Available online at www.scholarsresearchlibrary.com

Scholars Research Library

Scholars Research Library

Annals of Biological Research, 2012, 3 (12):5786-5793
(http://scholarsresearchlibrary.com/archive.html)

Annals of Biological Research
Scholars Research Library
ISSN 0976-1233
CODEN (USA): ABRNBW

The study of weight changes in two varieties of rapeseed under the influence of different dosages of gamma ray and PEG at early seedling stage

Meisam Radfar

Dep. of plant breeding, Faculty of Agriculture, Ardabil Branch, Islamic Azad University, Ardabil, Iran.

ABSTRACT

In order to develop nuclear energy uses in improvement of plants to dry condition and to investigate the effect of Gamma ray (800, 1000, 1200 gry and control) and PEG (-3, -6, -9 bar and control) on the length of root and shoot in two varieties of rapeseed (Sarigol and RGS003), an experiment was conducted in CRD design in factorial way at laboratory condition with 3 replication in 2011. The obtained results indicated that gamma ray had a significant effect on total fresh weight and turgescence weight ($p<0.01$). Drought stress affected significantly all 4 investigated traits (TFW, TW, TDW and RWC) with ($p<0.01$). The mutual effect of variety and PEG on total fresh weight and turgescence weight was significant ($p<0.05$) and this effect on RWC was significant, too ($p<0.01$). The obtained results revealed that the mutual effect of gamma ray with drought stress and variety on RWC was significant ($p<0.05$). The comparison of data mean showed that gamma ray on both traits namely TFW and TW led to weight loss and the highest weight is referred to the condition without application of gamma ray. The difference in TFW in 800, 1000, and 1200 gry dosages didn't prove to be significant. 800 gry dosage of gamma ray compared to other dosages indicated higher turgescence weight. The negative effect of drought stress on TFW was in a way that the highest TFW was observed in no stress condition and the least TFW was observed in -9 bar osmotic pressure. The drought stress had no effect on total dry weight of Sarigol, but increased the TDW of RGS003 so that the highest dry weight was observed in -3 bar osmotic pressure. The comparison of data mean indicated that the increase in negative osmotic pressure reduces the TW so that both varieties experience the highest weight in no stress condition and the least weight in -9 bar osmotic pressure. The application of ray had positive effect on increase of TDW in 800 and 1200 gry dosages.

Key words: drought stress, gamma ray, rapeseed, weight

INTRODUCTION

The rapeseed is considered as the fifth world vegetable oil supply after palm, soybean, cotton, and coconut and it involves the third biggest area under cultivation with 32 million hectare harvest level [1]. The oil extracted from edible rapeseeds has high quality. After extraction, rapeseed meal has a lot of protein and is appropriate for feeding animals [2]. The least needed temperature for germination is 4 º centigrade's so that in fall planting, the seedlings germinate after 6-12 days if the soil is humid. Rapeseed is sensitive to soil humid suction and any reduction in the humid leads to the decrease in germination percentage [3,4]

Application of corrective procedure named artificial mutation has been common in many countries and has been the focus of attention due to generation of new genetic material (mutant). This procedure has much potential that has reformed many plants successfully [5,6,7 and 8]. The artificial mutation induced for first time to *Drosophila Melanogaster* by Muller using x-ray in 1927. The plants reform through artificial mutation aroused a lot of interest in 1950s and 1960s [9,10].

At first, the researchers used x-ray to induce mutation but recently the application of gamma ray has been more common as a result of development of nuclear power plants and atomic centers. The most research activities of mutation in the world are conducted in cooperation with food and agriculture organization of the United Nations (FAO) and international atomic energy agency (IAEA) [11].

Physical mutants are divided into two groups namely ionizing beams and no ionizing beams [12]. Ionizing beams can be divided into electromagnetic beams and particle beams. The electromagnetic beams are light which are formed from nonfinite photons that move wave-like. Gamma ray and x-ray are examples of this group. Gamma ray can destroy the live cells but this danger can be controlled if the ray is used in a specified dosage and duration. The controlled gamma ray can induce genetic mutations to live cells. Gamma ray, in the case of penetration into genetic material of the cell, breaks the intermolecular bonds and inter-nucleotide bonds or even changes the structure and connection of atoms forming the material in DNA. Gamma ray has a short wave-length and higher frequency compared to other electromagnetic waves like visible light [13]. The seeds can withstand against heat and saltiness through Gamma irradiation and genetic and morphological changes. Lower dosage of gamma ray leads to stimulate the cell division and high dosage prevents the cell division due to formation of free radicals and system destruction [14].

