Adv. Hort. Sci., 2011 25(4): 260-263

Towards *in vitro* selection studies for salinity tolerance in Canino apricot cultivar. Effect of gamma irradiation on *in vitro* mutation and selection for salt-tolerance

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Key words: IBA, BAP, M3 and MS3 medium, mutation gamma ray, propagation, salt tolerance.

Abstract: *In vitro* mutation method was used to obtain salt-tolerant clone in apricot. Small propagules of Canino apricot cultivar were irradiated with gamma ray at doses of 0, 10, 25, 35, 50, 75 and 100 Gy. After 30 days from treatment, both the radio sensitivity and post-irradiation recovery were assessed as the number of proliferated shoots per explants, fresh weight of cultures, shoot length and productivity of irradiated explants. A sudden and sharp decrease in the survival percentage occurred with the dose 75 Gy, while the highest dose (100 Gy) was lethal for all propagules. A marked decline in the number of regenerated shoots per explant and fresh weight of produced cultures was associated with an increase of irradiation doses. Doses in the range of 10-75 Gy, which preserved high survival percentage of irradiated explants, seemed to be more suitable for *in vitro* mutation in Canino apricot cultivar. Irradiated shoots were exposed to different concentrations of NaCl which were added to the multiplication medium at the rates of 25, 50, 75, 100, 125 mM and after 30 days, vigorous shoots were selected from salinity treatments. In conclusion, apricot tissues exposed to different doses of gamma irradiation in the range of 10-75 Gy, followed by culturing the plantlets produced in a medium containing additional salts (ranging from 25 to 125 mM) can be considered a good method to identify the most tolerant mutants to salts in apricot cultivars.

1. Introduction

Salinity is a widespread problem around the world, especially in arid and semi-arid regions. Each year more and more land becomes non-productive due to salt accumulation. At least 25% of currently cultivated land throughout the world suffers from excess salinity (Bohnert and Jensen, 1996) and all major crop species are intolerant to salt (Fairbairn *et al*., 2000). The most economic and sustained way to overcome the problem of salt-stress is to develop salt-tolerant varieties (Frommer *et al*., 1999). *In vitro* culture has been widely used for the propagation and conservation of crop genetic resources in both agriculture and horticulture crops (Barakat and El-Lakany, 1992).

Mutation breeding programs using gamma irradiation on apricot buds were carried out by several investigators (Legave and Garcia, 1988; Ageeva, 1989; Gulcan and Aksoy, 1995). Legave and Garcia (1988) reported that bud sticks of five apricot cultivars were exposed to up to 70 Gy gamma rays and scored for bud survival and growth. The effect of gamma irradiation in the range 10-70 Gy on variation in the characters of apricot was also investigated (Ageeva, 1989). The varieties reacted in different ways to treatment. Induced mutations in apricot breeding were also investigated (Gulcan and Aksoy, 1995). Apricot (*Prunus armeniaca*) was treated with 0.3 KR gamma irradiation (source 60Co). Mutagenesis affected vigour, dry matter and vitamin C content, and the level of carotene in fruit. *In vitro* cultures of Japanese plum (*Prunus salicina*) cv. Shiro were also gamma-irradiated by Predieri and Gatti (2000). *In vitro* culture may offer potential for quick evaluation of germplasm against salt stress (Cano *et al*., 1998). Recently, Jain (2001) reported that tissue culture generates a wide range of genetic variation in plant species which can be incorporated into plant breeding programs. The effect of NaCl and $CaCl_2$ in *Prunus cerasifera* was investigated by Lucchesini and Vitagliano (1993). The *in vitro* response of peach cv. Redhaven and of the peach/almond hybrid rootstock GF677 to increasing concentrations of NaCl in the medium was reported (Biricolti and Pucci, 1995). The response to increasing rates of NaCl or $CaCl_2$ and proline on 'Mr.S 2/5' (*Prunus cerasifera*) peach rootstock cultured *in vitro* has also been reported (Dimassi-Theriou, 1998).

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Received for publication 12 January 2011
Accepted for publication 12 October 2011

Recently, increased sodium chloride (NaCl) salinity effects on bitter almond (*Amygdalus communis*) (*Prunus dulcis*) growth, cell osmolarity and nutrient acquisition were studied *in vitro* (Shibli *et al*., 2003) and it was found that elevating salinity from 0.0 (control) to 50, 75, 100 mM NaCl resulted in reductions in shoot growth (shoot height, shoot dry weight) and rooting (rooting percentage, root number, root length).

