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IN VITRO PROPAGATION OF SOME RARE ORNAMENTAL TREES *Salvadora persica* Wall.

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ABSTRACT

This work was carried out in the tissue culture laboratory of the Horticulture Research, Agricultural Research Center, Dokki Giza, Egypt during the period from 2004-2007.

The aim of this study was to establish an applicable protocol for the rapid micropropagation of *salvadora persica* Wall.

Sterilization treatments had a non significant effect on survival% of *Salvadora* explants. The effect of MS strength was significant only on the number of leaves, where normal MS strength resulted in the highest number in this regard. At the multiplication stage, BA levels affected fresh weight of shoots, number of shoots and number of leaves significantly. When BA was applied, the highest values of fresh weight of shoots were induced irrespective of BA concentration. With the number of shoots the highest values were a function of applying BA at either 2.5 or 5 ppm, while with number of leaves the highest values were confined to using BA at 5 ppm. NAA significantly affected shoot length, fresh weight of shoots and number of leaves. The highest values in this regard were a result of using NAA at 0.05 ppm. However, shoot length and number of leaves reached the highest rank at 0.01 NAA. The interaction between BA and NAA affected number of leaves significantly. The significantly highest number of leaves was a result of applying BA at 5 ppm and NAA at 0.01 ppm. At rooting stage, the medium substrate had a significant effect on shoot length, fresh weight of shoots, number of leaves, number of roots and root length. The highest values of these characters were a result of using cotton as a substrate. The effect of auxin type was significant on fresh weight of shoots, number of leaves, number of roots and root

length. Values of these records were higher in case of using NAA and lower when IBA was used. The interaction between medium substrate and auxin type significantly affected fresh weight of shoots, number of leaves, number of roots and root length. Nevertheless, cotton impregnated with NAA induced higher values of all these characters.

Key words: *Salvadora persica*, Murashige and Skoog [MS], Benzyladenine (BA) , 3 - indolebutyric acid (IBA) , 1-naphthalene acetic acid (NAA).

INTRODUCTION

Salvadora persica. is a genus of five species of trees or shrubs in the plant family Salvadoraceae, native to the Middle East. *Salvadora* plants are slow growing ones. They can be used as hedges, shrubs or small trees especially in the arid zones. *Salvadora* is extremely well-adapted to arid conditions, is salt tolerant and very drought resistant. It is adapted to alkaline or very saline soils, usually clay-rich, and soils without salt. It prefers clays, but is found on loams, black soils, and sand.

Young stems or branches of 3 to 5 mm diameter are cut repeatedly to produce short stems that are harvested for toothbrushes. Siwak, leaves and bark have available drug for tooth.

Roots are prepared as a salve and rubbed on the face for headaches. They are used for general body and back pains, chest diseases, and stomach aches. Seeds are used as a tonic and seed oil is used on the skin for rheumatism.

Ahmed *et al.* (2008) reported that seeds of *Salvadora persica* L. ("toothbrush tree" or "siwak") were collected from wild plants grown at Gabal Elba, a mountain in East South Egypt on the coast of the Red Sea. The seeds germinate after one month. Phytochemical screening revealed the occurrence of carbohydrates and/or glycosides, sterols, terpenes, flavonoids and alkaloids. The alcohol extract possesses antimicrobial activity against *Proteus vulgaris*. The herb contained 0.16% volatile oil in which heptadecene gamma-carbonic acid is the major. In a protocol for *in vitro* propagation of *S. persica* L. MS medium supplemented with 0.5 of each NAA and BA gave the best results for proliferated shoots and MS supplemented with IBA for rooting.

Salvadora plant readily germinates from seed as pretreatment is not necessary. Seeds exhibit no dormancy but the fruit pulp contains germination inhibitors which should be removed before sowing. Seed can be stored for about 1 month. Seedlings must remain in the nursery for 3 years prior planting,

The aim of this study was to establish an applicable protocol for the rapid micropropagation of *Salvadora persica*.

Gayathri and Gopal (2007) reported that the best establishment of buds from explants of *Amaryllus* and *Zephyranthes* (Fam. Amaryllidaceae) were obtained on a medium containing 3 or 5 ppm BAP and 1.0 or 0.5 ppm NAA, respectively.

Madhavilatha and Singh (2008) claimed that shoot proliferation from nodal explants of *Withania somnifera* (Fam. Solanaceae) was greatest in MS medium supplemented with BAP (2.0 ppm) and IAA (1.0 ppm).

