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Full Length Research Paper

Radio sensitivity studies of morpho-physiological characteristics in some Iranian rice varieties (*Oryza sativa* L.) in M_1 generation

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Gamma (γ) ionizing ray is a physical mutagen, producing variation in crop improvement and employing such as a complementary tool in breeding process. The γ-ray dosages are a critical issue, so it should be optimized. In this research, various doses of gamma radiation including 150, 250, 350 and 450 Gy applied on three rice varieties (Tarom-hashemi, Sange-tarom and Nemat), and their effects were evaluated on 50% lethal dose (LD_{50}), rate and percent of germination, radicle and plumule length at *in vitro*, including cluster analysis of dosage quantities at genotypes, range of variation (R), variation coefficient of treatment regarding to control dosage (CVt/CVnt). Euclidean similarity coefficient including UPGMA (Unweighted Pair Group Method with Arithmetic Mean) algorithm was used for cluster analysis. The results indicated that 250 and 350 Gy γ-rays have much more better effects to create variation rather than other doses and also clearly showed that γ-ray made novel variation in panicle sterility percentage, plant height, 1000 grain weight and fertile tillers on the studied varieties. Meanwhile the dendrogram of clustering classified into 3 groups on the base of gamma effects and control doses were placed in different groups. The LD_{50} values related to seed germination computed 350, 310 and 170 at Sange-tarom, Tarom-hashemi and Nemat respectively.

Key words: Gamma ray, rice, mutation, morpho-physiological characteristics.

INTRODUCTION

Rice as a staple food serves as the main source of calories consumed by human and occupies almost one-fifth of the total land area covered under cereals. Inducible mutation is a suitable source of producing variation in crop improvement (Domingo et al., 2007). Several improved mutant varieties with high economic value have been released via mutation breeding program (Din et al., 2004). Mutation induction with radiation was the most frequently used method to develop direct mutant varieties (Ahloowalia et al., 2001). Although more than 2500 mutant varieties are released worldwide, 64% of them related to the γ-ray, 22% to X-ray and the rest to the other sources treatment (Ahloowalia et al., 2004; Shu and Lagoda, 2007).

One of the reasons for the low yield is that the most cultivated rice are traditional cultivars that are tall, with long maturation period, prone to lodging and moreover susceptible to pest and disease. In order to address the above constraints, mutation breeding has been used to upgrade the well-adapted local varieties by altering one or two major traits, which limit their productivity or enhance their quality value. Semi-dwarfism and earliness are the characters most frequently described in released rice mutant cultivars, although other desired traits such as higher stem number, improved grain quality, blast tolerance, photoperiod insensitivity and salt tolerance are also common (Maluszynski et al., 1995). Girija and

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Table 1. Seed germination percentage of three rice varieties at γ-ray treatments in M_1.

Gamma dose(Gy) / Genotypes	0 (Control)	150	250	350	450	Mean
Tarom-hashemi	100	65	55	50	10	56
Sange-tarom	100	85	85	35	35	68
Nemat	100	55	35	20	5	43
Mean	100	68.3	58.3	35.0	16.6	-

Dhanavel (2009) reported that the usefulness of mutagens in mutation breeding depends not only on its mutagenic effectiveness (mutations per unit dose of mutagens), but also on its mutagenic efficiency or mutation in relation to undesirable changes like sterility, lethality, injury etc. The selection of effective and efficient mutagens is very essential to recover a high frequency and spectrum of desirable mutations (Solanki et al., 1994). Germination and survival was linearly decreasing with increase of radiation dose in case of γ-rays and fast neutrons (Din et al., 2004). In rice, to allow 60% survival of seeds (percentage of seeds germinated and developed to adult plants) and effective dosage of gamma ray generally ranged from 150 to 300 Gy (Rutger, 1992).

The present study was designed to determine best dosages for creating genetic variability in three Iranian rice varieties named Tarom-hashemi, Sange-tarom and Nemat and evaluation of morpho-physiological characteristics including seed germination characteristics in M_1 generation at different γ-ray dosages.

