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RESPONSE OF BITTER AND SWEET ALMOND TO SODIUM AZIDE AS CHEMICAL MUTAGENE

Gehan Y.R.*; M.D. El-Deeb[1] and I. Maksoud Ibrahim[2]

1. Dept. Plant Prod., Fac. Environ. Agric. Sci., Suez Canal Univ., Egypt.

2. Inst. Genetic Eng. and Biotechnol., Sadat City, Monofia Univ., Egypt.

ABSTRACT

Embryos, Shoot tips and one node cuttings from bitter and sweet almond are excised; sterilized, and cultured on Murashige & Skoog (MS) 1962 medium. Sodium azide for both proliferated bitter and sweet almond rootstocks was used. The obtained results showed that sodium azide induced the best results for mutation. Using embryo was the best explants in enhancing plant length at 2.5mM for 60 min of sweet almond in second sub culture. Also the best values of leaves number, shoot number, shoot length and leave per shoot at 2 mM for 60 min and the minimum value of stomata number was in 2mM for 60 min with shoot. The lowest leaf area was found in 1mM for 60 min with embryo.

Key words: Sodium azide, Chemical mutagene, bitter and sweet almond, *in vitro* stomata number.

INTRODUCTION

Peach is sensitive to drought stress, as it can't be cultivated under new reclaimed areas conditions. So, bitter and sweet almond can be used as rootstocks for peach cultivation under these areas. The peach (*Prunus persica* L.) is known as a species of *Prunus* native to China that bears an edible juicy fruit also called a peach. It is classified with the almond in the subgenus Amygdalus within the genus *Prunus*, Micropropagation offers the potential for mass production of own-rooted Peach, which may be useful as rootstock for virus indexing, and can accelerate screening for disease resistance. Shoot tips and one-node cuttings are the usual explants.

The large–scale commercial micropropagation of virus-free plants of the Peach rootstocks ´Istara´, ´GF677, ´Penta´, ´Tetra´, ´MrS´ ´Fire Cadman´, ´Barrierl´ Gensia´ and ´Julior´ has been reported (**Battistini and De Paoli 2002).** Mutation is a sudden heritable change in the genetic material at the gene or chromosome level. Such a change in the base sequence of DNA leads to a plant with altered characters called as mutant, the term coined by the Dutch botanist, Hugo de vries. The mutations have been so important in evolution that at one time these were considered to be the chief source of origin of new species.

It is now well established that ultimate source of new genes is the mutation which coupled with hybridization leads to creation of new genetic variation that is essential for the improvement as well as evolution of crop plants. Mutations may occur spontaneously or can be induced artificially. The tissue culture mutagenesis has following distinct advantages:

1- Many environmental (modifying) factors can be controlled to a greater extent

2-Using single cells/ protoplasts, the chance of diplomatic selection get minimized.

3-There are less chance of chimera formation if the regenerated plants have single cell origin. 4-Mutation frequency is generally high become each cell is indirect contact with the mutagen.

* **Corresponding author: Tel.:** +201007417921
E-mail address: gyousre@yahoo.com

5- *In vitro* selection for various biotic and a biotic factors can be effectively applied by plating mutagen treated cells on medium containing stress factors.

6- Millions of the cells (potential plants) can be screened in the single petri dish.

7- Use of haploid cell population obtained from anther/ pollen culture facilitates the detection and fixation of even recessive mutations. **(Chahal and Gosal, 2002).**

MATERIALS AND METHODS

Plant Material

This study was carried out in Plant Tissue Culture Laboratory in Faculty of Environmental Agricultural Sciences (FEAS), El-Arish, North Sinai, Suez Canal University (SCU) during the period from 2009 to 2015. Shoot tips and one–node cuttings were collected from mother plants (1-3 years old) during the active growth period (from March to July) in Rafah region, North Sinai. Also, mature seeds were collected from the same region of bitter and sweet almond (*Prunus amygdalus*).