Usman et *al.* (2005) cultured nodal and internodal segments from kinnow (*Citrus reticulata*) in MS medium supplemented with BA [benzyladenine] (0.1, 1 or 10 mg/litre). They mentioned that the number of shoots per explant increased with the increase in the concentration of BA up to 1 mg/litre; further increase in the concentration reduced number of shoots per explant. XiaoPing *et al.* (2007) remarked that segments of epicotyls of Dahong sweet orange were cultured on MS medium supplemented with BAP at 0, 1, 2, 3, 4, 5 and 6 ppm. The best BAP level was 1 ppm with an induction rate of 97.5%. Reis *et al.* (2008) informed that *Melissa officinalis* (Fam. Lamiaceae) shoots cultured in the MS medium containing 1 ppm BAP presented the highest shoot number. Venkateshwarlu (2008) stated that MS medium fortified with 1.0 mg benzyladenine /litre induced shoot buds on leaf segments of *Coccinia indica* (Fam. Cucurbitaceae). The number of shoots ranged from 1 to 3.

Aloufa *et al* (2003) stated that single node explants of *Ximenia americana* (Fam. Oleaceae) were cultured in MS medium containing 0.5-3.0 ppm BA. The number of shoots per explant increased with the increase in the level of BA. The maximum number of shoots was obtained with 2.25 ppm BA.

Ahmed *et al.* (2008) reported that for rooting of *Salvadora persica*, shoots were excised and transferred to MS medium supplemented with different concentrations of IBA of 0, 1, 2 and 3 mg/litre. One month later, shoots rooted in MS medium supplemented

with 3 mg/litre IBA. Singh and Goyal (2007) found that shoots of *Salvadora oleoides* developed *in vitro* were rooted on MS medium containing IBA at 3 mg/litre.

Jagadev *et al.* (2008) reported that for rooting of ginger (*Zingiber officinale*) shoots, MS supplemented with NAA (0.5 mg/litre) was found to be more effective and produced the maximum number of roots per shoot (13.3) and the maximum root length (2.0 cm). Yan *et al.* (2008) showed that 1/2MS supplemented with 0.6 mg NAA/litre was good for rooting of *Apocynum venetum*.

MATERIALS AND METHODS

This work was carried out in the Tissue Culture Laboratory of the Horticulture Research Institute, Agricultural Research Center, Doki, Giza, Egypt during the period from 2004-2007.

The aim of this study was to establish an applicable protocol for the rapid micropropagation of *Salvadora persica* Wall, Fam. Salvadoraceae.

Explant source

Lateral buds on young shoots were obtained from plants of *Salvadora persica* from the nursery of Timber Trees Dept., Horticulture Research Institute.

Explant preparation

As soon as these explants were obtained, they were brought to the tissue culture lab where they were cleaned by removing soil particles and dead parts, thoroughly washed up with a liquid soap and rinsed several times with tap water to rid them of surface contaminants as much as possible. They were then put in beakers of distilled water for sterilization treatments.

Glassware

Glass jars of 11.5 cm height × 6.5 cm diameter with their polypropylene caps were used during establishment, multiplication and rooting stages.

In order to avoid contamination problems, jars were washed then soaked in a detergent solution for 24 hours, sterilized with 10 % solution of sodium hypochlorite (NaOCl) for 2 hours, rinsed with distilled water and autoclaved at 1.05 kg/cm^2 and 121ºC for 25 minutes before being filled with 40 ml of the MS medium/jar.

Culture medium

Apart from experiment 2, where different strengths of Murashige and Skoog medium (1962) [MS] were used, half MS medium was used with some modifications. Content of calcium chloride ($CaCl_2.2H_2O$) in relation to the full strength were raised from 440 mg/litre, to 660 mg/litre, according to Shahin (2003) and Hashem and Saadawy (2003). The content of NH_4NO_3 was also raised from 1650 mg/litre in basic MS medium to 2475 mg/litre (50% increase) according to Hashem *et al.* (2005).

This medium contained, in addition to the prescribed salts and vitamins, 150 mg/litre citric acid, 100 mg/litre ascorbic acid, 100 mg/litre myoinositol, 20 g/l sucrose and 6.25 g/l agar. It was adjusted to 5.8 pH, poured in the jars and autoclaved at 121°C for 20 minutes under 1.05 kg/cm^2 pressure, left to cool and stored at 25±2ºC for one week before being used.