MATERIALS AND METHODS

Pure line seeds of Tarom-hashemi, Sange-tarom and Nemat varieties were irradiated by different γ-rays with 150, 250, 350 and 450 Gy of gamma rays from the ^{60}Co source at 13% moisture, at the Department of Agriculture Nuclear Research Institute, Karaj, Iran. Treated seeds along with control (non-radiation) were divided into two parts; part one was planted on seed nursery and after 21 days transferred to main paddy field for studying morpho-physiological characteristic behaviors and rest seeds; the second part grew at *in vitro* condition for testing germination, radicle and plumule length including LD_{50} (50% lethal dose) evaluation. Temperature and moisture of germinator for seed germination was 25°C and 75%, respectively.

Percentage of seed germination was studied at 3, 5 and 7 days period and radicle including plumule after 14 days period. Rate of germination was determined based on $Vg = (n_1.t_1 + n_2.t_2 + n_3.t_3)/N$ formula (Ehteshami and Chaeichi, 1998). V_g, n_i, t_1, t_2, t_3 and N related to germination rate, number of germinated seeds at different date and total germinated seeds, respectively. The experiment was done in Dashte Naz Agronomy Company placed in north of Iran. Morpho-physiological characteristics such as plant height, number of fertile tillers, panicle sterility percentage, panicle length, number of spikelet per panicle and 1000 grain weight were evaluated. Pollen fertility/sterility was evaluated microscopically by staining with 1% I_2-KI.

Furthermore, variation coefficient (CV), range of variation (R) and analysis of variation were done according to S^2t/S^2nt formula. Cluster analysis with standardized data operated to grouping γ-dosages effects using Euclidean similarity coefficient and unweighted pair group method of arithmetic (UPGMA) means algorithm. The morpho-physiological characteristic matrices were analyzed using NTSYS software version 2.02 (Rohlf, 2003). For standardizing data (z-scores), variables were subtracted from their mean, and divided by their standard deviation. The variance analysis and recording of data were done by SPSS version 17 and Excel software.

RESULTS AND DISCUSSION

The results indicated that the seed germination percentage of Nemat, Tarom-hashemi and Sange-tarom varieties reduced up to 5, 10 and 35% in 450 Gy by increasing γ-ray radiation (Table 1) and their LD_{50} were close to 170, 310 and 350 Gy respectively (Figure 1). The same results were also gotten by Din et al. (2004). It also revealed that the reduction of germination rate occurred from 13.65 (control) to 7.3 (450 Gy) in Nemat, 14.85 to 7.65 in Tarom-hashemi and 15.0 to 6.25 in Sange-tarom, but the rate of reduction is not uniform. Meanwhile, their radicle length at 14 days period also significantly reduced in all dosage treatments and ranged from 93 to 12, 156 to 87 and 176 to 57 mm (Figure 2). The same pattern is also observed for plumule means 49 to 7, 61 to 48 and 75 (control) to 34 mm at 450 Gy γ-rays. The results of dendrogram generated from cluster analysis indicated 3 distinct groups. Group 1 related to Nemat control variety (N0) as a subgroup, N3 at 350 Gy another subgroup but N1, N2 and N4 treatment at 150, 250 and 450 Gy near to each other in another subgroup (Figure 3).

Group 2 indicated Sange-tarom and Tarom-hashemi control (ST0 and TH0) near another subgroup means, Sange-tarom 150 and 250 Gy treatment. Group 3 belong to different dosages of γ-ray for Sange-tarom and Tarom-hashemi; means treatment of 350 and 450 Gy (ST3 and ST4), Sange-tarom in a subgroup and 250 and 350 Gy Tarom-hashemi in another subgroup, adjacent to Tarom-hashemi at 150 Gy (TH1). Results showed there is an interaction between genotypes and gamma dosages. Nemat as a high yield variety with 7400 kg/ha behaved differently in response to radiation from Tarom-hashemi and Sange-tarom as qualitative varieties with similar traits and also 3500 - 4000 kg/ha. It also revealed that control dosages (0 Gy) in all three genotypes were placed in different groups.

