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# An evaluation of genetic differentiation in rice mutants using semi-random markers and morphological characteristics

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**Abstract**

Mutation breeding has been used to develop numerous plant varieties in recent decades. This paper describes an investigation into the scope for using gamma irradiation to create genetic variation in three $M_2$ generation Iranian rice cultivars (Sange-tarom, Tarom-hashemi and Nemat), using intron-exon splice junctions (ISJ) and analysis of morphological traits to identify mutants. The $M_2$ genotypes were screened using 21 semi-random primers and 10 morphological traits. Of the 165 bands amplified by the semi-random primers, 67% were polymorphic. The average number of bands per primer and genotype were 12.3 and 11.0, respectively. The size of the amplified fragments ranged from 250 bp to 1.8 kbp. The most informative ISJ primers were $IT_{31-15}$ and $IT_{35-15}$. The genetic similarity between control and mutant plants ranged from 6 to 93%, 36.4 to 96.8% and 17.4 to 95% in the Nemat, Sange-tarom and Tarom-hashemi $M_2$ generations, respectively. Cluster analysis based on the Dice similarity coefficient using the UPGMA procedure grouped the mutant genotypes into four clusters. Morphological analyses of 97 selected genotypes with a maturation time of between 105 and 135 days and heights of 45 to 160 cm showed that there was enough variation to create promising mutant lines for breeding programs. The results obtained using the ISJ primers were consistent with those based on morphological analysis and have considerable potential for detecting mutants.

**Keywords:** Mutation breeding, ISJ technique, morphological analysis and rice.
**Abbreviations:** ISJ - intron-exon splice junctions, PCR- polymerase chain reaction, RAPD - random amplified polymorphic DNA, UPGMA- unweighted pair group method of arithmetic means, cv. - cultivar, ANOVA - analysis of variance, Gy- gray. SPSS - statistical package for social sciences.

## Introduction

Rice (*Oryza sativa* L.) belongs to the family *Graminae* and subfamily *Orazoidea* and is the staple food source for one third of the world's population. After wheat, rice is the second largest source of calories in the human diet and provides approximately 20% of the total calories consumed worldwide. It is a particularly important food source in Asia and provides more than two billion people with 60-70% of their daily energy requirements (Chakravarthi and Naraveni, 2006; FAO, 2008). Since the 1960s, several collections of mutant lines from different species have been isolated and successfully used in many different areas of plant biology and crop breeding (Domingo et al., 2007; Fu et al., 2008). Of the more than 2700 mutant varieties that have been released worldwide, 64% were created through exposure to gamma-rays, 22% through exposure to x-rays and the rest by other mutagenic treatments (Ahloowalia et al., 2004; Shu and Lagoda, 2007). Gamma (γ) rays are physical mutagens; gamma irradiation has proven to be a useful method for introducing new trait variations that may result in crop improvement and can be used as a complementary tool in plant breeding (Babaei et al., 2010). Induced rice mutants have been useful research tools for genetic and physiological assessments of yield-limiting factors in rice. Mutants have made it possible to identify critical elements for developing high yield potential varieties exhibiting desirable traits such as semi-dwarfism, early maturity, a greater number of panicles/plant and increased fertility. By 2003, 440 mutant rice varieties had been developed. Of these, 264 were produced by the direct application of mutagens and 176 were created by cross-breeding with induced mutants (Shehata et al., 2009). Mutants were traditionally identified on the basis of their morphological properties, but the development of new techniques based on DNA information has made this process quicker and more reliable. Because of its importance as a food crop, there is considerable interest in the development of molecular markers for rice to be used in genetic studies. Breeders therefore often adopt germplasm selection criteria that emphasize the importance of genetic diversity (Agrama and Eizenga 2008; Kiula et al., 2008; Agrama et al., 2009). The use of semi-random primers targeting intron-exon splice junctions (ISJ), which was first proposed by Weining and Langridge (1991) and developed by Rafalski et al. (1997), has proven to be very useful for analyzing cultivars and DNA 'fingerprinting' (Przetakiewicz et al., 2002). This approach is universally applicable in plants

