SD) for "percentage of fertility" and the highest mean and value for "Thousand seed weight" was due to population 230 Gy. In analysis of all traits such as number of full seeds, population 230 Gy was included as one distinct subset in comparison with other subsets (Table 5). Briefly, statistical analysis results of other traits haven't been represented in this research.

### 3.3 Morphological Parameter Evaluations of 29 Selective Mutant Lines Grown under Normal Conditions

Except line $TM_4$-220-10-4, other 28 mutant lines were earlier flowering by 5-11 days than control (95 days). This line was late flowering even for 1 day than control. Also, 14 lines were early flowering by 7 days or more in comparison with control. Height of 8 mutant lines had been decreased to 30 cm or more than control. Furthermore, $TM_3$-250-8-6 had least height among all lines and in comparison with control and was up to 103 cm. In normal condition, all of 29 selected lines had more yield than control on Kg/ hectare unit in the plot. Among them, 11 lines had yield of more than 5000 Kg/ hectare (Table 6).

**Table 2. Tolerance level of 25 selective $M_4$ generation mutant lines in three consecutive years of drought stress**

| Line name | First year | | Second year | | Third year | | |
|---|---|---|---|---|---|---|---|
| | **Tolerance on leaf rolling** | **Tolerance on spikelet fertility** | **Tolerance on leaf rolling** | **Tolerance on spikelet fertility** | **Tolerance on leaf rolling** | **Tolerance on spikelet fertility** | **Yield of line** |
| Control (under stress) | 9 | 9 | 9 | 9 | 9 | 9 | 148 |
| $TM_4$ - 220 – 10 - 4 | 0 | 5 | 1 | 3 | 1 | 1 | 1456 |
| $TM_4$ - 230 – 3 - 5 | 0 - 1 | 1 | 1 | 1 | 1 | 1 | 1649 |
| $TM_4$ - 230 – 4 - 1 | 0 - 1 | 1 | 0 | 0 | 1 | 1 | 1490 |
| $TM_4$ - 250 – 3 - 5 | 1 | 7 | 1 | 3 | 1 | 3 | 1008 |
| $TM_4$ - 250 – 8 - 6 | 1 | 7 | 1 | 1 | 1 | 1 | 1275 |
| $TM_4$ - 250 – 9 - 6 | 1 | 5 | 1 | 1 | 1 | 1 | 1387 |
| $TM_4$ - 250 – 10 - 7 | 3 | 5 | 1 | 5 | 1 | 1 | 1404 |
| $TM_4$ - 250 – 11 - 5 | 0 | 7 | 1 | 1 | 1 | 3 | 1050 |
| $TM_4$ - 250 – 15 - 5 | 0 | 5 | 1 | 1 | 1 | 1 | 1248 |
| $TM_4$ - 300 – 1 - 1 | 2.5 | 5 | 1 | 1 | 1 | 1 | 1955 |
| $TM_4$ - 300 – 6 - 1 | 0 | 3 | 0 | 1 | 1 | 3 | 936 |
| $TM_4$ - 300 – 8 - 1 | 1 | 7 | 1 | 3 | 1 | 1 | 1320 |
| $TM_4$ - 300 – 9 - 1 | 1 | 5 | 1 | 5 | 1 | 1 | 1365 |
| $TM_4$ - B – 2 - 1 | 2.5 | 7 | 1 | 1 | 1 | 1 | 1360 |
| $TM_4$ - B- 3 - 1 | 2.5 | 7 | 1 | 1 | 1 | 1 | 1288 |
| $TM_4$ - B- 5 - 1 | 0-1 | 7 | 1 | 3 | 1 | 3 | 936 |
| $TM_4$ - B- 6 - 1 | 0-1 | 7 | 1 | 1 | 1 | 1 | 1365 |
| $TM_4$ - B- 9 - 1 | 1.5 | 5 | 1 | 1 | 1 | 1 | 1224 |
| $TM_4$ - B- 11 -1 | 1 | 5 | 1 | 1 | 1 | 1 | 1360 |
| $TM_4$ - B- 12 - 1 | 1 | 5 | 1 | 1 | 1 | 1 | 1543 |
| $TM_4$ - B- 14 - 1 | 1 | 1 | 1 | 5 | 1 | 5 | 863 |
| $TM_4$ - B- 17 - 1 | 1 | 5 | 1 | 3 | 1 | 3 | 1109 |
| $TM_4$ - B- 18 - 1 | 1 | 7 | 1 | 3 | 1 | 3 | 1034 |
| $TM_4$ - B- 19 - 2 | 1 | 7 | 1 | 1 | 1 | 1 | 1394 |
| $TM_4$- B- 23 - 1 | 1 | 7 | 1 | 5 | 1 | 5 | 884 |