Explants Sterilization

Shoot tips, one–node cuttings and mature seeds were rinsed under running tap water with soap for 60 minutes to remove all the remaining detergent then washed with sterilized distilled water. The explants were soaked for 20 min in 20% commercial bleach of Clorox solution (5.25% NaOCl), then washed 3-4 times with sterilized distilled water to remove all traces of the disinfections. While, embryo left in water for 24 hour.

All sterilization steps had been done under aseptic conditions inside the culture cabinet (Laminar air flow hood) using sterilized instruments. The experiments were carried out as follows:

Chemical Mutagens

Shoots of Peach rootstocks were cultured on medium supplemented with different concentrations of sodium azide NaN_3 (0.00, 1.00, 1.50, 2.00, 2.50 mM) are applied to embryos and shoots for 30 and 60 minutes stimulated to obtain the most effective concentration which induces mutations.

Finger Prints using ISSR Marker

DNA Isolation Procedure

RESULTS AND DISCCUSIONS

Chemical Mutagens

Effect of Sodium Azide Concentrations

The effect of sodium azide concentration and duration on the survival of explants was assessed 30 days after treatment, control shoots (not treated with sodium azide) multiplied rapidly in subculture 1, producing more shoots during a 4 weeks subculture the highest value of survival was appeared after 30 min with embryos of bitter and sweet almond.

Also the results were affected by sodium azide concentration and duration as appeared in Table (2-a). The survival rates of shoots were affected by the concentration of sodium azid and the duration of treatment. The mortality with sodium azide low than colchicines. The survival rate increased for embryos of sweet almond with increasing the concentration and duration. While, shoots and embryos of bitter almond and shoot of sweet were decreased. Data in Table (2-b) reflect that embryo was significantly superior than either shoot in plant length, leaves number, leaf area, greening and reducing necrosis, while shoot tip increased both shoot number, leave shoot and stomata number parameters. Moreover, Table (2-c) shows that significany disappear between duration 30 and 60 min in improving all studied parameters under study i.e. plant length, leaves number, shoot number, leaf area and greening except leave shoot, stomata number and necrosis. Table (2-d) clarifies that using different concentrations of sodium azide showed more or less significant

Table (1): List of the primer names and their nucleotide sequences used in the study for ISSR procedure.

No.	Name	Sequence	No.	Name	Sequence
1	14A	5 CTC TCT CTC TCT CTC TTG 3`	4	HB-10	5´ GAG AGA GAG AGA CC 3`
2	44B	5` CTC TCT CTC TCT CTC TGC 3`	5	HB-11	5´ GTG TGT GTG TGT TGT CC 3`
3	HB-09	5` GTG TGT GTG TGT GC 3`	6	HB15	5´ GTG GTG GTG GC 3`

Table (2): Effect of different concentrations and durations of sodium azide on the survival rate of embryos and shoots tips explants of bitter and sweet almond rootstocks

Table (2-a): Effect of survival rate.

Root stock		Bitter almond		Sweet almond	
Explants		Embryos	Shoot tips	Embryos	Shoot tips
Duration	Concentration (mM)				
30	Control	100^{a}	100^{a}	100^{a}	100^{a}
	1	100^{a}	33.3^{i}	66.7^{e}	100^{a}
	1.5	100^{a}	33.3^{i}	66.7^{e}	100^{a}
	2	80^{c}	40^{h}	100^{a}	71.4^{d}
	2.5	60^{g}	33.3^{i}	33.3^{i}	100^{a}
60	1	60^{g}	33.3^{i}	100^{a}	83.3^{b}
	1.5	80^{c}	60^{g}	100^{a}	40^{h}
	2	66.7^{e}	40^{h}	66.7^{e}	62.5^{f}
	2.5	60^{g}	40^{h}	100^{a}	66.7^{e}

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table (2-b): Effect of explants.

Explants	Plant length	Leaves number	Shoot number	Leave shoot	Stomata No.	Leaf area	Greening	Necrosis
Embryo	3.057^{a}	14.967^{a}	0.133^{b}	0.667^{b}	14.124^{b}	0.404^{a}	3.967^{a}	0.533^{b}
shoot	2.279^{b}	12.414^{b}	0.448^{a}	2.414^{a}	18.839^{a}	0.413^{a}	4.276^{a}	0.724^{a}

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table (2-c): Effect of duration of sodium azide.