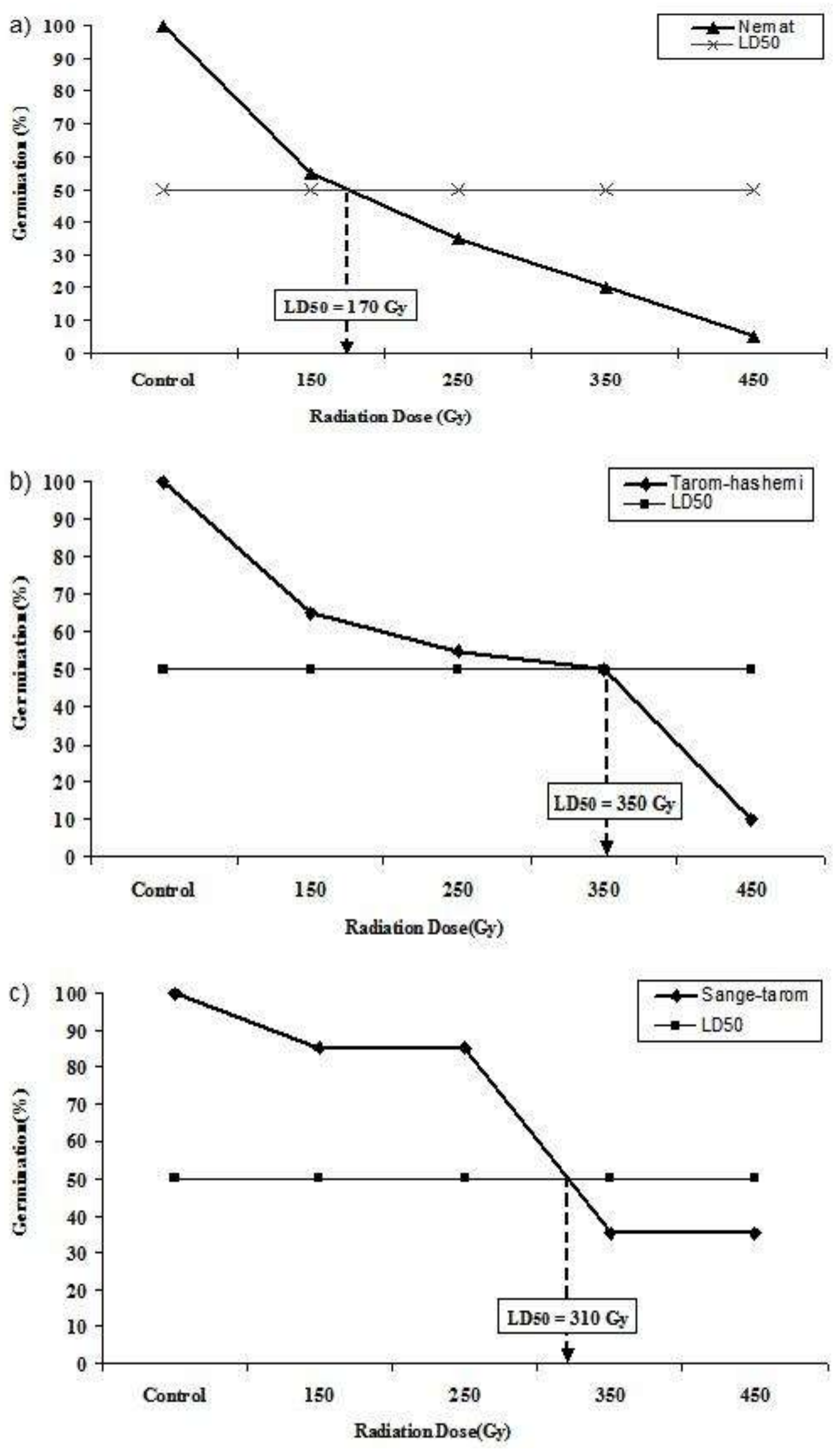

Figure 1. Seed germination percentage and LD_{50} of Nemat (a), Tarom-hashemi (b) and Sange-tarom (c) at different doses of γ-ray treatments.