** TM is abbreviation Tarom Mutant. Numbers 220, 230, 250 and 300 indicate different mutant rice populations. Bulk population is a mixture of four mutant populations that was investigated in field. Yield of lines is on number of full seeds/any plant. Bold numbers indicate yield levels of 17 high yield $M_4$ lines.*

**Table 3. Mean standard deviation (SD), standard error (SE), minimum and maximum of value- trait "number of full seeds" in $M_2$ generation rice populations**

| Population* | N | Mean | SD | SE | 95% Confidence Interval for Mean | | Minimum | Maximum |
|---|---|---|---|---|---|---|---|---|
| | | | | | Lower Bound | Upper Bound | | |
| 1 | 38 | 591.49 | 228.10877 | 37.00413 | 516.5141 | 666.4691 | 190.00 | 1331.68 |
| 2 | 18 | 1147 | 598.06962 | 140.966 | 849.5692 | 1444.3952 | 403.00 | 2017.00 |
| 3 | 46 | 642.56 | 260.05915 | 38.34363 | 565.3302 | 719.7863 | 155.00 | 1331.68 |
| 4 | 23 | 679.12 | 383.45480 | 79.95585 | 513.2982 | 844.9348 | 238.00 | 1786.00 |
| 5 | 24 | 564.20 | 285.03994 | 58.18353 | 443.8332 | 684.5568 | 188.00 | 1331.68 |
| Total | 149 | 683.49 | 374.53005 | 30.68270 | 622.8598 | 744.1254 | 155.00 | 2017.00 |

** Population (1) = 220Gy, Population (2) = 230Gy, Population (3) = 250Gy, Population (4) = 300Gy and Population (5) = Bulk. N represents number of plants within each group.*

**Table 4. ANOVA- trait "number of full seeds" in $M_2$ generation populations**

| | Sum of Squares | df | Mean Square | F | Sig.* |
|---|---|---|---|---|---|
| Between Groups | 4607531.471 | 4 | 1151882.868 | 10.269 | .000 |
| Within Groups | 1.615E7 | 144 | 112172.480 | | |
| Total | 2.076E7 | 148 | | | |

**P < 0.01 and data analysis was done by SPSS software version 16.0*

**Table 5. Mean comparison by Tukey HSD method- trait "number of full seeds" in $M_2$ generation populations**

| t | N | Subset for alpha = 0.05 | |
|---|---|---|---|
| | | 1 | 2 |
| 5 | 24 | 564.1950* | |
| 1 | 38 | 591.4916 | |
| 3 | 46 | 642.5583 | |
| 4 | 23 | 679.1165 | |
| 2 | 18 | | 1147 |
| Sig. | | .723 | 1.000 |