Duration	Plant length	Leaves number	Shoot number	Leave shoot	Stomata No.	Leaf area	Greening	Necrosis
30	2.633[a]	14.167[a]	0.233[a]	1.233[b]	18.022[a]	0.479[a]	3.967[a]	0.533[b]
60	2.717[a]	13.241[a]	0.344[a]	1.828[a]	14.806[b]	0.335[a]	4.276[a]	0.724[a]

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table (2-d): Effect of concentrations of sodium azide.

Conc.	Plant length	Leaves No.	Shoot No.	Leave shoot	Stomata No.	Leaf area	Greening	Necroses
Control	3.500[a]	17.250[a]	0.417[a]	2.750[a]	21.945[a]	0.499[a]	4.583[a]	0.417[a]
1 mM	2.600[ab]	12.833[b]	0.333[a]	1.417[a]	19.638[ab]	0.431[a]	4.417[a]	0.583[a]
1.5 mM	2.467[ab]	14.667[a]	0.250[a]	1.333[a]	14.305[bc]	0.286[b]	4.000[a]	1.000[a]
2 mM	2.675[ab]	11.750[b]	0.167[a]	0.500[b]	13.958[bc]	0.410[a]	3.917[a]	0.667[a]
2.5 mM	2.082[b]	11.909[b]	0.273[a]	1.636[a]	11.989[c]	0.419[a]	3.636[a]	0.454[a]

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table (2-e): Effect of the interaction between different concentrations and durations of sodium azide on embryos and shoots for bitter almond rootstock.

Explante	Duration	Conc.	Plant length (cm)	Leaf No.	Shoot No.	Leaf shoot	Stomata No.	Leaf area (cm^3)	Greening	Necroses
Embryo	30	Control	3.533[a]	14.667[abc]	0.000[b]	0.000[c]	20.503[a-d]	0.590[a]	4.667[a]	0.333[a]
		1	3.533[a]	17.333[abc]	0.333[a]	1.333[a]	24.777[ab]	0.513[a]	4.667[a]	0.333[a]
		1.5	3.300[a]	23.333[a]	0.000[b]	0.000[c]	15.610[a-f]	0.447[a]	3.667[ab]	1.333[a]
		2	3.300[a]	12.333[abc]	0.000[b]	0.000[c]	4.333[ef]	0.630[a]	3.333[ab]	0.000[b]
		2.5	0.667[b]	4.333[c]	0.000[b]	0.000[c]	3.890[f]	0.220[a]	1.667[c]	0.000[b]
	60	1	3.100[a]	13.333[abc]	0.000[b]	0.000[c]	19.833[a-d]	0.340[a]	4.000[ab]	1.000[a]
		1.5	3.067[a]	15.333[abc]	0.333[a]	2.000[ab]	12.890[a-f]	0.233[a]	3.667[ab]	1.333[a]
		2	4.000[a]	18.667[ab]	0.333[a]	1.333[ab]	6.943[def]	0.237[a]	5.000[a]	0.000[b]
		2.5	2.833[ab]	15.333[abc]	0.333[a]	2.000[ab]	13.847[a-f]	0.523[a]	4.333[a]	0.667[a]
Shoot	30	Control	3.600[a]	20.667[ab]	0.667[a]	4.667[ab]	25.943[a]	0.620[a]	4.333[a]	0.667[a]
		1	2.033[ab]	13.323[abc]	0.667[a]	2.000[ab]	22.110[abc]	0.533[a]	5.000[a]	0.000[b]
		1.5	1.867[ab]	10.667[abc]	0.333[a]	2.333[ab]	20.277[a-d]	0.350[a]	4.000[ab]	1.000[a]
		2	1.733[ab]	7.333[bc]	0.000[b]	0.000[c]	23.723[ab]	0.323[a]	3.667[ab]	1.333[a]
		2.5	2.767[ab]	17.667[ab]	0.333[a]	2.000[ab]	19.057[a-e]	0.570[a]	4.667[a]	0.333[a]
	60	1	1.733[ab]	7.333[bc]	0.333[a]	2.333[ab]	11.833[a-f]	0.337[a]	4.000[ab]	1.000[a]
		1.5	1.6333[ab]	9.333[bc]	0.333[a]	1.000[b]	8.443[c-f]	0.113[b]	4.667[a]	0.333[a]
		2	1.667[b]	8.667[bc]	0.333[a]	0.667[b]	20.833[a-d]	0.450[a]	3.667[ab]	1.333[a]
		2.5	2.050[ab]	9.500[bc]	0.500[a]	3.000[ab]	10.750[b-f]	0.335[a]	4.000[ab]	1.000[a]

