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Gamma irradiation affected phenotypic characteristics of almond (*Prunus amygdalus* L.)

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Abstract

'A200' and 'Sahand' are productive and late-blooming almond cultivars but their self-sterility traits make many problems in their pollination and fertilization. Therefore these cultivars selected to study for receiving to self-fertile with favorable characters mutants by means of gamma-radiation. This study was conducted to investigate the effects of different gamma irradiation period on some phenotypic characters of two almond cultivars including 'A200' and 'Sahand'. Experiment was carried out based on completely-randomized design in Sahand Horticultural Research station (Tabriz, Iran). Some shoots of the cultivars were irradiated 0, 5, 10, 30 and 150 minutes by 36 µC (Micro cowry) gamma-ray from Co^{60} and then the irradiated buds grafted on 'Azar' other almond cultivar seedlings. After four years from grafting time, some phenotypic characters such as growth vigor, branches angle (tree form), tree height, internodes distance, shoot and leaf density, shoot length, leaf color, petiole length, leaf width and length, bearing type (on spore or 1-year old shoots) were studied. Results showed that irradiation period had significant effects on growth vigor; internodes distance, shoot density, shoots length, leaf color, and bearing. Cultivars showed significant differences in internodes distance, tree height, shoot density, leaf length, leaf color, petiole length. Tree height, internodes distance, shoots density, shoot and leaf length as well as leaf color were significantly affected by interaction between cultivar and irradiation period. In addition, vigorous and wide angle branching by high green color were observed between plants of 'A200' cultivar which irradiated 150 minutes.

Keywords: Almond; Irradiation; Mutant; Phenotype

Abbreviations

LD - Leaf density; LL- Leaf length; LC - Leaf color; PL - Petiole length; TF - Tree form; TH - Tree height, SL - Shoot length; ID - Internodes distance; SD - Shoot density; DGIP - Different gamma irradiation periods

Introduction

Inducing mutation in plants with different methods such as irradiation and chemical substances has been used by many researchers in biotechnology and breeding programs.

Gamma irradiation has been used as an effective method, which can greatly induce high mutation numbers and modify physiological characteristics to create new mutants with improved properties (Ahloowalia and Maluszynski 2001, Bermejo et al 2011, Predieri, 2001).

Free radicals created by gamma irradiation can cause changes in organization, utility and metabolism of plant cells, which resultantly produce superior amounts of commercially useful changes and develop economically important varieties and cultivars (Kim et al., 2012; Wi et al., 2007). Many useful mutant varieties and cultivars were obtained by gamma irradiation at vegetative propagated plants (Ahloowalia and Maluszynski, 2001; Predieri, 2001).

Almond is one of the oldest crops in the world. Iran is ranked 4th, among almond-producing countries (www.fao.org). The most cultivars of almond are monogenic–gametophitic and self–incompatible and therefore have problems about pollination and fertilization. So obtaining self–fertile cultivars with favorable physiological and morphological traits would be very useful for breeders and producers. The induction of useful mutation as a mean for cultivar improvement has a special importance in breeding of fruit trees (Glenn et al 2008, Janick et al 1996, IAEA 2001, Britt 1996, Predieri 2001, Wi et al 2007).

In this context, irradiation has been applied by many researchers for different purposes including improvement of deciduous tree fruits such as dwarf growth habits and compact growth types in cherry and apple, self–compatibility in almond, resistance to diseases and pests, attractiveness and high quality of fruit (Janick et al, 1996, Lapins, 1965, 1971; Monostra, 1994). Monastra et al. (1994) irradiated the scions of 'Fascionello' cultivar of almond by 3 KR Gamma–ray from Co^{60} and achieved a new mutant cultivar 'Supernova' with late–flowering, high shelling percentage and self-fertility. A similar program carried out by gamma radiation on 'Ferragnes' cultivar and mutants with reduced vigor and very late-flowering were obtained (Le Gava and Garcia 1987, 1992).

Kim et al 2012 obtained three Citrus mutants by gamma irradiation with improved fruit quality and phenolic composition, such as total phenolics, total flavonoids, flavonoid distribution and D-limonene.

In addition, Sharafi and Motallebi-Azar (2011), studied gamma irradiation influences on some biological traits in two almond cultivars and observed incredibly different effects in both cultivars. They showed that long exposition periods of irradiation have significant positive effects on resistance to disease agent *Polystigma occharaceum*, mite, leaf area and flower density, but did not have significant effect on bearing type, resistance to aphids, blooming and leaf bud break dates in spring.