Generally, stress is the result of abnormal trend of physiological processes which is originated from the effect of one or more biological and non-biological factors and may lead to growth failure, low efficiency, and death of a part of plant or whole plant [15]. But from biological view point, any change in environmental condition which makes the plant to have efficiency lower than normal and optimal condition can be called stress. The stress, as the most important limiting factor, plays a main role in decreasing the crops production all over the world [16].

Drought is one of the most important abiotic stresses which influence many products in different parts of the world. A large amount of efficiency reduction and products reduction is as a result of drought stress. The drought stress occurs in the plant when the received water is less than water loss. This may results from either water over loss or reduction in the absorption or both cases [17]. The drought stress affects different aspects of plant growth and causes reduction and delay of germination, reduction of shoots growth, and reduction of dry matter production. The reduction of osmotic potential and water potential, turgescence removing, and stoma closure as well as growth failure are symptoms of water stress [18].

PEG is a flexible and nontoxic polymer and can cause negative osmotic pressure. Also, this substance has no tendency to react with chemicals and biological substances. This property has made PEG the most useful molecule to create negative osmotic pressure in biochemical experiments (especially osmotic stress) [19,20 and 21].

MATERIALS AND METHODS

In this research, two varieties of rapeseed namely Sarigol and RGS003 were selected for the experiment. Both varieties are spring hybrids of rapeseed. The reason for this selection is symmetry between spring planting and drought seasons of crop year which was in relation with the goal of study.

At first, the seeds were irradiated by Gamma ray due to easiness of irradiation and more effect. The dosage was identified according to predetermined range for rapeseed [13] and 3 dosages 800, 1000, 1200 gry were chosen. The seeds of both varieties were divided into 3 parts and were placed in paper package. One package of each variety was kept as control. The packages, considering the specifications written on each box, were delivered to Atomic Energy Organization for Gamma irradiation. After Gamma irradiation whose dosage is calculated regarding time and distance from irradiation source, the seeds were transferred to the laboratory for cultivation and examination. Using Vanthoff and Michel formula [22]. The required amount of PEG for providing solutions with -3, -6, and -9 pressure was computed and was prepared through solving the solid material in distilled water and increasing to 1000 cc in order to irrigate the cultivation environment.

Equation1: $\psi_s = -(1.18\times10^{-2})C - (1.8\times10^{-4})C^2 + (2.67\times10^{-4})CT + (8.39\times10^{-7})C^2T$ In this formula, **C** is the required PEG gram /water kg and **T** stands for the environment temperature (usually 25 ċ). Osmotic pressure is mega Pascal (each mega Pascal equals to 10 bars).

The packages including the seeds irradiated with 3 dosages and control seeds which each one has been divided into 4 parts for application of PEG treatment formed 16 packages for every variety. The seeds inside each package were randomly re-divided into 3 sections. These 3 sections were indicator of three repetitions for each treatment. The

above division has been planned and performed according to CRD design in factorial way with 3 treatments and 3 repetitions.

96 pottery dishes were washed with 96 degree ethanol alcohol to be prepared for planting the seeds. The same number of circular filter papers were provided and washed with 96 degree ethanol alcohol. Some 70% ethanol alcohols was prepared through diluting 96 degree alcohol in order to wash the seeds. The seeds firstly were soaked into alcohol for 7 seconds and were washed with distilled water. Afterwards 20 seeds were randomly selected and were placed in pottery dish on the dried filter paper.

After above-mentioned stages, the irrigation of cultivation environment with distilled water for environments including control seeds and with dosage-based PEG for pre-divided environments were started. Pottery dishes were transferred to incubator machine with 27 ċ temperatures. In order to study the indices of rapeseed seedling, total fresh weight (TFW) of all seeds in each pottery dish was measured with milligram accuracy by digital scale and TFW was calculated for each one. To measure the turgescence weight (TW), the seedlings were floated in distilled water for 24 hr and weighing was done in the same way. Finally, the samples were transferred to incubator and were kept in 50º centigrade for 48 hr. The total dry weight (TDW) was recorded on the basis of individuals. The index of relative water content (RWC) for each pottery was computed and recorded using below formula [23].

$$\text{RWC } (\%) = \frac{(TFW - TDW)}{(TW - TDW)} \times 100$$

These figures as the final data were analyzed using three-factor factorial experiment based on CRD design. Mean comparison for significant traits were made using Duncan multiple range test with 5% probability. Also, to investigate the relationship between indices, Pearson correlation coefficient was used and indices namely Y_s (performance in stress condition) and Y_p (performance in without stress condition) were used to compare sensitivity of measured traits in conditions with and without stress and the data were displayed in diagram form.