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Photo (1): Effect of the interaction between different concentrations and durations of sodium azid on embryos and shoots for bitter almond rootstock.

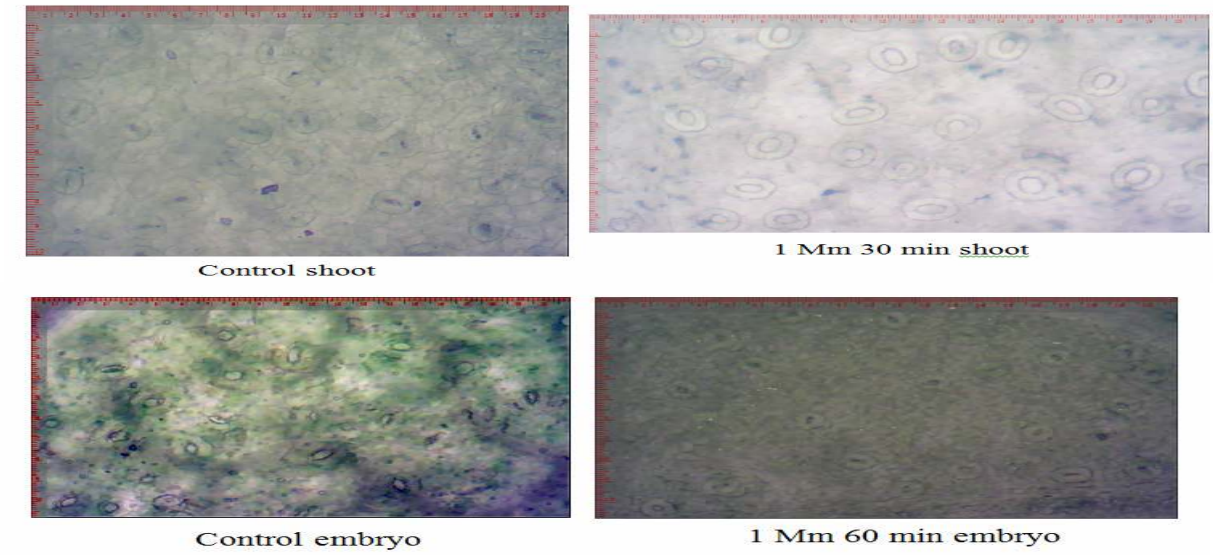

Photo (2): Effect of the interaction between different concentrations and durations of sodium azide on embryos and shoots for bitter almond rootstock.

differences with different parameters under study, i.e. plant length, leaves number, leave shoot, stomata number, leaf area while significancy among different concentrations of sodium azide were disappeared when shoot number, greening and necrosis parameters were considered. However Table (2-e) reflected the interaction of explants type, duration and concentration of sodium azide, it is appear that different combinations had more or less significant differences with different parameters under study.