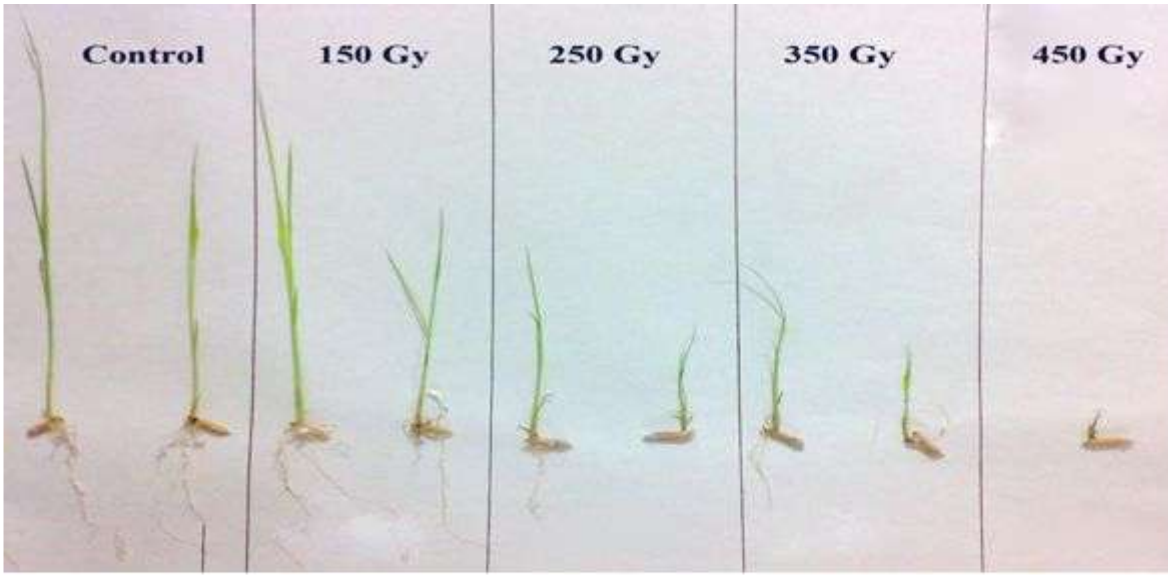

Figure 2. The effect of γ-rays on plumule and radicle length of Nemat variety at 14 days period.