**Table 1.** Primer properties showing the number of amplified bands and polymorphism in the ISJ analysis.

| primer | Nucleotide sequence 5′→3′ | No. of Nucleotide | Melting point | Total bands | Polymorphic bands | Polymorphism (%) |
|---|---|---|---|---|---|---|
| $IT_{31-15}$ | GAAGCCGCAGGTAAG | 15 | 48 | 15 | 12 | 86.6% |
| $IT_{32-15}$ | GACTCGCCAGGTAAG | 15 | 48 | 13 | 10 | 76.9% |
| $IT_{33-15}$ | GATGCCCCAGGTAAG | 15 | 48 | 8 | 4 | 50% |
| $IT_{34-15}$ | GCGGCATCAGGTAAG | 15 | 48 | 12 | 7 | 58.3% |
| $IT_{35-15}$ | CGAAGCCCAGGTAAG | 15 | 48 | 14 | 10 | 71.4% |
| $IT_{36-15}$ | GTCGACCCAGGTAAG | 15 | 48 | 15 | 9 | 60% |
| $ET_{4-18}$ | ACTTACCTGCCTGCCGAG | 18 | 58 | 7 | 3 | 42.8% |
| $ET_{31-15}$ | ACTTACCTGGGCCAG | 15 | 48 | 11 | 8 | 72.7% |
| $ET_{32-15}$ | ACTTACCTGGGCACG | 15 | 48 | 13 | 7 | 53.8% |
| $ET_{34-15}$ | ACCTACCTGGGCGAG | 15 | 50 | 13 | 8 | 61.5% |

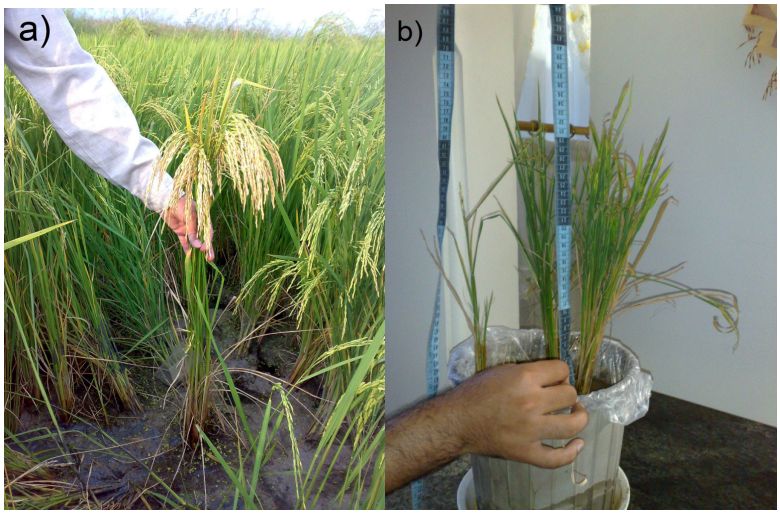


**Fig 1.** Two distinctive mutants from the $M_2$ population; a) early maturing genotype (107 days) and b) infertile dwarf (45 cm) genotype.

because it relies on primers based on the consensus plant ISJ sequences (7- 9 bases in length), which are necessary for effective splicing (Rafalski et al., 1997). The primers' lengths are then increased by adding additional bases at random to the 3' or 5' ends; this raises their annealing temperatures, yielding more reproducible PCR results. A key advantage of semi-specific primers is that they do not target the heterochromatic regions of the plant genome (Przetakiewicz et al., 2002). The technique has been employed in studies on several monocotyledonous and dicotyledonous plants, all of which have confirmed the versatility of these markers (Gawel et al., 2002). Notably, semi-random markers have been successfully used for cultivar analysis in a number of plant species including rye (Rafalski et al., 2002), maize (Rafalski et al., 2001), wheat and triticale (Gawel et al., 2002), *Solanum tuberosum* L. (Przetakiewicz et al., 2002), potato (Przetakiewicz et al., 2007), and the common bean (Marotti et al., 2007). The main objective of the study described in this paper was to use molecular (ISJ) markers and morphological trait analysis to evaluate the level of genetic variation induced by gamma irradiation in the $M_2$ populations of three Iranian rice cultivars. Semi-specific markers (ISJ) were used because previous studies that relied on RAPD molecular markers have shown poor reproducibility.

## Results

### *Morphological analysis*

ANOVA analysis of the Nemat cultivar indicated significant differences ($P<0.01$) in plant height between the different γ-ray doses relative to the control. A similar result was obtained for the proportion of unfilled seeds in the panicle and the yield per plant at the 99% confidence level ($P<0.01$), and also for total number of tillers, 1000-grain weight and the number of fertile tillers at the 95% confidence interval ($P<0.05$). No significant differences were found in the panicle length or the number of filled seeds per panicle between any of the gamma doses. While the ANOVA did not identify any between-cultivar differences in the early maturation traits, mean comparisons between treatments and the control were significant at the 150, 250 and 350 Gy doses at the 95% ($P<0.05$) confidence level (Table 2). Some of the plants in the Nemat $M_2$ generation exhibited distinctive genotypes, and some mutants derived from the Nemat cultivar underwent differentiation at 107 days maturity, when the plants' heights were 45-70 cm; conversely, the controls matured at 140 days and at heights of 100 cm (Fig. 1a and b). In the Sange-tarom cultivar, the only trait for which ANOVA indicated a significant difference related to the different