Data of Table (3-a) reflect that embryo was significantly superior than either shoot tip in leaves number, shoot number and leave shoot. However, shoot tip was significantly surpassed embryo explants when reducing necrosis and leaf area were concerned. Moreover, Table (3-b) shows

that duration 60 min was significantly superior than 30 min in roving plant length, leaves number, shoot number and reducing necrosis while 30 min was significantly increased stomata number. The results indicated that significance was lacked between duration 30 and 60 min in leave shoot, leaf area and greening. it is noticed from Table (3-C) that the best leaves number, shoot number, leave shoot and stomata number at 2.5 mM concentration (21.083, 0.917, 3.583 and 19.138 respectively) of sweet almond for sodium azide. While, the greatest plant length (3.867 cm) was found in 2 mM. Culturing of shoot tip and embryo on MS medium control was significantly superior than the other media in plant length, leaves number and stomata number. While, shoot number, leave shoot, leaf area, greening and necrosis showed non-significant. It is clear from Table (3-d) that embryo was the best explants combined with duration 60 min and with 2 mM for plant length (5.33) leaf number (30.33) while the best shoot numbers and leaves/ shoot at 2.5 mM concentration (1.667-7.33) of sweet almond for sodium azid in subculture1 for stomata number, The lowest value was 8.333 which recorded in 2mM at 30 min with shoots. Also the leaf area recorded the lowest valued was (0.350) in 1mM at 30 min with embryos. In contrast in Egypt, sweet almond were found to have a stomata frequency and size lower than that of bitter almonds **(Guirguis *et al*., 1995).**

Table (3-a): Effect of explant, duration and concentrations of sodium azide on explant development of for sweet almond rootstock.

Explants	Plant length	Leaves Number	Shoot number	Leave shoot	Stomata No.	Leaf area	Greening	Necroses
Embryo	3.535[a]	23.400[a]	0.900[a]	3.163[a]	15.572[a]	0.527[b]	4.733[a]	0.267[b]
shoot	3.327[a]	14.533[b]	0.233[b]	1.050[b]	15.205[a]	1.123[a]	4.533[a]	0.467[a]

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table (3-b): Effect of different Duration of Sodium azide on embryo and shoot for sweet almond rootstock.

Duration	Plant length	Leaves No.	Shoot No.	Leave shoot	Stomata No.	Leaf area	Greening	Necroses
30	3.203[b]	17.667[b]	0.533[b]	2.083[a]	16.027[a]	0.831[a]	4.533[a]	0.467[a]
60	3.658[a]	20.267[a]	0.600[a]	2.130[a]	14.750[b]	0.818[a]	4.733[a]	0.267[b]

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table (3-c): Effect of different concentrations of Sodium azide.

Conc.	Plant length	Leaves No.	Shoot No.	Leave shoot	Stomata No.	Leaf area	Greening	Necroses
Control	3.012b	17.333b	0.000c	0.000c	17.917ab	1.063a	4.667a	0.333a
1 mM	3.725ab	17.667b	0.500ab	1.350b	13.473c	0.822a	4.583a	0.417a
1.5 mM	3.058b	19.083a	0.667ab	2.583a	14.666bc	0.552b	4.750a	0.250a
2 mM	3.867a	19.667a	0.750ab	3.017a	11.750c	0.842a	4.750a	0.250a
2.5 mM	3.492ab	21.083a	0.917a	3.583a	19..138a	0.845a	4.417a	0.583a

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Table (3-d): Effect of the interaction between different concentrations and durations of sodium azide on embryos and shoots for sweet almond rootstock.