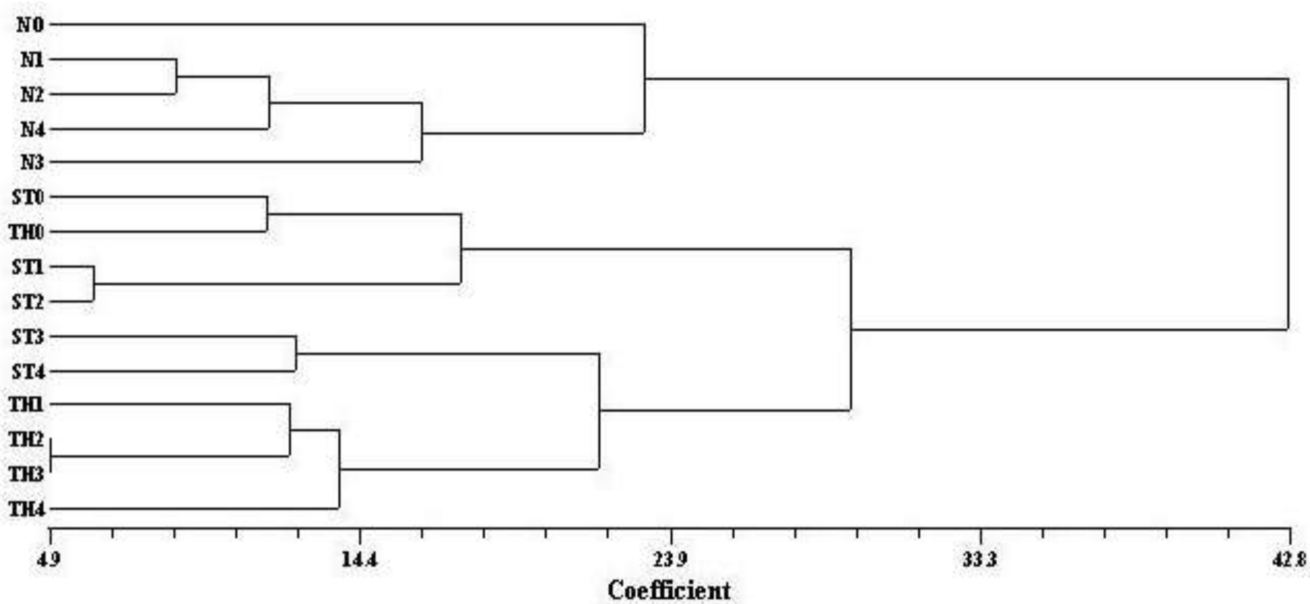

Figure 3. Grouping of different doses of γ radiation based on some morpho-physiological traits in Nemat (Ni), Sange-tarom (STi) and Tarom-hashemi (THi) by cluster analysis.

Variance analysis of morpho-physiological characteristics of 5 treatments consisting 0 (control), 150, 250, 350 and 450 Gy gamma radiation, indicated significant variation ($P < 0.01, 0.05$) for panicle sterility percentage and plant height in all genotypes. Thousand-grain weight of Tarom-hashemi at 450 Gy including number of spikelet/panicle and number of fertile tillers for Nemat at 350 Gy also indicated significant variation ($P < 0.01$), but panicle length was not significant in any variety or γ-ray dosage (Table 2).

Evaluation of variation indexes (CVt/CVnt, R and F) showed that the maximum variation coefficient ratio (CVt/CVnt) related to sterility at 250 Gy for Nemat while the maximum variation range belongs to Tarom-hashemi at 450 Gy (Table 3). The range of variation (R) in Tarom-hashemi and Sange-tarom are bigger than Nemat.

Table 2. Study of gamma mutagenic effect on some important morpho-physiological rice traits via ANOVA and LSD analysis.

Trait / Variety	Dose (Gy)	Plant height (cm) Mean ± Standard deviation	No. of productive tiller Mean ± Standard deviation	Panicle sterility percentage Mean ± Standard deviation	panicle length (cm) Mean ± Standard deviation	No. of spikelet per panicle Mean ± Standard deviation	1000 grain weight (g) Mean ± Standard deviation
Tarom-hashemi	0	154.5±6.5	20.0±2.76	7.72±2.33	29.6±2.5	9.0±1.0	27.0±0.8
	150	141.9 ±9.43 **	17.86±2.8 ^{ns}	50.9 ±26.7 **	26.8±2.17 ^{ns}	8.6 ± 0.89 ^{ns}	26.3±0.8 ^{ns}
	250	139.8±3.91 **	20.71±4.15 ^{ns}	69.49±18.4 **	30.8±4.09 ^{ns}	8.2±0.84 ^{ns}	25.8 ± 0.8 ^{ns}
	350	141.2 ± 7.37 **	20.8 ± 4.32 ^{ns}	54.17±31.26 **	31.4±2.51 ^{ns}	9.0±1.23 ^{ns}	27.0±0.5 ^{ns}
	450	132.25±4.27 **	18.33±4.16 ^{ns}	85.2±30.3 **	28.8±3.89 ^{ns}	8.6±1.5 ^{ns}	24.3±0.6 **
Sange-tarom	0	155.1±7.4	20.6±1.15	7.5±2.92	26.0±1.58	8.4±0.89	25.16±3.29
	150	149.7 ± 9.11 ^{ns}	24.0±3.0 ^{ns}	23.22±13.54 *	28.0±1.0 ^{ns}	9.33 ± 1.52 ^{ns}	24.0±1.0 ^{ns}
	250	146.0±8.83 ^{ns}	25.67±6.8 ^{ns}	50.99±8.42 **	27.3±2.51 ^{ns}	9.0 ±1.0 ^{ns}	24.5±1.0 ^{ns}
	350	142.1 ± 12.21**	23.6±4.04 ^{ns}	14.98±4.66 ^{ns}	27.6±2.07 ^{ns}	7.8±0.45 ^{ns}	25.0±1.1 ^{ns}
	450	129.75±8.22 **	20.0±5.66 ^{ns}	77.5±12.7 **	27.3±3.21 ^{ns}	8.67±2.08 ^{ns}	23.5±0.5 ^{ns}
Nemat	0	132.3±6.44	22.2±5.7	5.42±1.02	32.0±1.58	10.6±0.89	29.8±0.72
	150	120.2±1.75 **	19.83±3.37 ^{ns}	16.35±10.04 *	32.0±2.0 ^{ns}	9.33±1.15 ^{ns}	30.7±1.5 ^{ns}
	250	115.7±3.4 **	17.25±5.19 ^{ns}	18.66±10.08 *	34.3±0.58 ^{ns}	11.0±0.01 ^{ns}	31.5±0.5 ^{ns}
	350	117.13±2.53 **	15.0±2.68 **	10.46±9.15 ^{ns}	32.3±2.08 ^{ns}	8.33 ± 1.15 **	30.5±1.32 ^{ns}
	450	118.2±4.05 **	21.17±2.48 ^{ns}	12.75±1.48 ^{ns}	30.0±1.15 ^{ns}	9.67±1.15 ^{ns}	32.0±2.65 ^{ns}