**Table 2.** The ANOVA results for some important morphological traits of the Nemat cultivar ($M_2$) after irradiation with different γ-ray doses.

| Source of variation/mean comparison | Degrees of freedom | Variance/Mean of traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant height (cm) | Early maturity (days) | No. of total tillers | No. of fertile tillers | Panicle length (cm) | No. of seed in panicle | No. of filled seed in panicle | No. of unfilled seed in panicle | 1000 grain weight (gr) | Yield per plant (gr) |
| Treatment | 4 | 48.5** | 121.6ns | 10.9* | 7.7* | 1.3ns | 251.6ns | 63.9ns | 453.9** | 2.8* | 49.8** |
| Block | 2 | 57.2** | 44.8ns | 15.2* | 11.7* | 1.3ns | 86.7ns | 27.8ns | 16.5ns | 1.3ns | 157.8** |
| Exper. Error | 8 | 5.3 | 38.6 | 2.0 | 1.58 | 0.6 | 69.7 | 43.4 | 46.8 | 0.6 | 6.4 |
| Variation Coefficient of control | | 2.4 | 7.4 | 14.6 | 21.5 | 5.1 | 7.1 | 8.9 | 24.0 | 1.2 | 26.7 |
| Mean comparison between treatment and control (0 Gy) | 150 Gy | 110.0* | 119.3* | 13.0* | 11.3ns | 28.6ns | 122.0ns | 88.3ns | 34.0** | 29.4** | 29.8 |
| | 250 Gy | 115.2** | 118.0* | 12.7* | 12.0ns | 29.1ns | 128.7* | 94.7ns | 34.0** | 29.8* | 35.4 |
| | 350 Gy | 113.7** | 118.7* | 14.3** | 14.0** | 29.1ns | 127.7* | 92.7ns | 35.0** | 29.9* | 40.1** |
| | 450 Gy | 114.5** | 122.3ns | 14.3** | 12.7* | 30.1ns | 125.7* | 86.0ns | 39.7** | 31.2ns | 34.1ns |

**Table 3.** The ANOVA results for some important morphological traits of the Sange-tarom cultivar ($M_2$) after irradiation with different γ-ray doses.

| Source of variation/mean comparison | Degrees of freedom | Variance/Mean of traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant height (cm) | Early maturity (days) | No. of total tillers | No. of fertile tillers | Panicle length (cm) | No. of seed in panicle | No. of filled seeds in panicle | No. of unfilled seed in panicle | 1000 grain weight (gr) | Yield per plant (gr) |
| Treatment | 4 | 309.3* | 18.6ns | 6.2ns | 2.4ns | 4.5ns | 233.7ns | 150.6ns | 63.8ns | 4.7ns | 78.6ns |
| Block | 2 | 748.6** | 38.6ns | 77.3ns | 52.5ns | 7.2ns | 95.0ns | 115.4ns | 13.3ns | 3.2ns | 498.3ns |
| Exper. Error | 8 | 64.9 | 43.5 | 29.4 | 16.9 | 2.8 | 160.3 | 237.1 | 50.8 | 2.1 | 160.8 |
| Variation Coefficient of control | | 10.3 | 0.6 | 12.1 | 19.2 | 3.9 | 4.4 | 3.0 | 17.3 | 1.0 | 17.8 |
| Mean comparison between treatment and control (0 Gy) | 150 Gy | 109.7** | 108.7ns | 12.3ns | 9.3ns | 24.8ns | 93.0ns | 79.0ns | 14.0ns | 24.9ns | 19.3ns |
| | 250 Gy | 114.0* | 114.7ns | 14.0ns | 10.7ns | 26.6ns | 107.0ns | 97.3ns | 9.7ns | 25.9ns | 28.7ns |
| | 350 Gy | 113.8* | 111.7ns | 11.3ns | 9.3ns | 24.2ns | 89.7ns | 78.7ns | 10.3ns | 23.0ns | 16.3ns |
| | 450 Gy | 124.7ns | 109.7ns | 15.0ns | 11.0ns | 27.1ns | 102.0ns | 83.3ns | 18.7ns | 26.1ns | 26.8ns |