Explant	Duration	Conc.	Plant length (cm)	leaf No.	Shoot No.	Leaf/ shoot	Stomata No.	leaf area (cm^3)	Greening	Necrosis
Embryo	30	Control	2.867^{cde}	18.000^{a-d}	0.000c	0.000^{d}	22.222^{ab}	0.417^{efg}	4.667^{a}	0.333^{b}
		1	4.233^{abc}	23.000^{a-d}	0.333b	2.667^{abc}	10.723^{efg}	0.350^{fg}	4.667^{a}	0.333^{b}
		1.5	3.433^{b-e}	25.000^{abc}	1.333a	4.000^{abc}	17.110^{b-f}	0.450^{efg}	4.667^{a}	0.333^{b}
		2	2.500^{de}	19.000^{a-d}	1.333a	6.500^{ab}	12.833^{c-g}	0.927^{b-f}	4.667^{a}	0.333^{b}
		2.5	3.033^{b-e}	20.000^{a-d}	1.333a	2.667^{abc}	13.720^{c-g}	0.477^{efg}	4.000^{a}	1.000^{a}
	60	1	3.467^{b-e}	23.000^{a-d}	1.000^{a}	1.567^{bc}	10.447^{efg}	0.530^{efg}	5.000^{a}	0.000^{c}
		1.5	3.600^{b-e}	27.333^{ab}	1.000^{a}	4.667^{abc}	18.333^{b-f}	0.420^{efg}	5.000^{a}	0.000^{c}
		2	5.333^{a}	30.333^{a}	1.000^{a}	2.233^{bc}	12.500^{d-g}	0.323^{g}	5.000^{a}	0.000^{c}
		2.5	4.033^{a-d}	30.333^{a}	1.667^{a}	7.333^{a}	16.610^{b-g}	0.960^{b-e}	5.000^{a}	0.000^{c}
Shoot	30	Control	3.167^{b-e}	16.667^{bcd}	0.000^{c}	0.000^{d}	14.110^{b-g}	1.7100^{a}	4.667^{a}	0.333^{b}
		1	4.100^{a-d}	12.667^{cd}	0.000^{c}	0.000^{d}	20.500^{a-d}	0.993^{b-e}	4.667^{a}	0.333^{b}
		1.5	2.300^{e}	12.000^{d}	0.333^{b}	1.667^{bc}	13.110^{c-g}	0.570^{d-g}	5.000^{a}	0.000^{c}
		2	3.033^{b-e}	14.000^{cd}	0.333^{b}	1.333^{bc}	8.333^{fg}	1.270^{bcd}	4.333^{a}	0.667^{a}
		2.5	3.367^{b-e}	16.333^{bcd}	0.333^{b}	2.000^{bc}	27.610^{a}	1.293^{abc}	4.000^{a}	1.000^{a}
	60	1	3.100^{b-e}	12.000^{d}	0.667^{a}	1.167^{c}	12.223^{d-g}	1.417^{ab}	4.000^{a}	1.000^{a}
		1.5	2.900^{b-e}	12.000^{d}	0.000^{c}	0.000^{d}	10.110^{fg}	0.767^{c-g}	4.333^{a}	0.667^{a}
		2	4.600^{ab}	15.333^{bcd}	0.333^{b}	2.000^{bc}	13.333^{c-g}	0.990^{b-e}	5.000^{a}	0.000^{c}
		2.5	3.533^{b-e}	17.667^{bcd}	0.333^{b}	2.333^{abc}	18.610^{b-e}	0.650^{d-g}	4.667^{a}	0.333^{b}

Means followed by the same letter within each column are not significantly different at 0.05 level of probability according to Duncan's multiple range test.

Photo (3): Effect of the interaction between different concentrations and durations of sodium azide on embryos and shoots for sweet almond rootstock.

