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Full Length Research Paper

The effects of chemical and physical mutagens on morphological and cytological characters of barley (Iranian cv. Nosrat)

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Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing beneficial variations for practical plant breeding. In the current study, dry grains of barley (*Hordeum vulgaris* L., cv. Nosrat, 2n = 2x = 14) were exposed to physical and chemical mutagens. The results of Duncan multiple rang test showed that treatments of gamma ray (control (0), 200 and 320 Gy) had highest germination percentage and formed class a. The lowest germination percentage belonged to ethyl methane sulfanate (0.7%). Mean comparison of radical length trait showed that the highest radical length belonged to gamma ray control, 200 Gy and sodium azide (SA) 0.5 mM and treatments of gamma ray 700 Gy, 1200 Gy and ethylmethane sulphonate (EMS) 0.7% had lowest radical length. Mean comparison of plumule length trait showed that treatment of SA 1 mM had highest plumule length. Cytogenetic studies have shown chromosome breakage in some induced barley seed by high dose of 700 and 1200 Gy. On the basis on growth traits analysis, sodium azide 0.5, 1 and 5 mM, ethyl methane sulfanate 0.1% and gamma ray 200 and 320 Gy are appropriate mutagens. However, it needs more consideration on mutagen, dose of mutagens and trait to have best breeding program by mutagenesis.

Key words: Barley, physical and chemical mutagen, gamma radiation, sodium azide, ethyl methane sulphonate.

INTRODUCTION

Agricultural biotechnology plays important role in economic development and it can solve so many problems in the world. As regard the growing of population, we need to produce more food from biological environment though its' capacity is limited and bound to decrease. Now the main part of food materials is obtained from few plants and genetic improving of these plants is an important subject. Introduction of stable and adaptable varieties for every climate condition is one of the major strategies for overcoming food production limitation. In mutation breeding, the enhancement of the genetic variation is made through the influence of mutagens. Despite the advantages and limitations of this method, it has been applied for improving numerous improved cultivars, in different crops (Ando and Montalvan, 2001).

Mutation refers to the change in a DNA sequence, which may involve only few bases or the large-scale chromosome abnormality. It can be induced either spontaneously or artificially both in seed and vegetative propagated crops. Induced mutations have recently become the subject of biotechnology and molecular investigation leading to description of the structure and function of related genes. Induced mutations are highly effective in enhancing natural genetic resources and have been used in developing beneficial variations for practical plant breeding purpose and novel crop cultivars (Lee and lee, 2002). During the last seven decades, more than 2252 mutant varieties have been officially released

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in the world (Maluszynski, et al., 2000). Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanut and cow pea, which are seed propagated (Khan et al., 2009). In 1928, the Swedish geneticists Nilsson-Ehle and Gustaffsson experiments with X-rays and UV-irradiations using diploid barley species resulted in several valuable mutants with novel characters like: high-yielding, early maturity, lodging resistance and with changed ecological adaption (lundqvist, 2009). Early flowering and high yielding mutants in *Ocimum sanctum* line was induced by physical (gamma rays) and chemical mutagens: sodium azide (SA) and ethylmethane sulphonate (EMS) (Nasare and Choudhary, 2011). Since the early 20th century, barley (2n=2x=14, *Hordeum vulgare*) has been a model for investigating the effects of physical and chemical mutagens and for exploring the potential of mutation breeding in crop improvement. As a consequence, extensive and well characterized collections of morphological and developmental mutants have been assembled which is representing a valuable resource for exploring a wide range of complex and fundamental biological processes (Druka et al., 2011). Barley, the diploid and self-fertilizing crop with few but large chromosome and sufficient seeds from single plants is a novel plant for mutation experiments and researchers are working on barley mutation for more than 90 years.

Mutant plants are sometimes developed because of chromosome mutation which can take a number of forms: changes in whole set of chromosome and changes in the number of individual chromosome (Toole and Toole, 2004). Changes in chromosome number may involve even larger mutations, where segments of the DNA within chromosomes break and then rearrange.

In the present study, we examined the effects of different chemical and physical mutagens on seeds of barley species (cv. Nosrat) to find out the efficiency of different chemical and physical mutagens on growth and morphological traits such as germination percentage, emergence percentage, plant height, stem length, node number, inter node distance, flag leaf length, flag leaf width, number of spikelet per spike, spike length and awn length.