The objective of the present work was to obtain salt-tolerant clone (s) in apricot using an *in vitro* mutation method.

2. Materials and Methods

The present work was carried out in the Biotechnology Laboratory, Crop Science Department, Faculty of Agriculture, Alexandria University from 2001 to 2005. Small propagules of Canino apricot cultivar initiated from *in vitro* culture by the follwing protocol: two explants shoot tips and single node cutting; of 0.5-1 cm in length was used. Shoot tips and single node were soaked in 100 mg/l ascorbic acid +150 mg/l citric acid for 30 min. Explants were dried for 30 min. before they were immersed in fungicide Ridomil (1 g/l solution) for 30 min. and then washed with distilled water, then soaked in Clorox (7% Sodium hypochlorite) for 7 min and washed with sterile distilled water three times. The explants were aseptically excised and placed in Jar containing 50-60 ml of culture medium. Each Jar contained one explant, considered as one replication. Cultures were incubated at 25±2°C under 16 hour's illumination (2000 lux, day light fluorescent tubes). In order to check the phenol oxidation and to establish the explants with free from phenol, the explants were cultured on two medium (MS and M_3,) supplemented with four PVP concentrations (0.0, 40.0, 80.0, 160.0 mg/l). The effect of five medium protocols was examined: modified woody plant medium (M_3) (Perez-Tornero *et al*., 2000), MS medium (MS_1) and MS medium (Murashige and Skoog, 1962) modified by reducing KNO_3 and NH_4NO_3 by 25%, 50% and 75% which were designated MS_2 , MS_3 and MS_4, respectively. All media were supplemented with 3.0% sucrose, 4 mg/l adenine sulfate, 160 mg/l PVP, 0.4 mg/l BAP and 0.01 mg/l IBA. The pH of the media was adjusted to 5.7 by using 1.0 N HCl or 1.0 N NaOH and agar was added after adjusting the pH. The best two media (M_3 and MS_4) for proliferation were used to test the optimum effect of three types of cytokinins benzyladenine (BAP), Kiniten and isopentenyladenine (2iP) and their concentration on shoot tip proliferation, four different concentration of cytokinin 0.2, 0.4, 0.6 and 0.8 mg/l for each type using M_3. Also, four different concentration of cytokinin 0.5, 1.0, 2.0 and 4.0 mg/l for each type using MS_4. The proliferation was evaluated six weeks after the beginning of the experiment and the number of shoots, (longer than 5 mm) per explant, their length and productivity (number of shoots x the average shoot length) were recorded. Shoots of apricot derived from the shoot tips multiplication were cultured on M_3 medium Perez-Tornero et *al*. (2000). The medium was supplement with either NAA (0.0, 0.5, 1.0, 2.0 mg/l) or IBA (0.0, 2.0, 4.0, 6.0 mg/l) were employed.

In vitro *mutation and selection for salt-tolerance*

Effect of gamma irradiation on in vitro *shoot culture*. Small propagules of Canino cultivar initiated from *in vitro* culture (Fig. 1) were irradiated in a gamma cell with a cobalt^{60} source at the Middle-East Regional Radioisotopes centre for Arab Countries, El-dokki, Giza with 10, 25, 35, 50, 75 and 100 Gy doses. The irradiated propagules were removed from the jar and recultured on a fresh proliferation medium. After six weeks incubation, impact of the irradiation was assessed by determinating the number of shoots, the fresh weight of shoot multiplication, shoot length and productivity.

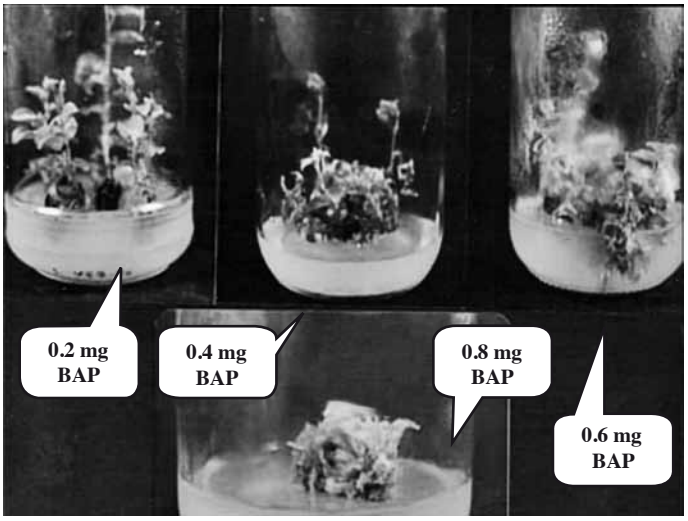

Fig. 1 - *In vitro* micro propagation of apricot cv. Canino on M_3 medium derived from shoot tip explants.