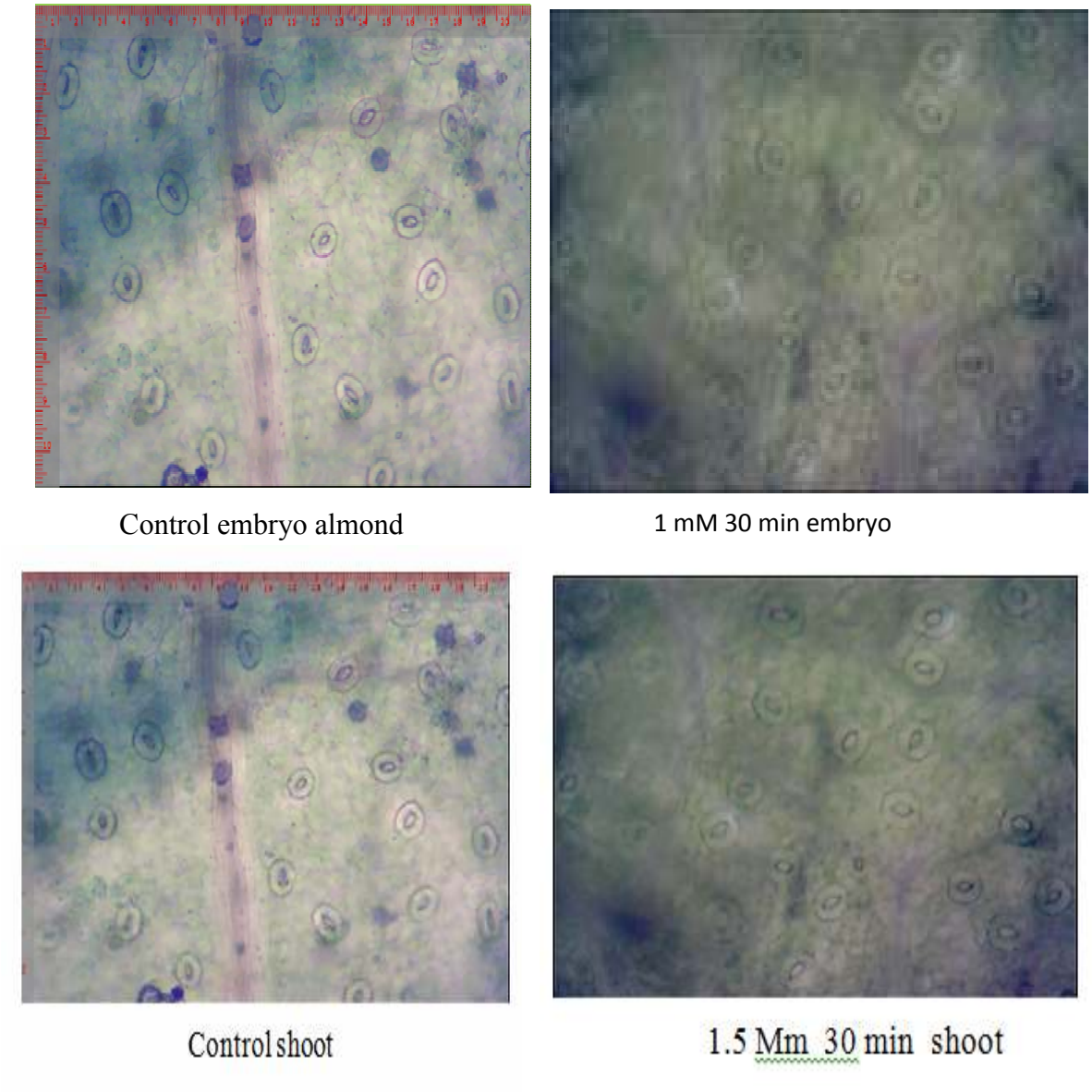

Photo (4): Effect of the interaction between different concentrations and durations of sodium azide on embryos and shoots for sweet almond rootstock.

Molecular Studies

Molecular Level of Polymorphic Bands

Data in Table (4) illustrating the ISSR profile of bitter almond. A total of 31 amplicons– amplified fragments (ranged from 300 to 1300 bp) were generated by the tested primers with an average number of six amplicons per primer. ISSR primers HB15 exhibited the highest number of fragments seven amplicons, followed by primer HB09 and HB10 which generated six amplicons for each. While primer 14A exhibit the lowest number of fragments three amplicons.

According ISSR profile of sweet almond. A total of 27 amplicons–amplified fragments (ranged from 300 to 800 bp) were generated by the tested primers with an average number of six amplicons per primer. ISSR primers HB15 exhibited the highest number of fragments six amplicons, followed by primer HB11 which generated five amplicons for each.

Table (4): ISSR analysis from the DNAs of bitter almond via six primers.