The most important almond breeding program in Iran is obtaining cultivars with the appropriate traits such as late-blooming, frost resistance, self-fertility, resistance to diseases and pests, high yielding and good growth habit. In this regard, to evaluate the effect of gamma irradiation on the phenotypic characteristics of almond this experiment was conducted and effects of 36 μC gamma–ray from co^{60} at different gamma irradiation periods (DGIP) on some parameters such as self-fertility and morphological characteristics of two almond productive and late–blooming cultivars such as 'A200', a Spanish cultivar and adapted to climate of northwest Iran and 'Sahand', an indigenous cultivar were studied.

Materials and methods

One-year old shoots of almond cultivars such as 'A200' and 'Sahand' were separated to 5 groups and irradiated by 36 μC gamma-ray from Co^{60} at 0 , 5, 10, 30, and 150 min at nuclear partition of Science Faculty, University of Tabriz (Iran) and then irradiated buds grafted on 'Azar' seedlings in Sahand Horticulture Research Station nursery at Azarshahr (Iran). After 1-year growth at nursery 267 irradiated trees translocated to main garden and planted based on completely randomized design in sandy-loam soil in 4 west-east rows. All of environmental conditions and horticultural practices (irrigation, nutrition and etc) for all genotypes were the same. After three years those trees had been passed juvenility period and bloomed were selected and parameters such as Leaf density (LD), leaf length (LL), leaf color (LC), petiole length (PL), tree form (TF) tree height (TH), shoot length (SL), internodes distance (ID) and shoot density (SD) noted for all genotypes according to almond descriptor. Tree form was determined based on main branches angle to weeping =9, dropping =7, spreading =5, uprigt=3 and extremely upright=1. Leaf density grouped to low=3, medium=5 and high=7 according to leaf number based on shoot length and shoot density grouped to very density=9, density=7, medium=5 and thin=3. Statistical analysis was carried out by SAS software and comparison of means carried out by Duncan's New Multiple Range Test.

Results and discussion

Analysis of variance showed that parameters such as leaf length, leaf color, tree height, shoot length and internodes distance were significantly affected by different gamma irradiation periods (DGIP), but LD and PL traits did not significantly affected by DGIP. In addition, significant difference was observed between two cultivars only for LL, LC, PL, TH, ID, and SD characters. The DGIP and cultivar interaction treatment were significant only for LL, LW, LC, TH, and SL traits (Table 1).

Table 1. Analysis of variance for studied phenotypic characteristics. Sources of variations (SOV), Different gamma irradiation periods (DGIP), Cultivar (CUL), Leaf density (LD), leaf length (LL), leaf color (LC), petioles length (PL).

SOV	Df	LD	LL	LW	LC	PL
DGIP	4	615.61ns	26.25**	4.03**	6.81**	0.77ns
CUL	1	339.49ns	152.95**	0.39ns	87.6**	7.27**
DGIP x CUL	4	600.14ns	11.79**	1.36**	1.97**	0.28ns
Error	257	1066.8	1.42	0.22	0.10	0.72

** and *: Significant at $p\leq0.01$ and $P\leq0.05$% levels, respectively; ns: Non significant.

Table 1. continued.

SOV	Df	TF	TH	SL	ID	SD
DGIP	4	3.58*	1.16**	1502.3**	0.64**	50.77*
CUL	1	1.05ns	5.42**	222.11ns	0.38*	43.86*
DGIP x CUL	4	1.15ns	0.35**	670.39**	0.19**	8.35*
Error	257	0.77	0.11	92.4	0.04	1.93

Comparisons of means indicated that LD, LL, LW, LC, PL, SL, ID and SD were increased by increasing GIP, so that the highest value of mentioned characteristics was observed in 150 minute. However DGIP hadn't significant effect on TF and TH in compared to that of the control (Table 2).

Table 2. Comparison of means for studied phenotypic characteristics. Different gamma irradiation period (DGIP), Leaf density (LD), leaf length (LL), leaf color (LC), petioles length (PL), Tree form (TF), tree height (TH), shoot length (SL), internodes distances (ID) and shoot density (SD).

GIP	LD	LL	LW	LC	PL	TF	TH	SL	ID	SD
Ctrl	4.2a	7.14b	2.56b	1.73b	1.68a	4.46ab	2.36ab	24.46bc	1.18b	5b
5 min	3.71a	5.91d	2.20c	1.4d	1.53a	4.82a	2.13c	20.44c	1.12b	3.75c
10 min	9.08a	6.43c	2.41c	1.56c	1.78a	4.83a	2.25bc	26.06b	1.17b	4.43b
30 min	4.63a	6.95c	2.58b	2.23a	1.59a	4.69ab	2.46a	34.34a	1.20b	4.91b
150 min	11.08a	7.58a	2.96a	2.08a	1.84a	4.27b	2.26bc	34.54a	1.4a	6.34a

Same letters show no difference among DGIP in each column.