MATERIALS AND METHODS

Plant materials

This research was carried out in 2011 at Agriculture College of Shahid Bahonar University of Kerman-Iran. The seeds of barley were received from Agriculture Research Institute of Kerman, by Ravari.

Physical mutagens: Gamma ray with 200, 350, 700 and 1200; chemical mutagen: SA and EMS.

Mutagenesis experiments

1. For radiation purpose, 1.0 kg seed cylinder (12 cm diameter x 14 cm height) that is considered to maintain uniformity of purposed dosages was applied and each dosage three times from all angle were radiated. The radiation dosages were measured with a Fricke Dosimeter basis on (ASTM, 2007).
2. Presoaked seeds (12 h, in water) were treated with freshly prepared 0.5, 1 and 5 mM of sodium azide solution for 8 h at 25 ± 2°C. Immediately after the treatment, the seeds were washed thoroughly in running water to reduce the residual effect of the mutagen on the seed coat and were dried for next steps (Ando and Montalvan, 2001).
3. Presoaked seeds (12 h, in water) were treated with 0.1, 0.3 and 0.7% of EMS for 2 h at 25 ± 2°C. Immediately after the treatment, the seeds were washed thoroughly in running water to reduce the residual effect of the mutagen on the seed coat and were used for next steps of experiment (Kumar and Chauhan, 1980).

Greenhouse and field experiments

20 treated seeds were placed on three Whatman No. 1 filter papers in a disposable, 9 cm diameter petridish. 3 ml of distilled water was added to each Petri dish. Then, they were wrapped in parafilm foil to reduce water loss and were incubated in darkness at room temperature (approximately 25°C) for two weeks in a completely randomized design (CRD) with five replications. The germination rate (%) was measure for 24 h interval for one week. Then the fresh and dried weights of roots and shoots for seedlings in all petridishes were measured. Then the potentially mutant seeds were planted in a randomized completely block design (RCBD) with three repetition in the field in 2011 in the research field of Shahid Bahonar University of Kerman. The following traits such as emergence percentage (%), emergence speed, plant height (cm), stem length (cm), node number, inter node distance (cm), flag leaf length (cm), flag leaf width (cm), spike length (cm), no. of spikelet per spike and length (cm) were measure and then analyzed. Simple correlation was used for investigation of relationship between traits and their influence on each other. In this study phenotypic correlation coefficient for nine traits calculated. In order to group the traits and treatments, cluster analysis was used based on unweighted pair group method with arithmetic mean (UPGMA) method and with use of average linkage between groups in squared Euclidean distance.

Cytogenetic investigations

In order to chromosomes investigation, 20 seeds of different treatments were planted inside plate for root production. Root tips were pre-treated in cold water for 24 h and were fixed in 3:1 ethanol: acetic acid solution for 24 h then rinsed and transferred to 70% alcohol and stored in a refrigerator until they were used. For preparation, root tips were hydrolyzed in 1 N HCl for 6 min at 65°C in water bath and then stained in an acetocarmin solution (45% glacial acetic acid is melted and cooled till 50°C and then 1 g acetocarmin was added and this mix is melted for 10 min) for 1 h. Chromosome spreads were made by using the squash technique (Dille and King, 1983; Dille et al., 1986).

RESULTS

Morphologic traits

The correlation coefficients for pair traits (Table 1) including internode distance and stem length (r = 0.872), no. of spikelet per spike and internode

Table 1. Correlation coefficients among morphologic traits of barley M1.

Parameter	Plant height	Stem length	Node number per stem	Inter node distance	Flag leaf length	Flag leaf width	No. spikelet per spike	Spike length	length
Plant height	1								
Stem length	0.661*	1							
Node number	0.000	0.000	1						
Inter node distance	0.669*	0.872**	0.000	1					
Flag leaf length	-0.317	-0.062	0.000	0.060	1				
Flag leaf width	-0.660*	-0.623*	0.000	-0.424	0.633	1			
no. spikelet per spike	0.161	0.530	0.000	0.692*	0.597	0.264	1		
Spike length	0.025	0.229	0.000	0.400	0.642*	0.474	0.637*	1	
length	0.267	-0.292	0.000	-0.075	0.441	0.404	0.100	0.416	1

distance (r = 0.692) and internode distance and plant height (r = 0.669) were positively significant and this coefficient were negatively significant for flag leaf width and plant height (r = -0.660) and pair traits flag leaf width and stem length (r = -0.623).