Plant growth regulators

In both establishment and multiplication stages, one type of cytokinins and one type of auxins, incorporated in the media, were used. These were benzyladenine (6-benzylaminopurine), referred to for short as "BA", and 1-naphthalene acetic acid (NAA).

Different concentrations of certain auxins were added to the media in the rooting stage according to the layout of the experiment. These auxins were 3-indolebutyric acid (IBA) and 1-naphthalene acetic acid (NAA).

This investigation comprised 4 experiments

Establishment Stage:

Experiment 1

Effect of sterilization treatments

Determination of the best sterilization treatment was the aim of this experiment. Surface sterilization for *Salvadora* explants was carried out by either one of the following treatments:

1- Sodium hypochlorite (NaClO) solution (about 0.75% active ingredient "Cl"). This solution was prepared by diluting 15 cm^3 of commercial bleach (Clorox, 52.5g Cl/l) with adistilled water to 100 cm^3 final volume. Immersion lasted for 12 minutes.

2- Mercuric chloride (MC), chemical formula of which is $HgCl_2$, at 500 ppm for 6 minutes.

3- A combined treatment of sodium hypochlorite (NaClO) followed by mercuric chloride (MC), both as detailed above.

All these treatments were immediately followed by rinsing in sterile distilled water for three successive times.

Explants were inoculated on ½MS medium under aseptic conditions using a laminar airflow cabinet. Jars were incubated at 25/20°C (day/night) ±2°C, 70% relative humidity. Two fluorescent tubes/shelf were installed at 30 cm above explants to provide light intensity of 2200-2400 lux at explant level.

Total number of treatments was three for *Salvadora*, in a completely randomized experiment, Each treatment comprised 3 replicates. Each replicate contained 5 jars with 1 explant/jar.

Experiment 2

Effect of MS strength

Four strengths of MS medium salts, i.e. ½MS, 1MS, 1½MS and 2MS were prepared. Each liter according to Gayathri and Gopal (2007) who supplied the medium with 3-5 ppm BAP to get the best establishment of buds from explants of *Amaryllus* and *Zephyranthes*. Other components of the media such as sucrose, agar, boric acid, copper sulfate and calcium chloride were the same as mentioned above. Explants of *Salvadora* were inoculated on these media. Each one of these MS strengths (treatments) were replicated 3 times with 5 jars in each replicate. These treatments were arranged in a completely randomized design.

Total number of treatments was 4 for *Salvadora* in a completely randomized experiment, . Each treatment comprised 3 replicates. Each replicate contained 5 jars with 1 explant/jar.

Multiplication stage

Experiment 3

Effect of BA and NAA on multiplication

A complete randomized design, with treatments disposed in factorial arrangement was carried out to study the effect of two factors on the multiplication process, i.e. NAA (at 0, 0.01 and 0.05 ppm), as a main plot and BA (at 0, 2.5, 5 and 10 ppm) as a subplot. The outcome of these combinations represented 12 treatments, each included 3 replicates, with 5 jars of one explant, in each replicate.

Rooting Stage

Experiment 4

Effect of auxins on rooting

Shoots of plant were inoculated on the rooting media. These media were supported in different ways. *Three* supporting matrices or substrates were used separately in this experiment with ½ MS solution. These substrates were agar, as the usual gelling agent, cotton and sand. Either IBA or NAA was added at 5 ppm to the above-mentioned media. The 6 combinations (treatments) of these two factors, auxin type (as main plot) and substrate (as subplot), were arranged in a complete randomized design, with treatments disposed in factorial arrangement. Each treatment comprised 3 replicates, with 5 jars in each replicate.

Data were statistically analyzed due to the methods described by to Snedecor and Cochran (1980). using L.S.D for comparing between means of treatments.

RESULTS AND DISCUSSION

Establishment stage

Experiment 1: Sterilization: Effect of sterilants on *Savadora* explants Table (1)

Survival percentage

Although the effect of sterilizing agent on survival percentage was found to be insignificant, the combined treatment with the two sterilants resulted in higher survival percentage (63.85%) compared to using either MC or clorox as they resulted in 59.22% and 54.99, respectively, with the last one being the lowest.

Discarded explants percentage

The percentage of discarded explants due to contamination or death of the explant was not significantly influenced with the sterilizing agent. However, the outcome of the combined treatment gave less discarded explants (26.15%) than either clorox or MC which resulted in 35.01 and 30.79%, respectively.

Table1. Effect of sterilant type on *Savadora* explants

Sterilant type	Survival %	Discarded %
Clorox	54.99	35.01
Mercuric chloride	59.22	30.79
Combined treatment	63.85	26.15
L.S.D at 5%	N.S.	N.S.