*, ** and ns are significance level in 5%, 1% and non significant in 5% statistical level respectively. ANOVA = Analysis of variance, LSD = Least significant difference.

Table 3. Evaluation of CVt/CVnt, R and F related to some important morpho-physiological rice traits treated by gamma (γ) ray.

Trait / Variety	Dose (Gy)	Plant height (cm)			No. of productive tillers			Panicle sterility percentage			Panicle length (cm)			No. of spikelet per panicle			1000 grain weight (g)		
		CV_t/CV_{nt}	R	F	CV_t/CV_{nt}	R	F	CV_t/CV_{nt}	R	F	CV_t/CV_{nt}	R	F	CV_t/CV_{nt}	R	F	CV_t/CV_{nt}	R	F
Tarom-hashemi	150	1.58	33	2.1 ^{ns}	1.13	7	1.02 ^{ns}	1.73	43	74.6 **	0.95	5	0.75 ^{ns}	0.94	2	0.8 ^{ns}	0.9	0.2	0.78 ^{ns}
	250	0.66	13	0.36 ^{ns}	1.45	13	2.25 ^{ns}	0.88	26	35.5 **	1.56	11	2.65 ^{ns}	0.92	2	0.7 ^{ns}	0.92	0.2	0.78 ^{ns}
	350	1.24	24	1.29 ^{ns}	1.5	9	2.44 ^{ns}	1.91	38	102 **	0.94	6	1.0 ^{ns}	1.22	3	1.5 ^{ns}	0.58	0.1	0.33 ^{ns}
	450	0.77	13	0.43 ^{ns}	1.64	8	2.26 ^{ns}	1.17	49	95.9 **	1.6	10	2.41 ^{ns}	1.59	4	2.3 ^{ns}	0.74	0.1	0.44 ^{ns}
Sange-tarom	150	1.26	23	1.49 ^{ns}	2.24	6	6.75 ^{ns}	1.5	27	10.7 *	0.59	2	0.4 ^{ns}	1.54	3	2.92 ^{ns}	1.5	27	18.4 ^{ns}
	250	1.26	18	1.4 ^{ns}	3.75	13	34.8 *	0.42	17	4.15 *	1.51	5	2.53 ^{ns}	1.04	2	1.25 ^{ns}	0.42	17	8.28 ^{ns}
	350	1.78	35	2.67 ^{ns}	3.06	9	12.2 ^{ns}	0.8	11	1.27 ^{ns}	1.24	5	1.72 ^{ns}	0.54	1	0.25 ^{ns}	0.8	11	2.53 ^{ns}
	450	1.31	17	1.21 ^{ns}	3.08	8	24.00 *	0.42	22	9.45 *	1.93	6	4.13 ^{ns}	2.26	4	5.42 ^{ns}	0.42	22	18.9 ^{ns}

Table 3 Cont.