The results of cluster analysis showed two main groups for investigated traits (Figure 1). Traits of node number, flag leaf length, seed per spike, spike length, flag leaf weight and internode distance formed group 1 and traits of stem length and plant height formed group 2.

Results of cluster analysis for all treatments showed three main groups (Figure 2). Treatments including SA (0.5 mM) and control for gamma ray treatments formed group 1, treatment of SA (1 mM) formed group 2, treatments of gamma ray 200 and 320 Gy, EMS (0.1 %), SA (5 mM) and control for SA and EMS formed group 3.

Variance analysis of traits

Variance analysis for traits: radicle length, plumule length, germination percent and germination rate showed that there were significant difference ($p > 0.05$ or $p > 0.01$) between treatments and there were significant differences ($p > 0.05$ or $p > 0.01$) between blocks for some traits (Table 2).

Result from Duncan multiple rang test showed that treatments of gamma ray control, that is, gamma ray 200 and 320 Gy had highest germination percentage and formed class a. The lowest germination percentage belonged to treatment of EMS (0.7%) which had a germination percentage less than 20% (Table 3). Mean comparison of radicle length trait showed that the highest radicle length belonged to control of gamma ray, 200 Gy and SA 0.5 mM and treatments of gamma ray 700 Gy, 1200 Gy and EMS 0.7% had lowest radicle length (Table 3). Mean comparison of plumule length trait showed that treatment of SA 1 mM had highest plumule length and lowest plumule length belonged to treatments of gamma ray 700 Gy, 1200 Gy and EMS 0.7% (Table 3). Mean comparison of germination rate trait showed that treatments of control of gamma ray, that is, gamma ray 320 and 2000 Gy had highest germination rate and the lowest rate was found in treatment EMS 0.7% (Table 3).

The mean comparisons basis on Duncan multiple rang test showed that gamma ray 0 Gy, that is, gamma ray 200 and 320 Gy, SA (0.5, 1 and 5 mM), EMS (control and 0.1%) had highest emergence percentage and formed class a. The lowest emergence percentage belonged to EMS (0.7%) which had an emergence percentage less than 20% (Figure 3). Mean comparison of emergence rate trait showed that gamma ray 0 and 200 Gy, SA (control, 1 and 5 mM) and control of EMS had highest emergence rate and lowest rate belonged to EMS 0.7% (Figure 4).

Cytology

Cytology studies have shown that chromosome number of barley plant had not change in induced plant than control though there was chromosome breakage in some induced barley seed by high dose of 700 and 1200 Gy (Figure 5).

DISSCUSION

It is known that various rays and chemical matters have positive or negative effects on living organisms. These effects can occur both spontaneously