Table 3. Comparison of means in cultivars for studied traits. Leaf density (LD), leaf length (LL), leaf color (LC), petioles length (PL), Tree form (TF), tree height (TH), shoot length (SL), internodes distances (ID) and shoot density (SD).

Cultivar	LD	LL	LW	LC	PL	TF	TH	SL	ID	SD
Sahand	8.96a	6.04b	2.51a	1.16b	1.48b	4.71a	2.48a	25.95a	1.2b	4.47b
A200	5.37a	7.73a	2.58a	2.48a	1.91a	4.57a	2.12b	27.07a	1.2a	5.36a

Same letters show no difference among cultivars in each column.

Significant differences were observed between cultivars in regard to some studied characters, which have been shown in Table 3. As it can been seen except for TH, other traits in 'A200' were higher than 'Sahand' cultivar.

Table 4. Comparison of means for interaction between different gamma irradiation periods (DGIP) and cultivars. Leaf density (LD), leaf length (LL), leaf color (LC), petioles length (PL), Tree form (TF), tree height (TH), shoot length (SL), internodes distances (ID) and shoot density (SD).

Cultivar x DGIP	LL	LW	LC	TH	SL	ID	SD
Sahand							
Control	6.03d	2.45cda	1f	2.46ab	16.83d	1.05d	4c
5 min	5.48e	2.25ef	1f	2.3b	21.96cd	1.11d	3.66c
10 min	6.1d	2.57bc	1f	2.57a	26.32bc	1.08d	4.48c
30 min	6.08d	2.52bcd	1.39d	2.58a	20.18d	1.16cd	4.15c
150 min	6.42cd	2.7b	1.97a	2.44ab	37.53a	1.45a	5.68b
A200							
Control	8.4b	2.68bc	2.57b	2.3b	29.55b	1.27bc	5.66b
5 min	6.75c	2.12f	2c	1.87c	18.16d	1.15cd	3.88c
10 min	6.75c	2.3dfe	1.95c	2.02c	25.88a	1.24bc	4.44c
30 min	6.92b	2.34bc	1.97a	2.39ab	29b	1.25bc	5.76b
150 min	9.38a	3.2a	3a	2.05c	31.25b	1.34ab	7a

Same letters show no difference among cultivar X Different gamma irradiation period (DGIP) in each column.

Comparison of means for DGIP and cultivar interaction indicated that the highest values of LL, LW, LC, ID, and SD were observed in 150 min irradiation in both cultivars. With increasing GIP, TH decreased in 'A200' but did not differ in 'Sahand' cultivar. However different GIP didn't affect SL in 'A200' but in 'Sahand' cultivar this parameter increased with increasing GIP (Table 4).

Studies that have been done on the effects of irradiation on plants have shown that Ionizing radiations with different periods and doses resulted to various effects in plants depend on species and cultivars (FAO/IAEA, 2009, IAEA 2001, Britt 1996).

In our experiment shoots of two almond cultivars 'A200' and 'Sahand' irradiated by 36 µC gamma–ray with different exposure times. Our results showed that increasing gamma radiation exposure time resulted in increase of some parameters such as leaf length, leaf weight, leaf color; internodes distance and shoot density in both cultivars. As our experiment, it was shown that cherry 'lambert' and 'compact lambert' mutated cultivars which irradiated by X-ray had 1/4–1/5 size of normal tree and bigger leaves (Janick et al 1996, Lapins, 1971). In addition, Legava and Garacia (1987, 1992) achieved dwarf mutant of 'Ferragnes' almond cultivar using gamma radiation similar to our results especially in 'A200'. Several reports have been made that show the role of gamma irradiation in inducing mutations in fruit trees such as apricot (by leaf shape changes), apple (by dwarf traits), fig, pistachio, pomegranate, almond and fijoa (by dwarf traits) (Glenn et al 2008, Janick et al 1996, Lapins, 1965, 1971 IAEA 2001, Britt 1996). In this regard, our study also showed that gamma irradiation affected some biological traits in two almond cultivars and were observed incredibly different effects in both cultivar (Sharafi and Motallebi-Azar, 2011).