All percentages were transformed according to Snedecor and Cochran (1980)

Experiment 2: Starting: Effect of MS strength on *Salvadora persica* explants. Table (2)

Shoot length.

Though the effect of MS strength on shoot length was insignificant, a certain trend could be observed. As MS strength increased from 1/2 to 1/1, shoot length increased from 1.22 (cm) to the highest record, i.e. 1.40 cm.However further increase in the same regard to 1½MS and 2MS urged shoot length to decrease to 1.32 and 1.26 cm, respectively.

Shoot fresh weight (g)

Shoot fresh weight was not significantly influenced by MS strength. Apart from this fact, the heaviest shoots (0.66 g) were those grown on normal MS medium. Weight of shoots grown on other MS media levels,of ½, 1½ and 2MS media was almost the same, (0.47, 0.45 and 0.46 g, respectively.

Number of shoots

Although the influence was insignificant, the highest number of shoots (9.20 shoots) was produced on the normal MS strength, while 7.0, 6.4 and 7.0 shoots were found on ½, 1½ and 2 MS media, respectively).

Number of leaves

The effect of MS strength on number of leaves was significant. As MS strength was increased from half strength to full strength, number of leaves significantly increased from 14.00 to the highest significant record, i.e. 23.33 leaves. Raising strength to 1½ MS had a significant negative effect on this charcter, reducing it to 12.67 leaves. Further increase in MS strength to 2 MS decreased number of leaves, however insignificantly, to the lowest record, i.e. 10.33 leaves.

Table 2. Effect of MS strength on *Salvadora* explants

MS strength	Shoot height (cm)	Shoot weight (g)	No. of shoots	No. of leaves
½ MS	1.22	0.47	7.00	14.00
MS	1.40	0.66	9.20	23.33
1½ MS	1.32	0.45	6.40	12.67
2 MS	1.26	0.46	7.00	10.33
L. S. D. at 5%	N.S.	N.S.	N.S.	2.75

In this concern, Singh and Goyal (2007) found that shoots of *Salvadora oleoides* developed *in vitro* were rooted on MS medium containing IBA at 3 mg/litre. Ahmed *et al.* (2008) reported that for rooting of *Salvadora persica*, shoots were excised and transferred to MS medium supplemented with different concentrations of IBA of 0, 1, 2 and 3 mg/litre. One month later, shoots rooted in MS medium supplemented with 3 mg/litre IBA.

Madhavilatha and Singh (2008) claimed that regenerated shoots of *Withania somnifera* (Fam. Solanaceae) showed optimum rooting in half-strength MS medium containing IBA (2 ppm).

Yan *et al.* (2008) showed that 1/2MS supplemented with 0.6 mg NAA/litre was good for rooting of *Apocynum venetum*.

Multiplication stage

Experiment 3: Effect of BA and NAA on *Savadora persica*:

Shoot length Table (3)

BA

The effect of BA application on shoot length of *Salvadora* explants was found to be insignificant. However, a stimulative effect could be noticed when BA concentration was raised from 0 to 2.5 and 5 ppm, shoot length increased from 1.19 and 1.27 to 1.34 cm, respectively. Further increase in BA level to 10 ppm had a negative influence on this character, reducing it to 1.15 cm.

NAA

The effect of NAA on shoot length was significant. The presence of NAA had a promotive effect on this parameter. NAA at 0.01 ppm gave the longest shoots (1.43 cm) compared to the shortest ones (0.98 cm) in the absence of NAA. However, further increase in NAA level to 0.05 ppm resulted in a decline in shoot length to 1.29 cm.

Interaction between BA and NAA

Anon significant effect was exerted by the interaction between BA and NAA on shoot length of *Salvadora*. as insignificant, differences were noticed between the different treatments. The tallest shoots (1.55 cm) belonged to explants grown on media supplemented with BA at 5 ppm and NAA at 0.01 ppm, while the shortest ones (0.80 cm) were those grown on media free of plant growth regulators or those supplemented with only BA at 10 ppm.

Table 3. Effect of BA and NAA on shoot length (cm) of *Salvadora persica*

BA conc. (ppm)	NAA conc. (ppm) 0.0	0.01	0.05	Mean
0.0	0.80	1.47	1.30	1.19
2.5	1.15	1.40	1.25	1.27
5.0	1.18	1.55	1.28	1.34
10.0	0.80	1.30	1.34	1.15
Mean	0.98	1.43	1.29	
L.S.D. BA at 5%	N.S.			
L.S.D. NAA at 5%	0.25			
L.S.D. BA x NAA at 5%	N.S.			