Nemat	150	0.3	6	0.07 ns	0.66	10	0.34 ns	3.24	20	95.2 **	1.26	4	1.6 ns	1.47	2	1.67 ns	2.06	3	4.49 ns
	250	0.6	10	0.28 ns	1.16	11	0.82 ns	2.85	20	96 **	0.34	1	0.13 ns	0	0	0 ns	0.66	1	0.48 ns
	350	0.4	8	0.15 ns	0.69	7	0.22 ns	4.61	18	79.1 **	1.3	4	1.73 ns	1.64	2	1.67 ns	1.79	2.5	3.37 ns
	450	0.7	15	0.39 ns	0.45	7	0.19 ns	0.61	2.8	2.08 ns	0.77	2	0.53 ns	1.42	2	1.67 ns	3.42	5	13.5 ns

*, ** and ns are significance level in 5%, 1% and non-significant in 5%, respectively. CVt , CVnt, R and F are coefficient variation of treatment and control, range of treatments and variance of treatment per variance of control, respectively.

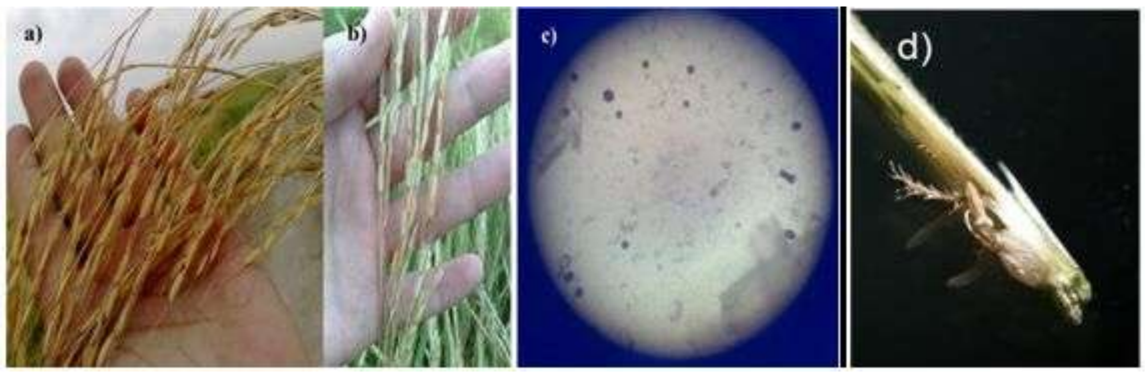

Figure 4. Comparison of panicle sterility in control and Tarom-hashemi variety treated at 350 Gy (a and b); (c) pollen-sterility and (d) abnormal female reproductive organ in Nemat at 250 Gy.

Variation range of panicle sterility generally in all three cultivars was the most in comparison with other traits and after that, plant height of Tarom-hashemi in 150 and 350 Gy and plant height of Sange-tarom in 350 Gy are in the next ranks. Highest value of F (treatment variance per control variance = S^2t/S^2nt) was related to panicle sterility in Tarom-hashemi 350 Gy. In general, panicle sterility showed the most reaction to gamma radiations compared with other characteristics (Figures 4a and b). Pollen grains viability of γ treated samples studied using KI-I_2 staining solution, showed sterile pollen grains more than 90% with none circular, colorless or light color pollens, so it can cause male sterility in panicles which may enable us to produce male sterile plants applying hybrid rice production (Figure 4c). Study of female reproductive organs in some treated plants revealed that gamma radiation made abnormal shape in stigma, style or ovary with creating woody or shorter organs or losing their feathery and fleshy tissues (Figure 4d).

Conclusion

The result of cluster and variance analysis for agronomy traits including the comparison of index, variation coefficient of varieties at 250 and 350 gamma ray dosages have shown better performance in this study. Similar results were also observed by Cheema and Atta (2003). Dendrogram analysis as well as LD_{50} quantities indicated interaction between genotypes and γ-ray dosages. So further study, should focus on 150 to 350 Gy with 50 Gy interval including elimination of 450 Gy from experiment due to negative impact on seed germination and range of variation.

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