Primer Code	Total amplified fragments	Length range (bp)	Polymorphic Bands	Polymorphis (%)	Monomorphic Bands	Monomorphic (%)
14A	3	340-620	1	33.33	2	66.66
44B	5	340-600	2	40	3	60
HB09	6	360-920	3	50	3	50
HB10	6	420-920	2	33.33	4	66.66
HB11	4	300-820	2	50	2	50
HB15	7	500-1300	3	42.86	4	57.14
Total	31	300-1300	13	41.94	18	58.06

Table (5): ISSR analysis from the DNAs of sweet almond via six primers.

Primer Code	Total amplified fragments	Length range (bp)	Polymorphic Bands	Polymorphism (%)	Monomorphic Bands	Monomorphic (%)
14A	4	380-700	1	25	3	75
44B	4	300-600	1	25	3	75
HB09	4	300-700	1	25	3	75
HB10	4	360-800	1	25	3	75
HB11	5	300-700	2	40	3	60
HB15	6	360-800	3	50	3	50
Total	27	300-800	9	33.33	18	66.66

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الملخص العربي

تأثير الصوديوم أزيد كمطفر كيميائي على أصل اللوز المر والحلو

جيهان يسرى رمضان، محمد دياب الديب[١]، إبراهيم عبد المقصود إبراهيم[٢]

١. قسم الإنتاج النباتي، كلية العلوم البيئية الزراعية بالعريش، جامعة قناة السويس، مصر.

٢. معهد بحوث الهندسة الوراثية والتكنولوجيا الحيوية بمدينة السادات، جامعة المنوفية، مصر.

أجريت هذه الدراسة في معمل زراعة الأنسجة النباتية - كلية العلوم الزراعية البيئية بالعريش - جامعة قناة السويس في الفترة من ٢٠٠٩ حتى ٢٠١٥م بهدف دراسة تأثير محدثات الطفرة (التشعيع والمطفرات الكيماوية) لإنتاج طفرات وتباينات وراثية وتأثيرها على السلوك الوراثي ودراسة مدى تحملها للملوحة والجفاف وكذا عمل بصمة وراثية لهذه النباتات. ومحاولة إيجاد طريقه قياسية لإنتاج نباتات متماثلة من أصول الخوخ اللوز المر والحلو *Prunus amygdalus* بأعداد كافية تمكننا من تلبية الرغبات المتزايدة لأصحاب المشاتل لهذه الأصول المرغوب فيها خاصة تحت ظروف الأراضي المستصلحة حديثاً. أخذت الأجزاء النباتية (الجنين - القمة النامية - العقلة ذات البرعم الواحد) من أصل اللوز المر والحلو وتم تعريضها لماء جارى مستمر لمدة ٦٠ دقيقة ثم تعقيمها بكحول ٧٠% لمده دقيقه ثم غمرها في محلول كلوركس بنسبة ٢٠% مع إضافة نقطتين من Tween-20 لمدة ٢٠ دقيقة تم غمرها في ماء مقطر معقم ٣ مرات لمدة ٥ دقائق في كل مرة، ثم زراعتها بعد ذلك تحت ظروف معقمة على بيئة موراشيجى وسكوج حرة وموراشيجى مضاف إليه ٦-بنزايل أمينو بيورين وعليه تم تطبيق بعض المعاملات المرتبطة بمراحل الإكثار المباشر باستخدام تقنيه زراعة الأنسجة وهي (مرحله التأسيس - التضاعف - الاستطالة - التجذير - الأقلمة) بالإضافة إلى تعريض النباتات لماده الصوديوم أزيد (١-١,٥-٢-٢,٥ مللي مول) ومدة التعريض كانت ٣٠-٦٠ دقيقة، الذي أعطى أعلى تغيرات في كل من أصل اللوز المر تركيز ٢ ملي مول لمدة ٦٠ دقيقه للأفرع و١,٥ ملي مول لمدة ٣٠ دقيقة للبذرة من الصوديوم أزيد واللوز الحلو ٢,٥ ملي مول لمدة ٣٠-٦٠ دقيقة للأفرع.

الكلمات الإسترشادية: الصوديوم أزيد، الطفرة الكيميائية، اللوز المر والحلو، معملياً، نسبة الثغور.

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