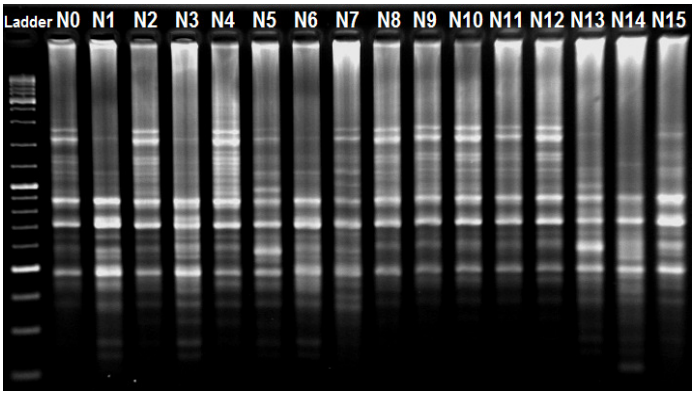


**Fig 2.** Semi-random agarose gel profile from the Nemat $M_2$ population using primer $IT_{31\text{-}15}$.

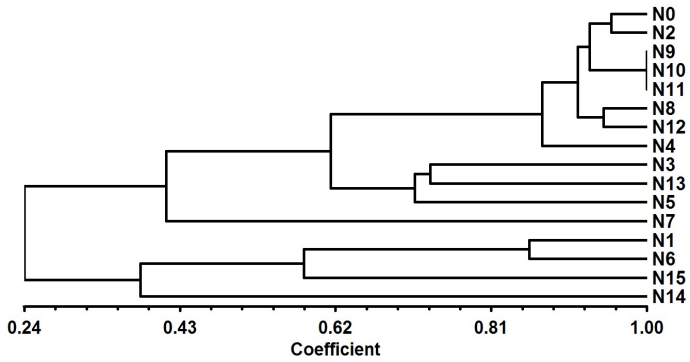


**Fig 3.** Dendrogram of 15 Nemat $M_2$ genotypes, constructed on the basis of ISJ marker analysis.

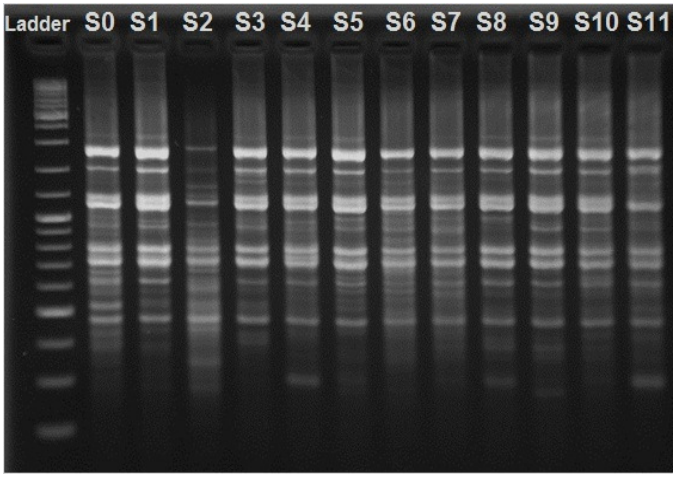


**Fig 4.** Semi-random agarose gel profile for the Sange-tarom $M_2$ population using primer $IT_{35\text{-}15}$.

levels of irradiation was plant height; varying the level of irradiation had no significant effect on the other traits examined. The heights of plants exposed to a dose of 150 Gy differed from the controls at the 99% level ($P<0.01$); those irradiated with doses of 250 and 350 Gy deviated from the controls at the 95% ($P<0.05$) confidence level (Table 3). In contrast, $M_2$ plants of the Tarom-hashemi cultivar that had been exposed to various doses of radiation exhibited numerous morphological differences compared to the control plants. Traits in which significant ($P<0.01$) differences between treated plants and controls were observed included plant height, growth period, panicle length, number of seeds per panicle, and yield per plant; no significant differences were observed for the other traits considered. However, the main objective of the present study was to examine the responses to irradiation in terms of plant height and growth period; of these traits, only the latter was affected by irradiation in the Tarom-hashemi cultivar. Thus, irradiation at a dose of 350 Gy resulted in a height difference that was significant at the 99% ($P<0.01$) confidence level; doses of 250 or 450 Gy yielded differences that were significant at the 95% confidence level (Table 4). In all of the mutants, the stem color (which was used as a specific index for identifying each cultivar) was unchanged from that of the parent cultivar (Table 5). Morphological analysis indicated that the T48 genotype had a small seed awn and that the T59 and T55 genotypes had no seed awn; as such, these three genotypes could be useful for future breeding programs. Interestingly, many mutant genotypes of the Tarom-hashemi cultivar either had no seed awn or had awns that were considerably shorter than those in the parent cultivars (Table 5). An awn is a negative trait in humid environments and rice breeders have tried to breed this trait out of rice in northern Iran.