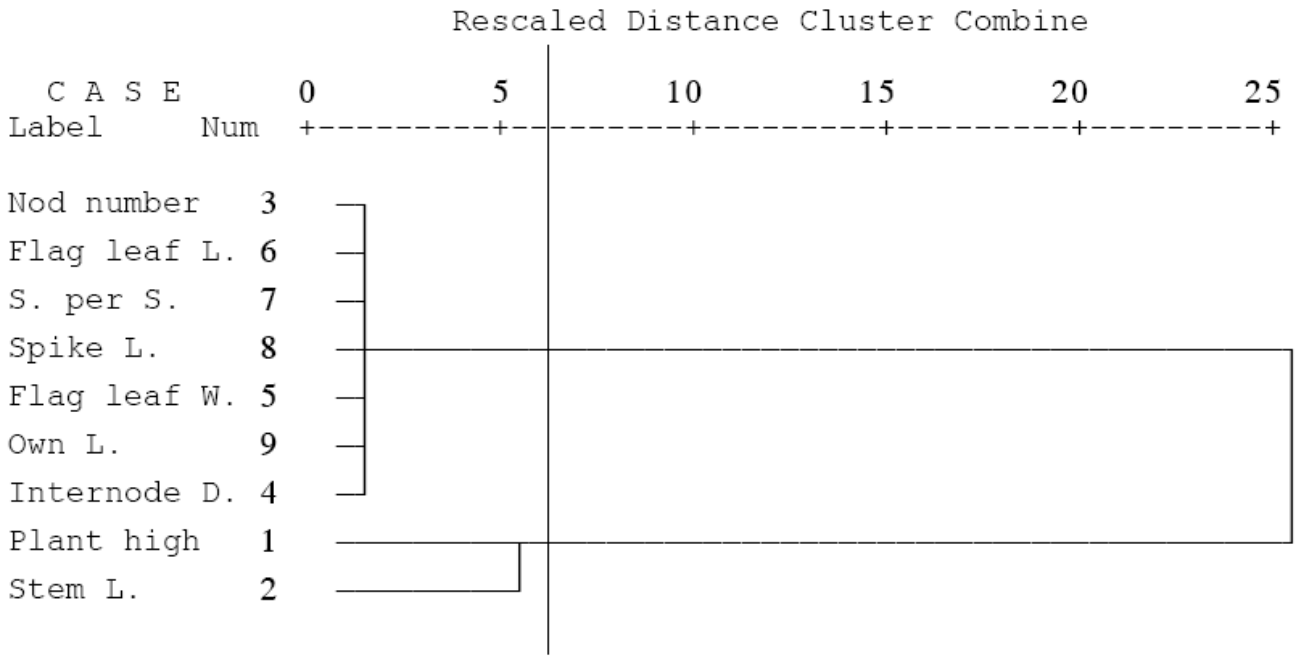

Figure 1. Dendrogram of cluster analysis based on nine agronomical traits by UPGMA method. UPGMA, Unweighted pair group method with arithmetic mean.

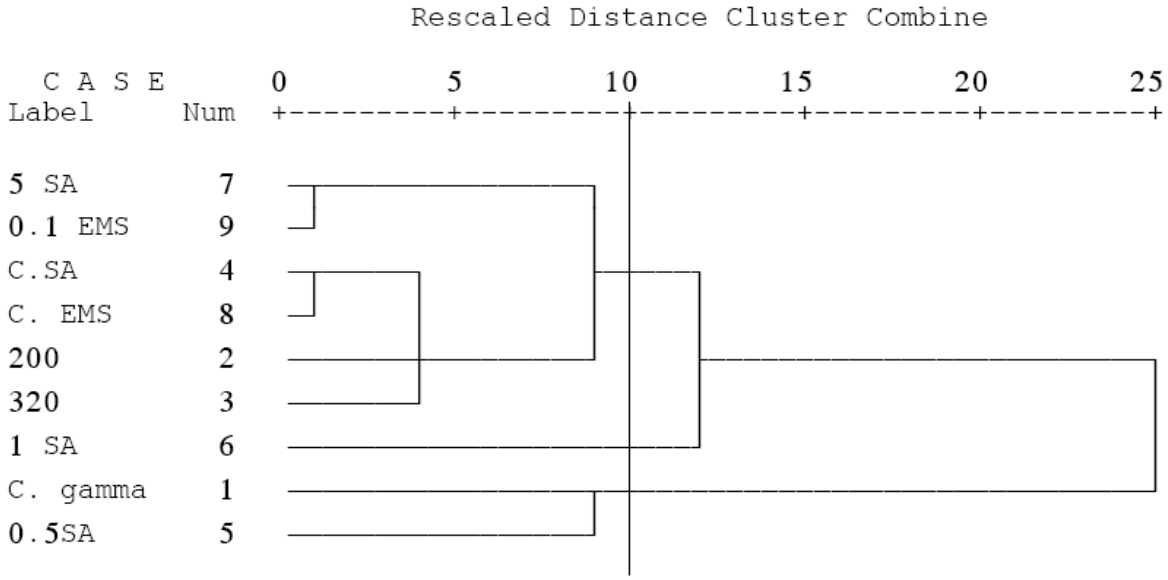

Figure 2. Dendrogram of cluster analysis for classify variables based on UPGMA method. UPGMA, Unweighted pair group method with arithmetic mean.

in nature and as artificially by mutagens. According to the results, increasing concentration of gamma ray (200, 320, 700 and 1200 Gy), SA solutions (0.5, 1.0 and 5.0 mM) and EMS solutions (0.1, 0.3 and 0.7 %) significantly decreased the radicle length, plumule length, germination percent and rate of barley seeds. These results are according to previous research.