Increasing gamma irradiation exposure time in our study resulted in decrease in tree height of 'A200', but had no effect on the parameter in 'Sahand'. Interestingly, shoot length of 'Sahand' was increased by increasing GIP but no difference observed compared to that of the controls in 'A200'. In addition, in our experiment leaf colure of 'A200' improved by increasing GIP, which had reverse effect on the parameter in 'Sahand'. In agreement with our findings such a result has been reported in 'F12/1' cherry cultivar which had mutants with darker heaves (Theater and Hedtrich, 1990).

According to our study Kim et al 2012 obtained three Citrus mutants by gamma irradiation of budstisticks in *Citrus unshiu* Marc cv Miyagawa with 120 Gy of cobalt (CO^{60}) with improved fruit quality and phenolic composition.

Our studies will continue on 267 gamma irradiated trees from 'A200' and 'Sahand' for obtaining the mutants with favorable traits for using in breeding and producing programs. It should be mentioned that, many genotypes (mutants) with late bloom, dwarf growing habit, pest and diseases tolerance have been found in 10 years study on the irradiated trees and will be introduced for breeding.

Conclusion

It was concluded that different gamma irradiation periods had significant effects on the studied parameters such as growth vigor, internodes distance, shoot density, shoot length, leaf color, and bearing. The cultivars were different regard to some the studied traits and interaction effects between cultivars and DIGP were significant on tree height, interned distance, shoot density, shoot and leaf length leaf color. However, positive effects were often observed in "A200" and negative ones observed more in the "Sahand" irradiated trees.

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References

Ahloowalia BS, Maluszynski M, 2001. Induced mutations-A new paradigm in plant breeding. Euphytica. 118: 167-73.

Bermejo A, Pardo J, Cano A, 2011. Influence of gamma irradiation on seedless citrus production pollen germination and fruit quality. Food Nutr Sci. 2: 169-80.

Britt, AB. 1996. DNA damage and repair in plants. Annual Review of Plant Physiology and Plant Molecular Biology, 47, 75–100.

FAO / IAEA, 2009. Induced Mutations in tropical fruit trees. Plant Breeding and Genetics Section, A-1400 Vienna, Austria, IAEA Vienna.

Glenn M , Wunsche J, McIvor I, Nissen B, George A, 2008. Ultraviolet radiation effects on fruit surface respiration and chlorophyll fluorescence. Journal of Horticultural Science & Biotechnology. 83(1): 43–50

IAEA, 2001. Improvement of Tropical and Subtropical Fruit Trees through Induced Mutations and Biotechnology. Vienna, Austria, 25-29 September. pp 1-89. -312.D2.RC.823.

Janik J, James N, Cummins S, Brown K, Hemmat M, 1996. Fruit Breed, Volume I: Tree and Tropical Fruits. John Wiley & Sons, Inc.

Kim MY, Soon Jm, Kim JH, Kim IJ, Lee HY, Lee DS, Lee YJ, Byun JH, Kim JH, Kim JY, Jeong SR, Kim JH, Moon SH, 20012. Changes in the phenolic composition of citrus fruits and leaves prepared by gamma irradiation of bud sticks. Life Science Journal. 9(3).1281- 1285.
Lapins KO, 1965. Compact mutants of apple by ionizing radiation, Can J plant Sci. 45(2): 117-124.
Lapins KO, 1971. Mutants of golden delicious apple induced by ionizing radiation. Can J Plant Sci. 5I: 123-131.
Lapins KO, 1971. Mutation frequencies in vegetative shoots derived from two zones of irradiated buds of sweet cheerry, *Prunus avium* L. Radiation Bot. 11(3): 199-200.
Legave JM, Garcia G, 1992. Premiers conclusions sure les effects mutagens afton ' irradiations de bourgeons du cultivar 'Ferragness' P. 85 – 88, 8 th colleque. Nimes. France. 1990.
Monastra F, Della SG, Fideghelli C, Ceuarta R, 1994. Comparison between two planting distances for three almond cultivars. I International Congress on Almond ISHS Acta hort. 373.
Predieri S, 2001. Mutation induction and tissue culture in improving fruits. Plant Cell Tiss Org. 64: 185-210.
Sharafi Y, Motallebi-Azar AR, 2011. Gamma irradiation influences on some biological traits in two almond (*Prunus amygdalus* L.,) cultivars. J Med Plant Res. 5(2): 255-258.
Theater C, Hedtrich R, 1990. Induction of dwarf f12/1 cherry rootstocks by in vitro mutagenesis. Acta Hort. 280: 367-374.
Wi SG, Chung BY, Kim JS, Kim JH, Baek MH, Lee JW, Kim YS, 2007. Effects of gamma irradiation on morphological changes and biological responses in plants. Micron. 38: 553-64.