In vitro *selection*. Small propagules of the cultivar (Canino) (Fig. 1) were irradiated in a gamma cell with cobalt ^{60} source Gy at a dose of 10, 25, 35, 50 and 75 Gy. The propagules were subcultured five times before *in vitro* selection for salt-tolerance. Individual shoots from irradiated cultures were grown on the proliferation 0.6 mg/l BAP M_3 medium supplemented with different concentrations of NaCl (25, 50, 75, 100 and 125 mM). After six weeks of incubation, the vigorous shoots were selected and transferred to fresh medium free from salt. Analysis of variance with SAS software (SAS Institute, 1988) was carried out. Treatment means were compared using the LSD test at 5% level probability. Data were analyzed as a factorial arrangement in Randomized Complete block design according to Steel and Torrie (1980).

3. Results and Discussion

Effect of gamma irradiation on in vitro *apricot culture*

The basic requirement for effective use of mutation induction in plant breeding programs is the analysis of radio sensitivity of the explant material (Walther and Sauer,

1986). Predieri (2001) reported that one of the first steps in mutagenic treatment is the estimation of the most appropriate dose to apply. The aim of the present work was to determine the radio-sensitivity of *in vitro* apricot culture, as assessed by the number of regenerated shoots, the fresh weight of shoot multiplication, the shoot length and productivity in order to select the suitable dose of gamma irradiation to conduct *in vitro* mutation for improvement.

The collected data, reported in Table 1, indicate that a clear decrease in *in vitro* traits occurred with increasing irradiation dose. Complete lethality (100% death) was observed with an irradiation dose higher than 75 GY (Fig. 2). Several other studies have been conducted on the radio-sensitivity of *in vitro* cultures of fruits, such as *Prunus avium* (Walther and Sauer, 1985), kiwifruits (Shen *et al*., 1990), grapevine (Lima da Silva and Doazan, 1995; Charbaji and Nabulsi, 1999) and *Prunus salicina* (Predieri and Gatti, 2000). Previously, Laneri *et al*. (1990), working with *Gerbera jamesonii*, stated that in a mutation breeding experiment, the dose chosen for the main experiment should result in the highest survival of irradiated explants and that a low inhibition of the rate of production of new shoots gives the highest efficiency in recovering useful mutants. In light of these studies, the results obtained in the present investigation suggest that doses of 10 Gy to 75 Gy seem to be the most suitable for inducing mutation for apricot improvement.

Table 1 - Effect of gamma irradiation six weeks after the treatment on *in vitro* apricot shoot

Gamma irradiation (gy) doses	Weight	Number of shoots	Shoot length	Productivity
Control	5.85 a	23.40 a	6.15 a	147.36 a
10	3.58 b	14.30 b	2.99 b	42.27 b
25	3.45 b	13.80 b	3.14 b	42.52 b
35	2.40 bc	9.60 bc	3.63 b	41.68 b
50	1.50 cd	6.00 cd	0.77 c	11.05 c
75	0.68 d	2.70 d	0.48 c	4.18 c

Means within a column or a row followed by the same letter(s) are not significantly different at the 0.05 level of probability.

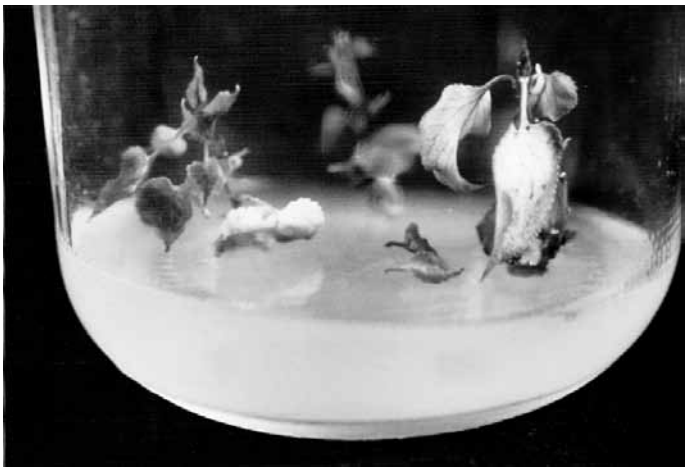

Fig. 2 - Effect of gamma irradiation 100 Gy, six weeks after the treatment on *in vitro* apricot shoot.