The results of Sarduei-Nasab et al. (2010) showed that, high gamma ray doses decrease emergence index compared with control treatment, and also, radiation has inhibitory effect on stem height and width. Also, the results of Eroglu et al. (2007) showed that, mitotic index at embryonic root tip of barley seedling decreased with increasing doses of gamma radiation.

Table 2. Results of variance analysis for radicle length, plumule length, germination percent and germination rate traits in a randomized completely block design.

Source of variation	Mean squared			Germination percent	Germination rate
	Degree of freedom	Radicle length	Plumule length		
Block	3	6.189^{ns}	2.171^{ns}	0.041*	3.929**
Treatment	12	18.33*	23.789**	0.174**	36.078**
Error	36	6.724	7.937	0.013	0.730

ns, * and ** show insignificant and significant differences in p > 0.05 and p > 0.01 respectively.

Table 3. Mean comparisons, effect of three different mutagens on radicle length, plumule length, germination percent and germination rate of barley species (cv. Nosrat).

Treatment	Radicle length (cm)	Plumule length (cm)	Germination percent (%)	Germination rate (%)
Ray control	7.125^{a}	7.9375^{ab}	88.75^{a}	87.91^{a}
200 Gy	7.35^{a}	6.84^{abc}	87.5^{a}	87.27^{a}
320 Gy	5.3375^{ab}	4.8775^{abcd}	77. 5^{a}	77.52^{a}
700 Gy	1.4975^{bc}	2.06^{d}	61.25^{ab}	56.32^{b}
1200 Gy	1.235^{bc}	1.435^{d}	41.25^{bc}	37.85^{cd}
SA Control	6.1725^{a}	8.0725^{a}	52.5^{bc}	42.44^{c}
0.5 SA	5.145^{abc}	7.855^{ab}	51.25^{bc}	39.59^{cd}
1 SA	3.885^{abc}	3.905^{abcd}	48.75^{bc}	36.11^{cde}
5 SA	3.19^{abc}	2.9325^{cd}	41.25^{bc}	28.34^{de}
EMS Control	3.9425^{abc}	4.705^{abcd}	40^{c}	34.7^{de}
0.1 EMS	3.155^{abc}	3.355^{bcd}	36.25^{c}	28.45^{de}
0.3 EMS	3.075^{abc}	3.14^{cd}	35^{c}	25.45^{e}
0.7 EMS	0.8325 ^{c}	1.6275^{d}	16.25^{d}	6.32^{f}

EMS, Ethylmethane sulphonate; SA, sodium azide.