***ISJ analysis***

Molecular genetics analyses were conducted using the intron-exon splices junction technique (ISJ) in parallel with the morphological study to investigate the genetic induction of variation as a result of γ-ray treatment. The semi-random primers (IT & ET types with 15-18 nucleotides) produced 165 bands in total, of which 111 (67%) were polymorphic (Table 1). The number of bands per primer ranged from 8 to 18; the average number of bands per primer and genotype were 12.3 and 11.0, respectively. The size of the amplified fragments ranged between 250 bp and 1.8 kbp. The highest and lowest numbers of bands were generated using primers $IT_{31\text{-}15}$ and $ET_{4\text{-}18}$, which yielded 15 and 7 bands and exhibited 86.6% and 42.8% polymorphism, respectively. As shown in figures 2, 4 and 6, the $IT_{31\text{-}15}$ and $IT_{35\text{-}15}$ primers were the most useful for detecting genotype variation in mutants. Experiments using these primers unambiguously highlighted genetic differences between the selected mutants and the control (non-irradiated) plants. The similarity indexes for the semi-random primer data calculated using the Dice similarity index indicated that the highest similarity (93%) occurred between the control (N0) and the N2, N9, N10, N11 and N12 Nemat mutants ($M_2$), and that the Nemat N1 mutant was the least similar to the control plants. The Sange-tarom mutants with the greatest similarity to the parent cultivar were S1 (96.8%), followed by S5 and S11 (93.7%); that with the lowest was S2 (36.4%).

**Table 4.** The ANOVA analysis for some important morphological traits of the Tarom-hashemi cultivar ($M_2$) after irradiation with different γ-ray doses.

| Source of variation/mean comparison | Degrees of freedom | Variance/Mean of traits | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Plant height (cm) | Early maturity (days) | No. of total tillers | No. of fertile tillers | Panicle length (cm) | No. of seed in panicle | No. of filled seeds in panicle | No. of unfilled seed in panicle | 1000 grain weight (gr) | Yield per plant (gr) |
| Treatment | 4 | 445.4 | 78.9* | 13.9 | 7.8 | 10.5* | 723.7* | 572.4 | 39.1 | 7.2 | 196.9* |
| Block | 2 | 141.2 | 25.8 | 3.3 | 5.4 | 0.9 | 154.1 | 236.5 | 50.5 | 9.9 | 121.5 |
| Exper. Error | 8 | 123.6 | 19.9 | 8.4 | 4.7 | 2.5 | 125.0 | 187.4 | 32.1 | 12.7 | 49.2 |
| Variation Coefficient of control | | 5.6 | 1.8 | 24.1 | 25.0 | 5.3 | 9.8 | 10.6 | 22.8 | 0.6 | 38.1 |
| Mean comparison between treatment and control (o Gy) | 150 Gy | 118.4* | 109.0ns | 14.7ns | 11.3ns | 29.1ns | 100.0** | 81.3* | 18.3ns | 25.5ns | 24.5 |
| | 250 Gy | 122.1* | 104.3* | 10.7ns | 8.3ns | 27.6** | 94.7** | 79.7* | 15.3ns | 25.2ns | 16.6* |
| | 350 Gy | 128.3ns | 101.7** | 9.8ns | 8.7ns | 28.9* | 105.3* | 93.0ns | 12.3ns | 21.9ns | 15.3** |
| | 450 Gy | 138.9ns | 104.3* | 10.3ns | 9.7ns | 31.4ns | 115.7ns | 101.0ns | 14.7ns | 25.0ns | 24.6ns |

*, ** and ns are the significance at the 5%, 1% levels and non significant in 5% statistical level, respectively.

**Table 5.** The characteristics of suitable selected genotypes ($M_2$) at different γ-rays levels compared with the control (N=Nemat, S=Sange-tarom and T=Tarome-hashemi cultivars)