RESULTS AND DISCUSSION

The study of data in table of variance analysis (**table 1**) indicates that gamma ray had a significant effect on TFW and TW ($p<0.01$). The drought stress significantly affected all four studied traits ($p<0.01$). The mutual effect of variety and PEG on TDW and TW was significant ($p<0.05$). Also the mutual effect of variety and PEG on RWC was significant ($p=<0.01$). The obtained data revealed that the mutual effect of gamma ray with drought stress and the mutual effect of gamma ray with drought stress and variety on the RWC proved to be significant ($p<0.05$).

Table l. Variance analysis of data related to measured traits of rapeseed

S.O.V.	df	MS			
		TFW	TDW	TW	RWC
Varieties	1	33900.17	8.17	10858.76	8.14
Gamma ray	3	63245.57 **	16.31	156331.57 **	105.35
Varieties × Gamma ray	3	7743.47	26.42	5220.01	159.97
PEG	3	930644.93 **	284.19 **	3358580.45 **	2835.57 **
Varieties × PEG	3	12207.22	109.08 *	42269.01 *	384.52 **
Gamma ray× PEG	9	4693.27	18.76	20812.87	182.75 *
Varieties × Gamma ray× PEG	9	13172.45	22.93	6631.59	205.61 *
Error	64	10354.85	33.25	11144.15	88.86
C.V.		18.34 %	11.29 %	15.86 %	12.13 %

**significant if p 5% ** Significant if p 1%*

The comparisons of data mean indicated that gamma ray on both TFW and TW led to weight loss and both traits increased in the case of no gamma ray application. The differences in TFW in 800, 1000, and 1200 gry dosages weren't significant. Dosage of 800 gry compared to other dosages led to more TW. In spite of this fact that the effect of different dosages of gamma ray on TFW was equal but it was observed that same seedlings didn't absorb the equal amount of water after being floated in distilled water. **Table 2** displays that the treatment of the seeds with dosage of 800 gry plays more important role considering the increase of absorption capability and can increase the TW. In this way, the seedlings can absorb and save more water.

Table 2. The mean of treatment combination of gamma ray in average condition of varieties and PEG considering TFW and TW

Gamma ray dosage (gry)	Mean	
	TFW (m gr)	TW (m gr)
Control	624.2 A	770.5 A
800	562.8 B	668 B
1000	521.5 B	601.8 C
1200	511 B	602.6 C

The comparisons of data mean indicated the negative effect of drought stress on TFW so that the most TFW was observed in no stress condition and the least TFW was observed in -9 bar osmotic pressure. Therefore, like gamma ray, different dosages can be used to creation of selection condition (**table 3**).

Table 3. The mean of treatment combination of PEG in average condition of varieties and gamma ray considering TFW

PEG dosage (bar)	Mean
	TFW (m gr)
control	964.9 A
-3	694.7 B
-6	397.1 C
-9	162.7 D

According to the data obtained from comparison of data mean (**figure 1**), it was determined that the drought stress has no effect on total dry weight of Sarigol but it increased the TDW in RGS003 so that the highest TDW was observed in -3 bar osmotic pressure.

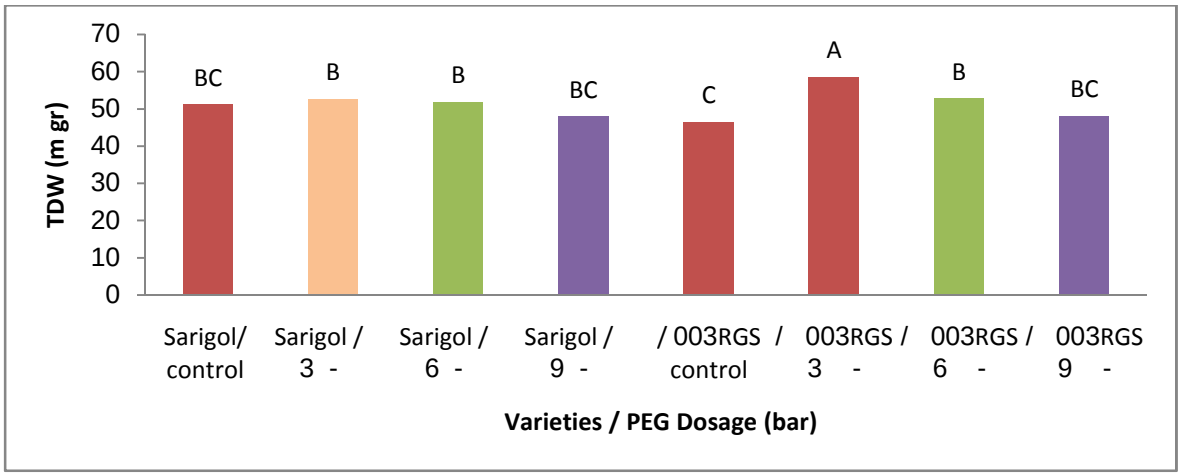

Figure 1. The comparison of treatment combination of PEG and rapeseed varieties considering TW in average condition of gamma ray

The comparison of data means (**figure 2**) indicates that the increase in negative osmotic pressure led to reduction of TW so that in both varieties, the highest weight was observed in no stress condition and least weight was observed in -9 bar osmotic pressure. Also, it was observed that RGS003 has more TW compared to Sarigol in -3 bar osmotic pressure.