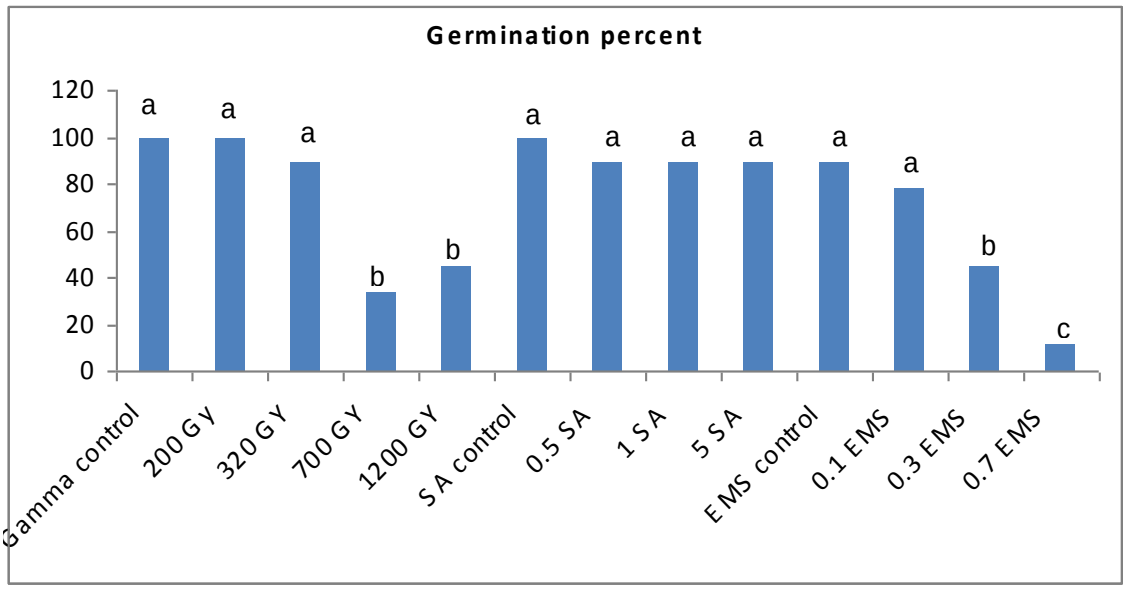

Figure 3. Mean comparisons of germination percent trait of treated barley species (cv. Nosrat) seed by chemical and physical mutagens based on Duncan multiple rang test.

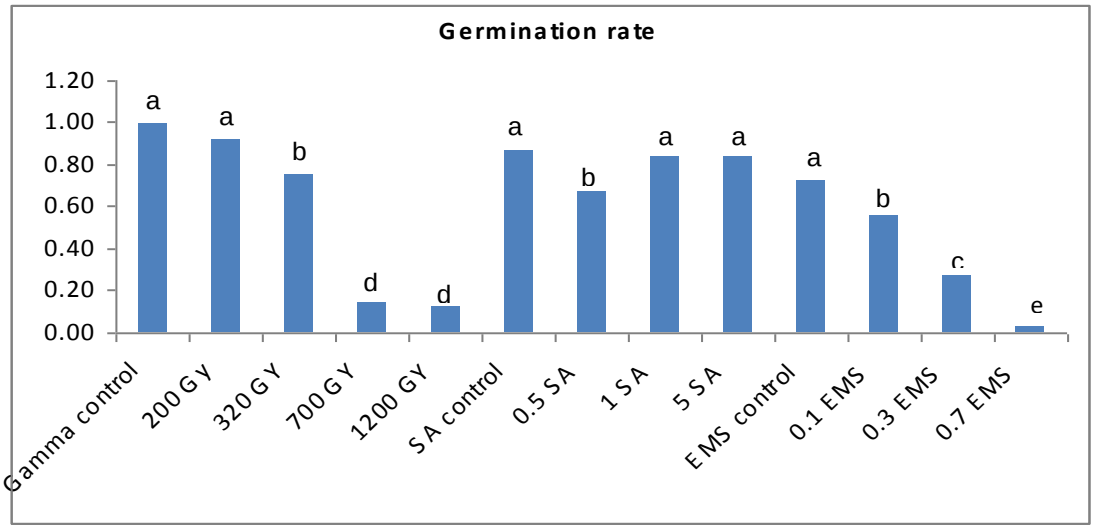

Figure 4. Mean comparisons of germination rate trait of treated barley species (cv: Nosrat) seed by chemical and physical mutagens basis on Duncan multiple rang test.