**Means for groups in homogeneous subsets are displayed. N represents number of plants within each group (subset).*

**Table 6. Characteristics of 29 selective $M_4$ generation mutant lines grown under normal conditions**

| Plot No. | Designation | H.D* | H.P | N.T | P.L | L.L | W.L | F.S | S.S | T.S | N.T×F.S | N.T×S.S | N.T×T.S | Y |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 101 | $TM_4$-220-10-4 | 96 | 115 | 19 | 22 | 27 | 0.80 | 98 | 24 | 122 | 1862 | 456 | 2318 | 4732 |
| 102 | $TM_4$-220-13-1 | 90 | 124 | 24 | 21 | 24 | 1.00 | 90 | 4 | 94 | 2160 | 96 | 2256 | 4952 |
| 103 | $TM_4$-220-15-2 | 89 | 113 | 16 | 28 | 20 | 1.00 | 91 | 5 | 96 | 1456 | 80 | 1536 | 5100 |
| 104 | $TM_4$-230-3-5 | 85 | 124 | 18 | 24 | 17 | 0.80 | 81 | 4 | 85 | 1458 | 72 | 1530 | 5400 |
| 105 | $TM_4$-230-4-1 | 86 | 128 | 18 | 20 | 26 | 1.10 | 86 | 3 | 89 | 1548 | 54 | 1602 | 6040 |
| 106 | $TM_4$-230-1-1 | 87 | 121 | 21 | 22 | 20 | 1.10 | 89 | 6 | 95 | 1869 | 126 | 1995 | 6108 |
| 107 | $TM_4$-230-1-2 | 89 | 120 | 17 | 26 | 22 | 1.00 | 55 | 6 | 61 | 935 | 102 | 1037 | 5720 |
| 108 | $TM_4$-250-2-1 | 90 | 112 | 15 | 20 | 22 | 0.80 | 85 | 26 | 111 | 1275 | 390 | 1665 | 4448 |
| 109 | $TM_4$-250-3-5 | 88 | 115 | 18 | 22 | 29 | 1.00 | 94 | 11 | 105 | 1692 | 198 | 1890 | 4092 |
| 110 | $TM_4$-250-8-6 | 90 | 103 | 17 | 22 | 28 | 0.70 | 65 | 5 | 70 | 1105 | 85 | 1190 | 4088 |
| 111 | $TM_4$-250-9-6 | 87 | 119 | 19 | 22 | 30 | 0.90 | 68 | 7 | 75 | 1292 | 133 | 1425 | 4728 |
| 112 | $TM_4$-250-9-7 | 85 | 120 | 16 | 26 | 27 | 1.00 | 69 | 6 | 75 | 1104 | 96 | 1200 | 4472 |
| 113 | $TM_4$-250-10-5 | 84 | 128 | 13 | 24 | 25 | 1.00 | 88 | 5 | 93 | 1144 | 65 | 1209 | 4212 |
| 114 | $TM_4$-250-10-6 | 85 | 121 | 17 | 22 | 20 | 1.00 | 76 | 4 | 80 | 1292 | 68 | 1360 | 5108 |
| 115 | $TM_4$-250-10-7 | 89 | 135 | 17 | 23 | 30 | 1.00 | 97 | 4 | 101 | 1649 | 68 | 1717 | 6000 |
| 116 | $TM_4$-250-11-5 | 90 | 128 | 13 | 26 | 27 | 1.00 | 115 | 8 | 123 | 1495 | 104 | 1599 | 5380 |
| 117 | $TM_4$-250-11-6 | 90 | 124 | 12 | 23 | 18 | 1.00 | 87 | 5 | 92 | 1044 | 60 | 1104 | 4200 |
| 118 | $TM_4$-250-15-5 | 88 | 120 | 16 | 19 | 21 | 0.80 | 72 | 8 | 80 | 1152 | 128 | 1280 | 4292 |
| 119 | $TM_4$-250-16-5 | 87 | 123 | 22 | 20 | 23 | 1.00 | 91 | 11 | 102 | 2002 | 242 | 2244 | 4100 |
| 120 | $TM_4$-250-17-5 | 89 | 126 | 18 | 26 | 22 | 1.10 | 80 | 7 | 87 | 1440 | 126 | 1566 | 4628 |
| 121 | $TM_4$-250-17-6 | 85 | 127 | 14 | 21 | 20 | 1.10 | 83 | 8 | 91 | 1162 | 112 | 1274 | 4624 |
| 122 | $TM_4$-300-1-1 | 89 | 116 | 11 | 23 | 22 | 0.90 | 90 | 7 | 97 | 990 | 77 | 1067 | 4388 |
| 123 | $TM_4$-300-2-1 | 90 | 130 | 21 | 22 | 17 | 0.80 | 96 | 7 | 103 | 2016 | 147 | 2163 | 4976 |
| 124 | $TM_4$-300-4-1 | 88 | 126 | 22 | 24 | 21 | 1.10 | 151 | 15 | 166 | 3322 | 330 | 3652 | 5024 |
| 125 | $TM_4$-300-4-2 | 84 | 114 | 14 | 23 | 20 | 1.00 | 115 | 14 | 129 | 1610 | 196 | 1806 | 5776 |
| 126 | $TM_4$-300-5-1 | 86 | 121 | 16 | 22 | 21 | 0.90 | 81 | 10 | 91 | 1296 | 160 | 1456 | 5852 |
| 127 | $TM_4$-300-6-1 | 89 | 130 | 19 | 22 | 18 | 0.80 | 70 | 19 | 89 | 1330 | 361 | 1691 | 4662 |
| 128 | $TM_4$-300-6-2 | 90 | 124 | 18 | 25 | 33 | 1.00 | 115 | 22 | 137 | 2070 | 396 | 2466 | 4000 |
| 129 | $TM_4$-300-8-1 | 90 | 116 | 21 | 20 | 19 | 1.00 | 99 | 9 | 108 | 2079 | 189 | 2268 | 4040 |
| 130 | Control | 95 | 147 | 13 | 27 | 35 | 1.10 | 107 | 11 | 118 | 1391 | 143 | 1534 | 3600 |