With respect to this point that TDW in Sarigol is same in stress condition, table 3 and figures 1 and 2 suggest that if drought stress is less severe, the TFW will be more and consequently there will be more water in rapeseed. But regarding fixed TDW, the reason for reduction of TW must be investigated in future studies.

The comparison of means pertained to different dosages of gamma ray in fixed dosages of PEG on RWC index in Sarigol didn't show any significant difference but application of different dosages of PEG in fixed dosages of gamma ray on RWC trait in Sarigol for 1000 and 1200 gry dosages showed difference. It means that increase in negative osmotic pressure led to decrease in RWC in 1000 and 1200 dosages as the highest RWC

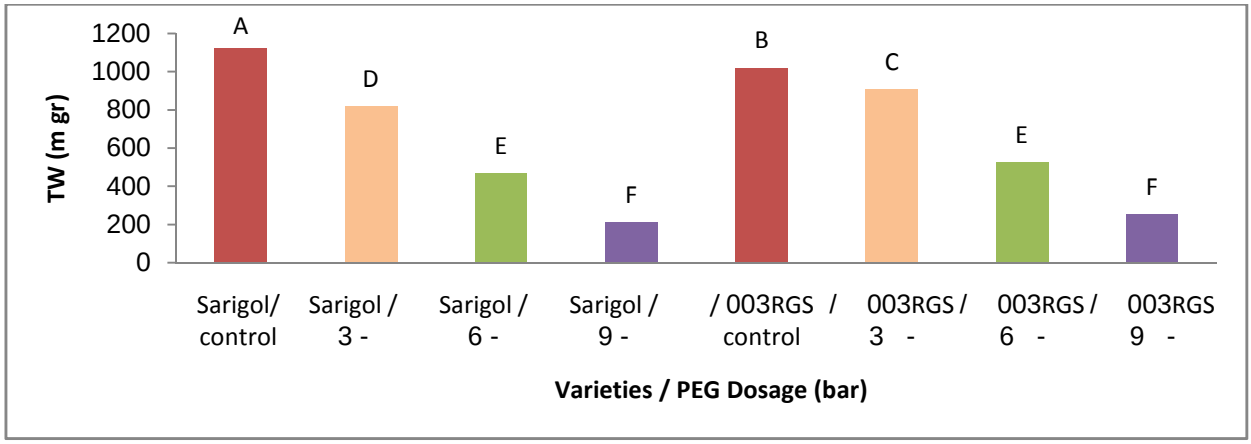

Figure 2. The comparison of treatment combination of PEG and rapeseed varieties considering TW in average condition of gamma ray

was observed in control dosage of PEG for both dosages of gamma ray and the least RWC was observed in -9 bar PEG for both dosages of gamma ray. It can be concluded that Sarigol in 1000 and 1200 gry dosages is sensitive to drought considering RWC trait (**figure 3**).