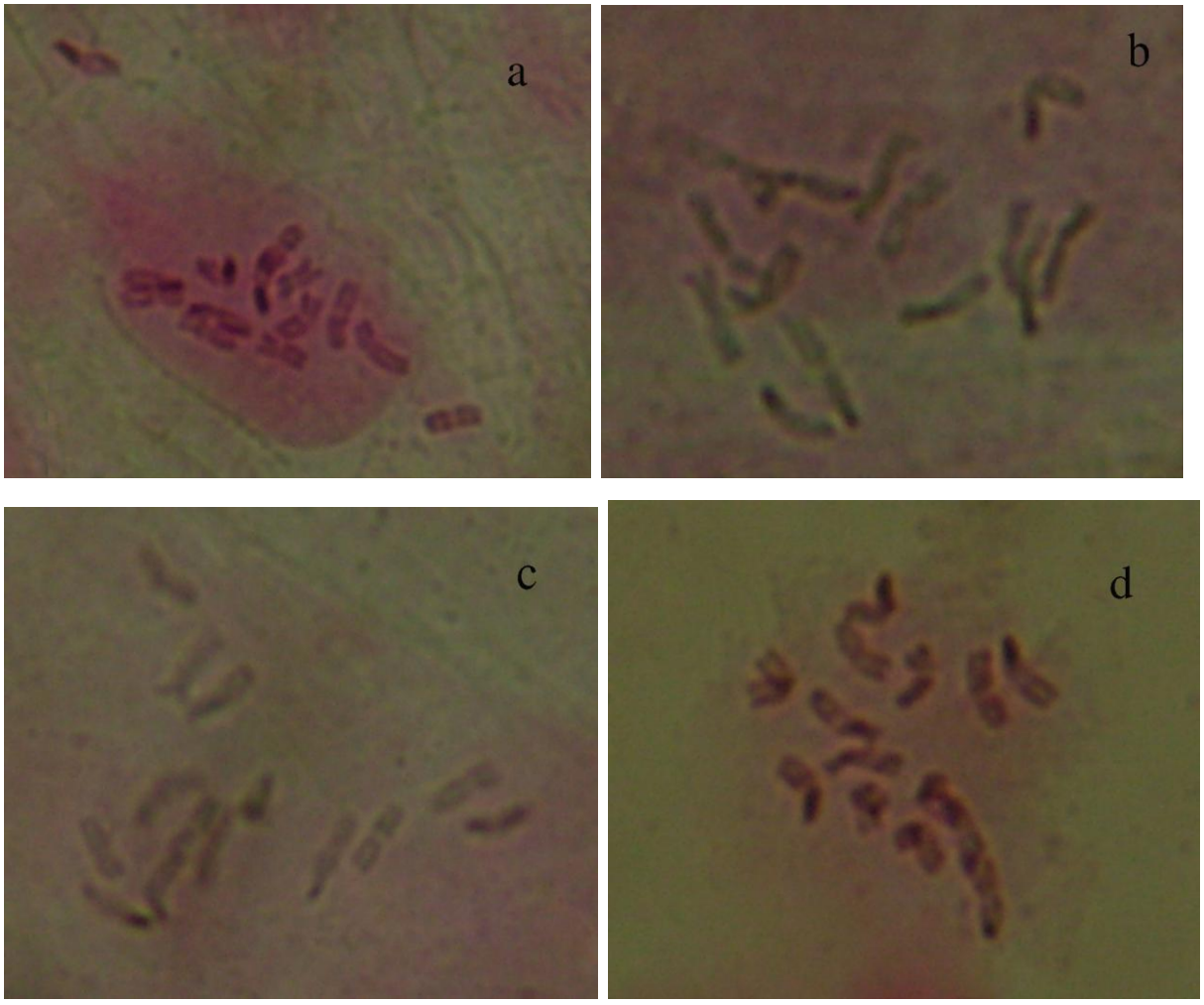

Figure 5. 14 chromosomes of barley species (cv. Nosrat). a, 200 Gy dose gamma ray; b, 700 Gy gamma ray; c, 5 SA (mM) SA; d, 0.7 EMS. EMS, Ethylmethane sulphonate; SA, sodium azide.

Ilbas et al. (2005) studied the morphological and cytogenetic effects of NaN_3 (SA) on barley seedling. Increasing concentrations of NaN_3 affected germination rates on days seven and 14 following application for 3 and 4 h. Length of the roots and leaves were affected by treatment with NaN_3 on day 14 of the germination period. Also, the mitotic index decreased compared to the untreated control.

Cytogenetic study shows that chromosome numbers of all induced seeds were not changed but in induced plants with high dose of mutagens including 700 and 1200 Gy of gamma ray there exist some chromosome breakages. The most recent study on gamma ray mutagen confirmed our result by induction of different chromosome aberration (Ballarini and Ottolenghi, 2003; Ballarini et al., 2002).

It should be noted that treatments including; 700 and 1200 GY of gamma ray and 0.7 EMS were fatal due to the fact that treated plants could not grow in green house and germinated in field conditions. The above results suggest that the mentioned doses of mutagens are not appropriate for breeding from mutagenesis. Based on the morphological and growth trait analysis, SA, EMS and gamma ray are appropriate mutagens but they are found in low doses as mentioned in the results of this study. However, there is need for more consideration of the doses of mutagens and for its trait to have the best breeding program by mutagenesis.

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