In vitro *mutation and selection for salt-tolerance in Canino apricot cultivar*

Mutation breeding can be employed as a promising technique that allows diversification of apricot. Induced mutations change only one or a few specific traits of an elite cultivar without undesired additional variations (Predieri, 2001). In fact Predieri concluded that the most suitable method may be mutation treatment and propagation of *in vitro* axillary shoots without passage through undifferentiated growth, and it can contribute to fruit improvements without upsetting the requirements of the fruit industry nor the consumers. Through *in vitro* selection, mutation with a useful agronomic trait, e.g. salt or drought tolerance or disease resistance, can be isolated in a short time (Jain, 2001).

The present work was conducted to obtain salt-tolerant clone(s) in apricot cv. Canino using *in vitro* shoot mutation. Small propagules were irradiated with 0, 10, 25, 35, 50, or 75 Gy and explants were multiplied for five subcultures. The generated irradiated shoots were subjected to a salt (NaCl) which was added to the medium with the concentrations 25, 50, 75, 100, or 125 mM. The number of vigorous shoots of cv. Canino showed marked differences in their *in vitro* salinity tolerance (Table 2). It is clear that the number of vigorous shoots decreased rapidly with increasing salinity. The highest number of vigorous shoots was obtained in medium supplemented with 25 mM selective agent of salinity when the propagules were exposed to 25 and 50 Gy, respectively (Table 2). From these results, it can be concluded that apricot cv. Canino tissues exposed to different doses of gamma irradiation in the range 10-75 Gy, followed by culturing in medium containing a higher concentration of additional salts (ranging from 25 to 100 mM) can be considered a good method to identify mutants in apricot cv. Canino which are the most tolerant to salts. FAO/IAEA (1997) reported that plant biotechnology in combination with mutation induction and conventional breeding might open new frontiers for obtaining salt-tolerance rice varieties. The application of mutation techniques in breeding has increased constantly over the past years. These techniques must be rapid to keep pace with the large quantity of breeding materials generated after mutagenesis. Screening under field conditions is difficult due

Table 2 - Effect of irradiation doses on the number of vigorous shoots of apricot cv. Canino, after six weeks from culturing in media containing different salt concentrations

Salt concentration mM	Irradiation dose (Gy)					
	0	10	25	50	75	Total
25	0	2	5	4	3	14
50	0	2	3	1	2	8
75	0	0	0	2	2	4
100	0	0	0	1	2	3
125	0	0	0	0	0	0
Total	0	4	8	8	9	29

to stress heterogeneity, presence of salt-related stress and the significant influence of environmental factors such as temperature, relative humidity and solar radiation. Genetic modification of crop plants to improve their salt-tolerance is a possible way of increasing production, especially for regions of the world where arable lands must be extended to marginal areas, and sometimes irrigated with saline water (Dorion *et al*., 1999).

References

AGEEVA N.G., 1989 - *Effect of gamma irradiation on variation in the characters of apricot*. - Bulletin' Gosudarstvennogo Nikitskogo Botanicheskogo, 68: 67-70.

BARAKAT M.N., EL-LAKANY M.H., 1992 - *Clonal propagation of* Acacia saligna *by shoot tip culture*. - Euphytica, 59: 103-107.

BIRICOLTI S., PUCCI S., 1995 - *Effects of increasing NaCl rates on 'Redhaven' peach and 'GF677' rootstock cultured* in vitro. - Adv. Hort. Sci., 9(2): 75-78.

BOHNERT H.J., JENSEN R.G., 1996 - *Metabolic engineering for increased salt tolerance. The next step*. - Australian Journal of Plant Physiology, 23: 661-667.

CANO E.A., PEREZ-ALFOCEA F., MORENO V., CARO M., BOLARIN M.C., 1998 - *Evaluation of salt tolerance in cultivated and wild tomato species through* in vitro *shoot apex culture*. - Plant Cell and Organ Culture, 53: 19-26.

CHARBAJI T., NABULSI I., 1999 - *Effect of low doses of gamma irradiation on* in vitro *growth of grapevine*. - Plant Cell, Tissue and Organ Culture, 57: 129-132.

DIMASSI-THERIOU K., 1998 - *Response of increasing rates of NaCl or $CaCl_2$ and proline on "Mr. 2/5" (*Prunus cerasifera*) peach rootstock cultured* in vitro. - Adv. Hort. Sci., 12(4): 169-174.