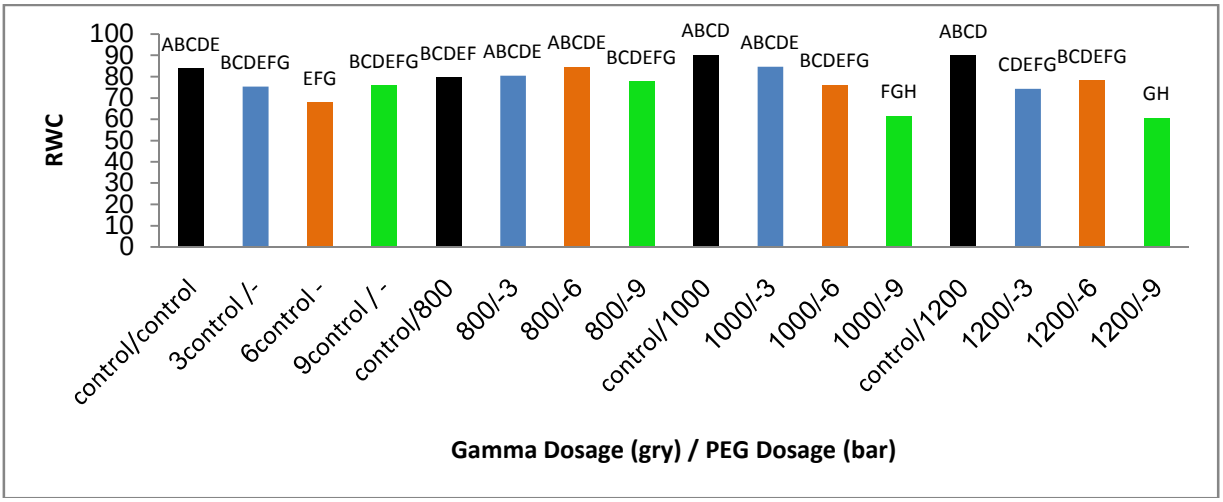

Figure 3. The mutual effect of gamma ray treatment and PEG treatment on RWC in Sarigol

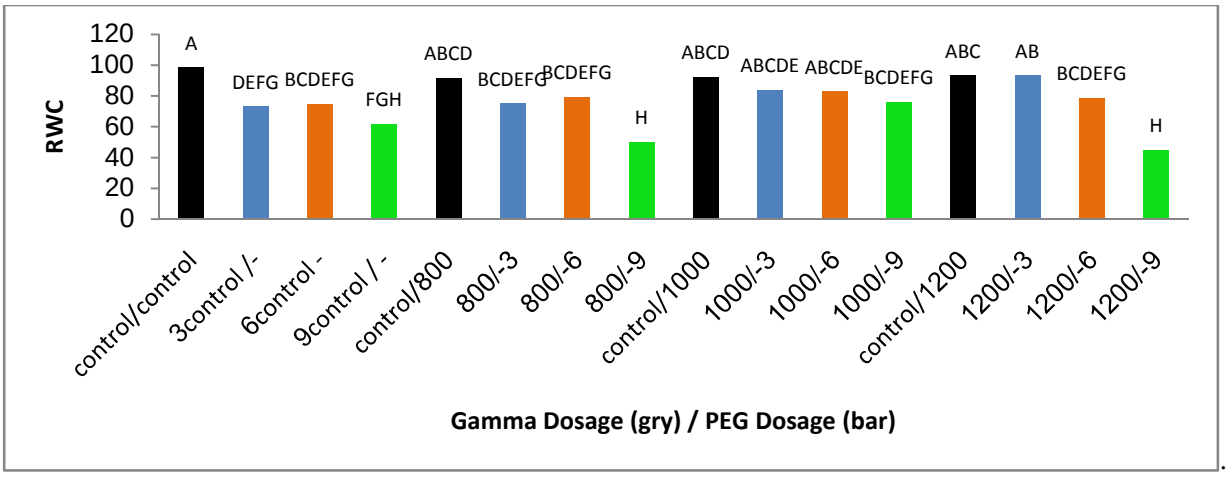

Figure 4. The mutual effect of gamma ray treatment and PEG treatment on RWC in RGS003

Different treatments of gamma ray in fixed dosages of PEG on RWC trait in RGS003 proved significant differences in -3 and -6 bar dosages that is the increase of gamma ray dosage led to changes in RWC for PEG dosages so that

1200 gry dosage in -3 bar pressure and 1000 gry dosage in -9 bar pressure yield the most RWC but different dosages of PEG in fixed dosages of gamma ray on RWC in RGS003 for control dosages, -3 and -9 bar showed significant difference in a manner that increase in negative osmotic pressure caused reduction in RWC. In this way, control dosage of PEG in control dosage of gamma ray showed the highest RWC and dosage of -9 bar PEG in 800 and 1200 gry gamma ray yield the least RWC. It can be concluded that RGS003 in 800 and 1200 gry dosages is more sensitive to drought in RWC trait. It must be added that increase in gamma ray on RWC made no change in Sarigol but it had positive effect on RGS003 and 1000 gry dosage increased this trait in -9 bar pressure and 1200 gry dosage increased the trait in -3 bar pressure (**figure 4**).

The data on correlation table (**table 4**) indicate that there is a positive correlation between TFW with index of RWC and TW ($p<0.01$). The TW is positively correlated with index of RWC ($p<0.01$). Based on the data on table 4, there is relationship between seedling weight with different modes of germination percentage and length of root and shoot.