| No. of plant * | Gamma doses (Gy) | Growth period (days) | Plant height (cm) | No. of tillers | | Pancile length (cm) | No. of seeds per panicle | | | Weight of 1000 Seeds (gr) | Yield per plant (gr) | Stem Color | Awn |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | total tiller | fertile tiller | | Total | Filled seed | unfilled seed | | | | |
| N0 | 0 (Control) | 140 | 100 | 16 | 15 | 30 | 105 | 98 | 7 | 30 | 44.1 | green | no |
| N29 | 150 | 112 | 106.0 | 15 | 14 | 27.0 | 158 | 100 | 58 | 30.6 | 42.8 | green | no |
| N33 | 150 | 105 | 115.0 | 15 | 15 | 29.0 | 173 | 135 | 38 | 30.8 | 62.4 | green | no |
| N143 | 150 | 110 | 143.0 | 14 | 14 | 30.0 | 127 | 97 | 30 | 31.8 | 43.2 | green | no |
| N99 | 250 | 109 | 115.0 | 13 | 13 | 28.0 | 145 | 107 | 38 | 29.5 | 41.0 | green | no |
| N79 | 350 | 106 | 118.0 | 15 | 15 | 30.0 | 136 | 121 | 15 | 32.1 | 58.3 | green | no |
| N152 | 450 | 108 | 146.0 | 23 | 13 | 31.0 | 102 | 75 | 27 | 35.0 | 34.1 | green | no |
| T0 | 0 (Control) | 118 | 140 | 14 | 12 | 27 | 97 | 92 | 5 | 25 | 27.6 | light green | long awn |
| T48 | 150 | 112 | 95.0 | 16 | 12 | 28.0 | 96 | 87 | 9 | 27.7 | 28.9 | light green | short awn |
| T59 | 150 | 120 | 88.0 | 30 | 14 | 25.0 | 72 | 64 | 8 | 26.6 | 23.8 | light green | no |
| T55 | 250 | 117 | 120.0 | 26 | 17 | 31.0 | 99 | 74 | 25 | 31.4 | 39.5 | light green | no |
| T11 | 350 | 103 | 132.0 | 15 | 12 | 28.0 | 144 | 124 | 20 | 25.6 | 38.1 | light green | long awn |
| S0 | 0 (Control) | 122 | 133 | 15 | 12 | 26 | 92 | 85 | 7 | 25 | 25.5 | light green | no |
| S74 | 150 | 101 | 103.0 | 21 | 11 | 26.0 | 90 | 87 | 3 | 24.1 | 23.1 | light green | no |
| S60 | 150 | 101 | 113.0 | 14 | 14 | 25.0 | 92 | 78 | 14 | 23.9 | 26.1 | light green | no |
| S61 | 250 | 112 | 110.0 | 13 | 7 | 27.0 | 127 | 116 | 11 | 25.4 | 20.6 | light green | no |
| S49 | 350 | 123 | 78.0 | 15 | 13 | 21.0 | 44 | 42 | 2 | 18.2 | 9.9 | light green | no |

* N0, S0 and T0 relate to non-irradiated samples of the Nemat, Sange-tarom and Tarome-hashemi cultivars, respectively. Ni, Si and Ti relate to the top selected rice mutants with specific characteristics in the $M_2$ population.

The Tarom-hashemi mutants with the greatest similarity to the parent cultivars were T4, T5, T7, T8 (95%); that with the lowest was T1 (17.4%) genotypes. The ISJ analysis yielded a dendrogram for the $M_2$ generation, Nemat mutants that separated them into four groups on the basis of the similarity analyses (58%) and their morphological distinctiveness (Fig. 3). The N1, N6 and N15 genotypes were placed in one distinctive group and N0, N2, N3, N4, N5, N8, N9, N10, N11, N12 and N13 into another group; these groups were separated from both N7 and N14. Very similar results were obtained for Sange-tarom mutants, for which the average similarity was 83%, and for the Tarom-hashemi mutants, which had an overall similarity of 80% (Fig. 5 and 7). The ISJ analysis-based dendrogram for the 12 selected Sange-tarom genotypes revealed them to be split into four groups, with the S2, S9 and S6 mutants each comprising a distinct cluster and another group containing the S0, S1, S5, S11, S7, S10, S3, S4, S8 and S12 genotypes (Fig. 5). Cluster analysis of the Tarom-hashemi genotypes showed that T1, T3 and T6 formed three separate groups, with a fourth group consisting of the T0, T2, T4, T5, T7, T8, T9 and T10 genotypes (Fig. 7).