**It is necessary to mention that number of plants in any plot was 16. HD, H.P, N.T, P.L, L.L, W.L, F.S, S.S, T.S, N.T×F.S, N.T×S.S, N.T×T.S and Y are abbreviation of heading date, height of plant, number of fertile tiller, panicle length, length of flag leaf, width of flag leaf, mean of number of full seeds in any spike, mean of number of empty seeds in any spike, mean of number of total seeds in any spike, number of full seeds in any plant, number of empty seeds in any plant, number of total seeds in any plant and yield of any line in plot (Kg/ hectare), respectively. Unit of H.T, P.L, L.L, W.L, F.S, S.S and T.S parameters is on centimeter (cm). Yield levels of selected 11 $M_4$ lines have been bolded.*

## 4. DISCUSSION

The optimum dose determination experiment and statistical analysis of data from the mutant populations in the field revealed that the plant population 230 Gy has the highest of values for all studied traits. This issue indicates that suitable dose of irradiation in induction of favorable mutations in landrace Tarom Mahalli is “230 Gy”. Above results is accordance with results from Cheema and Atta [8]. They found that $LD_{50}$ values for seed fertility were 238, 232 and 223 Gy for Basmati 37 Basmati Pak and Super Basmati, respectively [8]. In total, 23 tolerant mutant plants under drought stress were obtained after three generations of selection on mutants. In the third year of evaluation ($M_4$ generation), only 17 $M_4$ lines had high yield and were selected as resistant in comparison with control (Tarom) under drought stress condition. However, study of Tahmasebi Sarvestani indicated that water deficiency during vegetative, flowering and grain filling stages reduced grain yield by 21, 50 and 21% on average in four rice cultivars, Tarom, Kazar, Fajr and Nemat that are commonly cultivated in Mazandaran province, Iran, respectively [11]. Morphological parameter evaluations of 29 selected mutant lines grown under natural conditions indicated that 14 lines were early flowering by 7 days or more and height of 8 mutant lines had been decreased to 30 cm or more than control. Also, 11 lines had yield of more than 5000 Kg/ hectare. Wang reported that Yua feng Zao variety, which matures 45 days earlier than the original variety IR 8, was developed through gamma irradiation [16]. New variety still has high yield potential. Also, previous observations showed that mutation has significantly affected the short stature. For example, Hu mentioned the short stature plants have the advantage to utilize more inputs and possess resistance to lodging as compared to tall ones, hence produce better yield [1,2,17-21]. In rice, significant improvements through the use of induced mutations have been reported for high yield [22-25]. However, many traits have been studied for their use in breeding for drought tolerance in rice, only a few show evidence of their contribution to improved yield under drought [26]. We obtained several high yield drought tolerant lines by mutation breeding approach that this issue indicates to be unique this research.

## 5. CONCLUSION

Experiment of optimum dose determination and statistical analysis of data from mutant rice populations indicated that dose 230 Gy is suitable dose for mutation breeding of rice landraces. On obtained results from standard phenotypic evaluation systems of IRRI and study of morphological characters of mutant rice plants in different mutation generations under drought stress and normal conditions, 11 lines were selected for descent test and yield experiments in several regions and years. Also, 14 early flowering lines and 8 dwarf lines were identified from 29 selected mutant lines grown under natural conditions. Results of our study and studies of other researchers indicated that gamma irradiation has been successful in inducing genetic variability in rice and mutation breeding can be applied as useful tool in developing new cultivars with superior traits. This research can be helps to development of rice cultivation in drought periods or low falling regions of the world.

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## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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