Table 4. The correlation coefficient of studied traits in rapeseed

Traits	RWC	TW	TDW	TFW	Ger. P.	Root l.	Shoot l.
Shoot length	0.687 **	0.960 **	0.151	0.965 **	0.750 **	0.648 **	1
Root length	0.334	0.719 **	0.656 **	0.631 **	0.758 **	1	
Ger. Percent	0.648 **	0.787 **	0.462 **	0.767 **	1		
TFW	0.742 **	0.983 **	0.214	1			
TDW	0.091	0.298	1				
TW	0.647 **	1					
RWC	1						

**significant if p 5% ** Significant if p 1%*

The amount of traits in stress and no stress conditions were calculated based on (Y_s) and (Y_p) indices and illustrated in **figures 5, 6, 7, and 8**. These figures indicate that changes in amount of (Y_s) along with increase in gamma ray in fixed dosages of PEG and variety aren't steady. The positive effect of gamma ray in 800 and 1000 gry dosages on RWC in -3 and -6 bar dosages is visible. The positive effect of gamma ray with 1200 gry dosages on TDW in -9 bar pressure was observed. It can be mentioned that the application of 800, 1000, and 1200 gry dosages of gamma ray is appropriate in order to make changes in the seedling for selecting better plants.

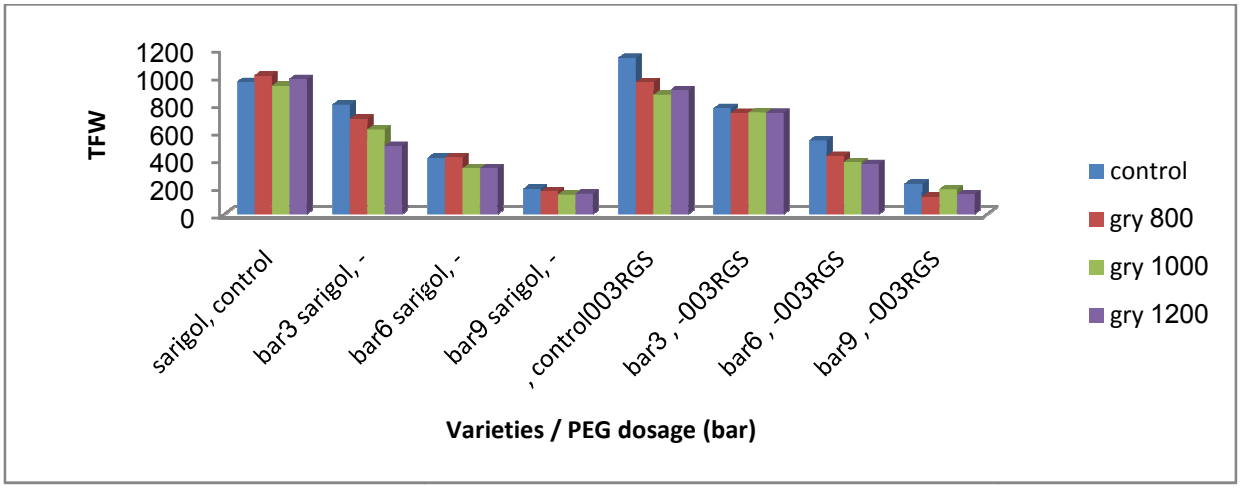

Figure 5. Degree of changes in the amount of (Y_s) and (Y_p) in fixed dosages of PEG on rapeseed varieties in gamma ray treatment on TFW condition

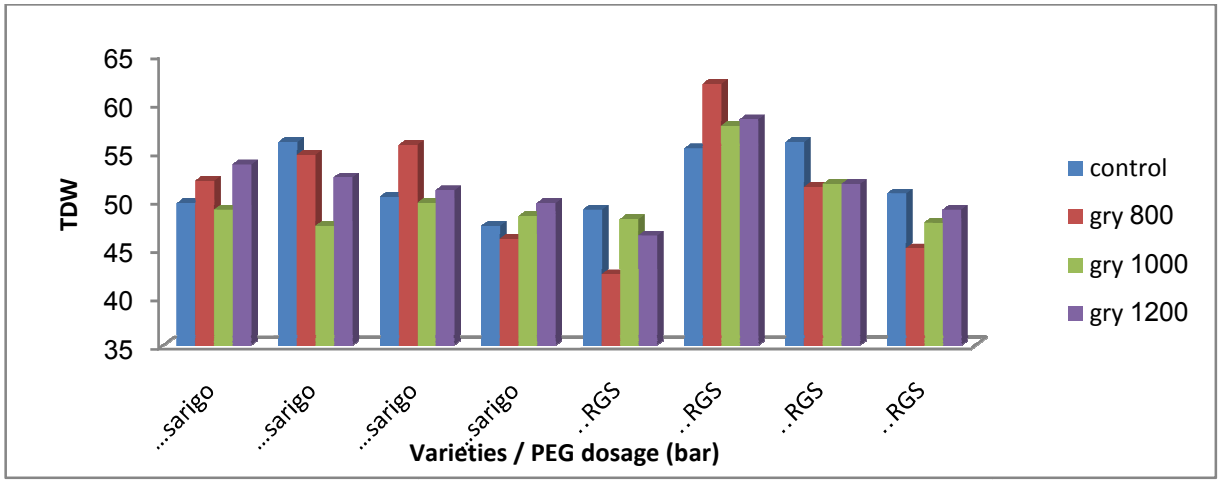

Figure 6. Degree of changes in the amount of (Y_s) and (Y_p) in fixed dosages of PEG on rapeseed varieties in gamma ray treatment on TDW condition