**Discussion**

Based on the statistical analysis and selected mutant genotypes, γ-ray doses of 150, 250 and 350 Gy generate more variation than doses of 450 Gy, with 150 Gy producing a particularly high level of diversity in all cultivars. Therefore, lower doses may be more useful than higher ones in future rice breeding programs. Of the 97 selected plants from the $M_2$ population that exhibited genetic differences relative to the parent cultivar, 48 (49.5%) derived from plants exposed to 150 Gy; the other doses examined (250, 350 and 450 Gy) caused genetic changes in 24 (24.7%), 22 (22.7%) and 3 (3.1%) plants, respectively (Fig. 8). Our results clearly confirm earlier findings for rice (Cheema and Atta, 2003; Domingo et al., 2007; Shereen et al., 2009). The selected mutant genotypes consisted of 27, 32 and 38 single plants from the Nemat, Sange-tarom and Tarom-hashemi cultivars, respectively. The results clearly demonstrate that different cultivars respond differently to gamma irradiation (Fig. 8). The mutants exhibited growth periods to maturation of between 105 and 140 days (in the case of the Nemat cultivar) and grew to heights of between 75 to 160 cm (in the Tarom-hashemi and Sange-tarom cultivars). The greatest variation was observed in plants derived from the Nemat cultivar; one of the mutants from this line was high yielding and exhibited other valuable traits in terms of growth period, plant height, and panicle sterility. Overall, the results obtained indicate that irradiation can introduce a significant level of genetic and morphological diversity in mutant offspring relative to that observed in the parents. This increase in genetic diversity was confirmed at the molecular level; similar results have been reported by Gomma et al. (1995), Abdul-Majeed (1997), Abdallah (2000), Domingo et al. (2007), Shereen et al. (2009), and Babaei (2010). Shehata et al. (2009) successfully developed ten mutant lines in an $M_5$ generation that possessed some desirable traits that were absent in the unmodified parent. The results from the present study demonstrate that γ-irradiation can generate a considerable amount of genetic variability and provide new avenues for crop improvement and diversification; in this respect, our results are consistent with those of workers such as Meheter et al. (1996), Bordoli and Talukder (1999), Elayaraja et al.

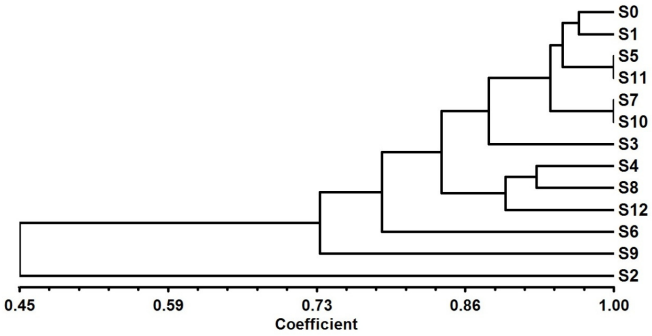


**Fig 5.** Dendrogram of 12 genotypes from the Sange-tarom $M_2$ population, constructed on the basis of ISJ analysis.

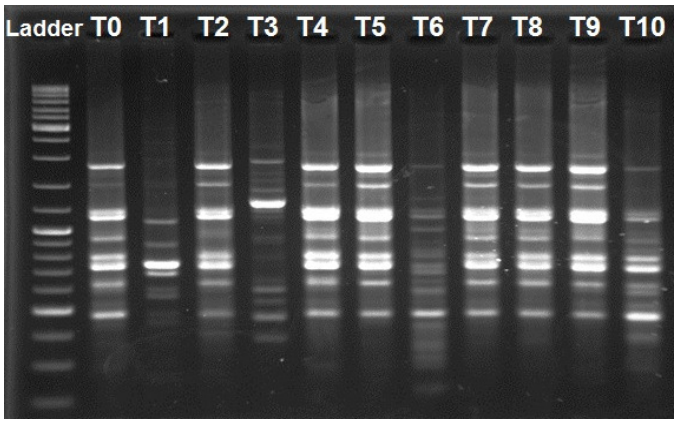


**Fig 6.** Semi-random agarose gel profile from the Tarom-hashemi $M_2$ population using primer $IT_{35\text{-}15}$.

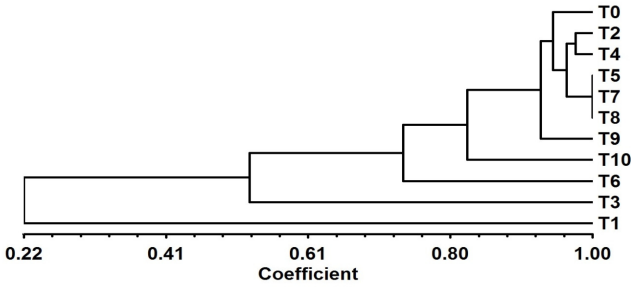


**Fig 7.** Dendrogram of 10 genotypes from the Tarom-hashemi $M_2$ population based on ISJ marker analysis.

(2005) and Luzi-Kihupi et al. (2009). The analysis of the DNA profiles, similarity coefficients and dendrogram clusters from the ISJ data in the $M_2$ population supported the morphological results. The results thus demonstrate the power of ISJ markers in varietal identification and for detecting mutants with high levels of reproducibility, indicating that they could easily replace RAPD markers in situations where the latter give rise to problems with reliability and reproducibility. Gawel et al. (2002) reported that the ISJ method is as cheap and fast as using RAPDs, and also generates more complex banding patterns with a higher