DORION N., WIES N., BURTEAUX A., BIGOT C., 1999 - *Protoplast and leaf explants culture of* Lycopersicon cheesmanii *and salt to lerance of protoplast-derived calli*. - Plant Cell, Tissue and Organ Culture, 56: 9-16.

FAIRBAIRN D.J., LIU W., SCHACHTMAN D.P., GOMEZ-GALLEGO S., DAY S., TEASDALE R.D., 2000 - *Characterization of two distinct HKT1- like potassium transporters from* Eucalyptus camaldulensis. - Plant Molecular Biology, 43: 515-525.

FAO/IAEA, 1997 - *Develop low cost tissue culture techniques for mutation induction*. - Plant Breeding Unit.

FROMMER W.B., LUDEWIG U., RENTSCH D., 1999 - *Taking transgenic plants with a pinch of salt*. - Science, 285: 1222-1223.

GULCAN R., AKSOY U., 1995 - *Utilization of induced mutations in apricot breeding has been investigated*. - Acta Horticulturae, 384: 231-236.

JAIN S.M., 2001 - *Tissue culture-derived variation in crop improvement*. - Euphytica, 118: 153-166.

LANERI U., FRANCONI R., ALTAVISTA P., 1990 - *Somatic mutagenesis of* Gerbera jamersonii *hybrid: irradiation and* in vitro *cultures*. - Acta Horticulturae, 280: 395-402.

LEGAVE J.M., GARCIA G., 1988 - *Radio sensitivity of apricot bud sticks exposed to strong gamma rays and nursery observations on the second vegetative generation from irradiated buds*. - Agronomy, 8(1): 55-59.

LIMA DA SILVA A., DOAZAN J.P., 1995 - *Gamma ray-mutagenesis on grapevine rootstocks cultivated* in vitro. - J. Int. Sci. de la Vigne et du Vin, 29: 1-9.

LUCCHESINI M., VITAGLIANO C., 1993 - *Effect of NaCl and $CaCl_2$ in* Prunus cerasifera *tissue culture*. - Acta Horticulturae, 336: 109-113.

MURASHIGE T., SKOOG F., 1962 - *A revised medium for rapid growth and bioassays with tobacco cultures*. - Physiol. Plant., 15: 473-497.

PEREZ-TORNERO O., LOPEZ J.M., EGEA J., BURGOS L., 2000 - *Effect of basal media and growth regulators on the* in vitro *propagation of apricot (*Prunus armenica *L.) cv. Canino*. - Journal of Horticultural Science & Biotechnology, 75(3): 283-286.

PREDIERI S., 2001 - *Mutation induction and tissue culture in improving fruits*. - Plant Cell, Tissue and Organ Culture, 64: 185-210.

PREDIERI S., GATTI E., 2000 - *Effects of gamma radiation on plum (*Prunus salicina *Lindl*.) 'Shiro'. - Adv. Hort. Sci., 14: 215-223.

PREDIERI S., GATTI E., 2000 - *Effects of gamma radiation on micro cuttings of plum (*Prunus salicina *Lindl.) 'Shiro'*. - Istituto di Ecofisiologia delle Piante Arboree da Frutto, CNR, 14(1): 7-11.

SHEN X.S., WAN J.Z., LUO W.Y., DING X.L., 1990 - *Preliminary results of using* in vitro *axillary and adventitious buds in mutation breeding of Chinese gooseberry*. - Euphytica, 49: 77-82.

SHIBLI R.A., SHATNAWI M.A., SWAIDAT I.Q., 2003 - *Growth, osmotic adjustment, and nutrient acquisition of bitter almond under induced sodium chloride salinity* in vitro. - Communications in soil science and plant analysis, 34(13/14): 1969-1979.

STEEL R.G.D. - TORRIE J.H., 1980 - *Principles and procedures of statistics: A biomaterical approach*. - McGrow Hill Book Co. Inc., New York, USA, pp. 196-201.

WALTHER F., SAUER A., 1985 - *Analysis of radio-sensitivity - a basic requirement for* in vitro *somatic mutagenesis*. I. Prunus avium *L*. - Acta Horticulture, 169: 97-104.

WALTHER F., SAUER A., 1986 - *Analysis of radio-sensitivity: A basic requirement for* in vitro *somatic mutagenesis. II*. Gerbera jamesonii. - Proc. Int. Symp. Nuclear Techniques and *in vitro* Culture for Plant Improvement. IAEA/FAO, Vienna, pp. 155-159.