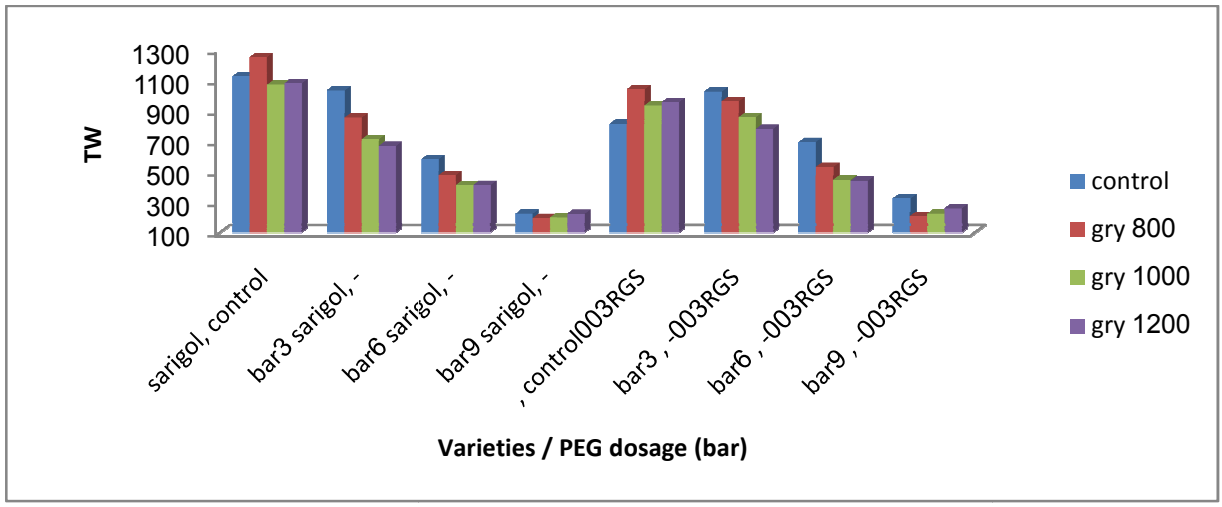

Figure 7. Degree of changes in the amount of (Y_s) and (Y_p) in fixed dosages of PEG on rapeseed varieties in gamma ray treatment on TW condition

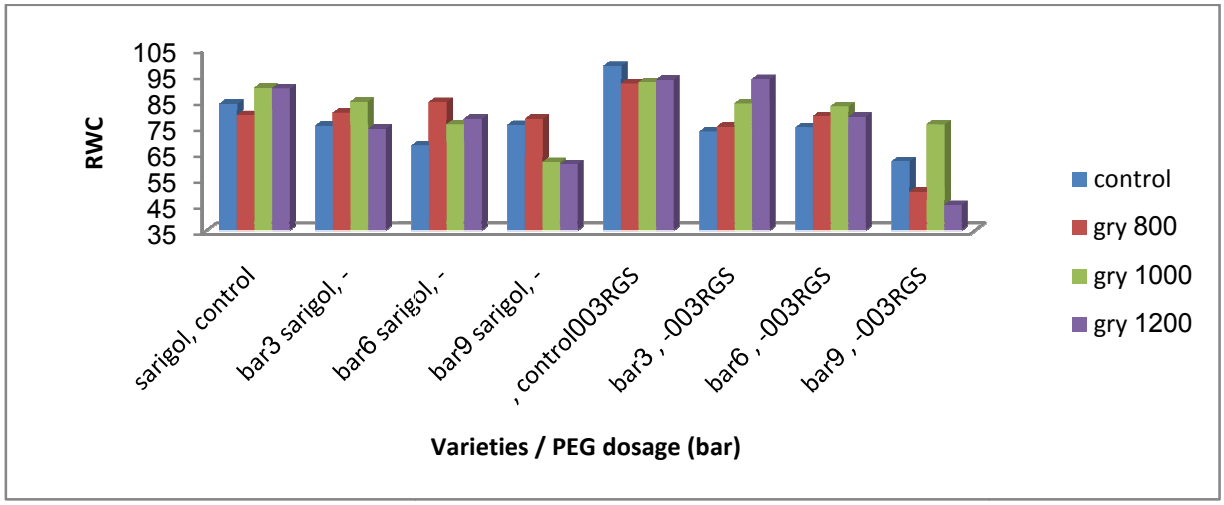

Figure 8. Degree of changes in the amount of (Y_s) and (Y_p) in fixed dosages of PEG on rapeseed varieties in gamma ray treatment on RWC condition

Acknowledgement
The authors appreciate from Dr. M. H. Fotokian for his support in supplying the seed and Mr. K. Aghahi from laboratory of Shahed University. We wish to thank from Prof. S. A. Mohammadi from Tabriz University.