degree of polymorphism. In the present study, the DNA banding patterns for the mutant Nemat, Sange-tarom and Tarom-hashemi cultivars (fig. 2, 4 and 6) indicated that the $IT_{31-15}$ and $IT_{35-15}$ primers were most useful for DNA profiling, discriminating between rice mutants, and detecting induced genetic variation. Hashemi et al. (2009), Samei et al. (2008) and Shehata et al. (2009) also reported similar results for ISJ primers.

## Materials and methods

### *Plant material and mutagenic treatment*

Rice seeds (*Oryza sativa* L.) from cv. Sange-tarom, Tarom-hashemi and Nemat were exposed to doses of γ radiation of 150, 250, 350, or 450 Gy at 13% moisture using a $Co^{60}$ source at the Department of Agriculture Nuclear Research Institute, Iran. The duration of the gamma irradiation treatment was between 0.5 and 2 hours, depending on the γ-ray dose. The radiated seeds were grown through to the $M_1$ and $M_2$ generations during 2008 and 2009.

### *Morphological study*

Treated seeds, along with controls that were not exposed to radiation were planted in a seed nursery and then transferred to a paddy field after 28 days. The morphological characteristics of the $M_1$ and $M_2$ generation plants were measured and recorded; the specific traits measured were the plants' heights (cm), number of fertile tillers, percentage of sterile panicles, panicle length (cm), number of spikelets per panicle, 1000 grain weight (gr), yield per plant (gr), stem color and the condition of the seed awn.

### *Molecular study*

DNA was extracted from fresh leaves of plants in the $M_2$ population based on the methods described by Dellaporta et al. (1983) with minor modifications. Twenty-one semi-random primers were tested and ten polymorphic primers were selected for the final analysis (Table 1). The amplification reaction was performed using the protocol described by Samei et al. (2008). PCR products were separated by electrophoresis on 1.8% agarose gels at 70V in 0.5×TBE buffer. Gels were stained with ethidium bromide (0.5mg/ml) and visualized in a gel documentation system.

### *Data analysis*

Variation in the morphological traits of the $M_2$ population was analyzed using a randomized complete block design (RCBD) with 3 replicates and mean separation by the least significant difference (LSD) method (Yazdi Samadi et al., 2008). Results obtained by Analysis of Variance (ANOVA) were examined at the 95% and 99% confidence levels ($P<0.05$, $P<0.01$), using the SPSS ver.17 software package. Semi-random reproducible fragments were scored as present or absent (1, 0). The ISJ matrices were then analyzed using the NTSYS ver.2.02 software package (Rohlf, 1998). The degree of similarity between different sets of genetic data was evaluated by calculating the Dice similarity index; similarity estimates were made using the UPGMA algorithm. The resulting data are presented as a dendrogram.

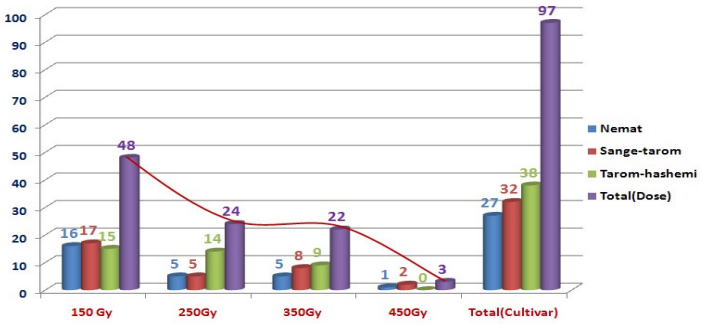


**Fig 8.** The number of selected genotypes in the $M_2$ populations (Y axis) derived from parents treated with different doses of γ-rays (X axis) from the Nemat, Sange-tarom and Tarom-hashemi cultivars. Selection was conducted on the basis of phenotypic deviation from the 'ideal' for the corresponding cultivar.

## Conclusion

The results reported herein demonstrate that mutation is a good tool for altering the characteristics of rice plants. In addition, it was shown that the ISJ technique is a powerful tool for identifying genetic diversity and can be combined with morphological studies to facilitate the detection of induced mutants. The results obtained illustrate the potential for producing promising rice lines with shorter growth periods and increased resistance to stem lodging compared to traditional rice cultivars, along with other desirable traits.

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