REFERENCES

[1] FAO., Data statistics. http://faostat.fao.org, **2010**.
[2] Shiranirad, A.H. and E. Dehshiri, Canola Guide (planting until harvesting), *Journal of Agricultural Education*. **2002**. Page 37.
[3] Nabavi, S., The investigation of the effects of drought stress during seeds growth on quality and growth of seeds. *Unpublished doctoral dissertation*, Science and research university, Tehran. **2006**.
[4] Radfar, M., S. Aharizad, S. A. Mohammadi and M. Zaefizadeh, The Effects of Gamma Ray Irradiation and PEG on Germination Of two Varieties of Rapeseed. *Annals of biological research*, **2012**. 3(4):1828-1832
[5] Farsi, M. and A. Bageri, Plant correction Principles. *Mashhad Jahad Daneshgahi Press*. **1998**.
[6] Filippetti, A., Improvement of grain yield in *Vicia faba* L. by using experimental mutagenesis. *Genetica-Agraria*. **1983**. 37: 53-67.
[7] Gaul, H., Critical analysis of the methods for determining the mutation frequency after seed treatment with mutagens. *Genetica-Agraria*. **1960**. 12: 297-318.
[8] International Atomic Energy Agency., Mutation in plant breeding. IAEA Publisher. **1966**.
[9] Drake, T. W., The molecular basis of mutation. *Hilden-Day Inc*. Pp. **1969**. 100-176.
[10] Srivastava, A. and V. P. Singh, Induced high yielding broad bean mutant. *Mutation Breeding Newsletter*, **1996**. 42:8-124.
[11] Kyin, S. M., O. A. Khin and S. Khin, Development of a short duration upland rice mutant line through anther culture of gamma irradiated plants. *Mutation breeding newsletter and reviews*. **2005**. 1: 13-14.
[12] Ahmadian, F. and K. Jafar Khorshidi, The guide for application of rapeseed meal to feed animals, birds, and aquatics. *Nagsh Gostarane Bahar Press*. **2009**. 7- 46.
[13] International Atomic Energy Agency., Manual on mutation breeding. Technical Reports Series NO. 119. *IAEA Publisher*. **1977**.
[14] Zaka. R., C. Chenal and T. M. Misset, Effect of low doses of short term gamma irradiation on growth and development through two generations of *Pisum sativam*. *Sci. of the Total Environment*. **2004**. 320: 121-129.
[15] Hekmat Shoar, H., Plant physiology under difficult condition. *Nikam Press*. **1993**.
[16] Haghjoo, M., M. Rustai, Z. Bamdad and B. Jahangiri, The effect of drought stress on 9 varieties of wheat during germination and growth of seedling in Fars Province stage. The articles in first national conference of Iran seeds technology and science, Gorgan, Iran. **2008**.
[17] Kuchaki, A. and A. Alizadeh, Agriculture principles in dry regions. *Razavi Press*. **1995**. p. 260.
[18] Singh, J. and A. L. Patel, Water statues, gaseous exchange, prolin accumulation and yield of wheat in response to water stress. *Annual of Biology Ludhiana*, **1996**. 12: 77-81.
[19] Al-Khayri, J. M. and A. M. Al-Baharany, Growth, water content and prolin accumulation in drought stressed callus of date palm. *Biologia Plantarum*, **2004**. 48(1): 105-108.
[20] Blum, A., Use of PEG to induce and control plant water deficit in experimental hydroponics culture. http://www.plantstress.com/methods/index.asp, **2005**.
[21] Macar, T. K., T. Ozlem and Y. Ekmekci, Effect of deficit induced by PEG and NaCl on chickpea (*Cicer arientinum*. L.) cultivars and lines at early seedling stage. *Gazi univ. J. Sci*., **2009.** 22(1): 5-14.
[22] Michel, B. E. and M. R. Kaufmann, The osmotic potential of polyethylene glycol 6000. *Plant Physiology*, **1973**. 51: 914-916.
[23] Ferrat, I. L. and C. J. Lovat, Relation between relative water content, Nitrogen pools and Growth of *phaseolus vulgaris* L. and *P. acutifolius* A. Gray during water deficit. *Crop Science*, **1999**. Vol. 39. 467-474.