Fresh weight of shoots Table (4)

BA

BA significantly affected fresh weight of shoots . The presence of BA in the media gave heavier shoots irrespective of BA level, compared to the BA at 0 ppm of control (0.06 g). BA at 2.5, 5 or 10 ppm resulted in shoots weighing 0.46, 0.47 and 0.44 g, respectively, with insignificant differences between the three records.

NAA

NAA at 0.05 ppm gave significantly the heaviest shoots (0.48 g), compared with the lightest ones (0.28 g) when no NAA was used or 0.33 g when it was used at 0.01 ppm.

Interaction between BA and NAA

The interaction between BA and NAA had a non significant effect on fresh weight of *Salvadora* shoots. Regardless of this fact,the lightest and heaviest shoots (0.06 and 0.64 g) were noticed on media

free of plant growth regulators, and media supplemented with BA at 10 ppm and NAA at 0.05 ppm, respectively.

Table 4. Effect of BA and NAA on fresh weight of shoots (g) of *Salvadora persica*

BA conc. (ppm)	NAA conc. (ppm) 0.0	0.01	0.05	Mean
0.0	0.06	0.11	0.10	0.09
2.5	0.49	0.29	0.60	0.46
5.0	0.31	0.52	0.58	0.47
10.0	0.26	0.41	0.64	0.44
Mean	0.28	0.33	0.48	
L.S.D. BA at 5%	0.17			
L.S.D. NAA at 5%	0.14			
L.S.D. BA x NAA at 5%	N.S.			

Number of shoots Table (5)

BA

Applying BA significantly affected the number of shoots. Explants grown on media free of BA produced significantly the least number of shoots (2.79 shoots). Incorporating BA in the media at 2.5 ppm induced significantly the highest record in this concern (9.66 shoots). The higher the level of BA increased beyond the abovementioned one, i.e. to 5 and 10 ppm, the lower the number of shoots was produced, i.e. 7.70 and 5.73 shoots, respectively.

NAA

A non significant effect was detected of NAA on the number of shoots. However, this character increased greatly to 7.12 and 7.32 shoots at NAA 0.01 and 0.05 ppm, compared to the corresponding record (4.98 shoots) in the absence of NAA.

Interaction between BA and NAA

The interaction between BA and NAA did not significantly affect number of shoots. However, the lowest number (2.80 shoots) resulted from using media void of plant growth regulators, while the highest one (12.33 shoots) was a result of using BA at 2.5 and NAA at 0.05 ppm

Table 5 Effect of BA and NAA on number of shoots of *Salvadora persica*

BA conc. (ppm)	NAA conc. (ppm) 0.0	0.01	0.05	Mean
0.0	2.80	3.33	2.25	2.79
2.5	8.83	7.83	12.33	9.66
5.0	5.60	10.00	7.50	7.70
10.0	2.67	7.33	7.20	5.73
Mean	4.98	7.12	7.32	
L.S.D. BA at 5%	2.67			
L.S.D. NAA at 5%	N.S.			
L.S.D. BA x NAA at 5%	N.S.			

In this respect, Aloufa *et al* (2003) stated that single node explants of *Ximenia Americana* (Fam. Oleaceae) were cultured in MS medium containing 0.5-3.0 ppm BA. The number of shoots per explant was increased with the increase in the level of BA. The maximum number of shoots was obtained with 2.25 ppm BA.

Reis *et al.* (2008) informed that *Melissa officinalis* (Fam. Lamiaceae) shoots cultured in the MS medium containing 1 ppm BAP presented the highest shoot number.

Usman *et al.* (2005) cultured nodal and internodal segments from Kinnow (*Citrus reticulata*) in MS medium supplemented with BA [benzyladenine] (0.1, 1 or 10 mg/litre). They mentioned that the number of shoots per explant increased with the increase in the concentration of BA up to 1 mg/litre; further increase in the concentration reduced the number of shoots per explant.

XiaoPing *et al.* (2007) remarked that segments of epicotyls of Dahong sweet orange were cultured on MS medium supplemented with BAP at 0, 1, 2, 3, 4, 5 and 6 ppm. The best BAP level was 1 ppm with an induction rate of 97.5%.

Number of leaves Table (6)

BA

The effect of BA application on number of leaves was significant. Number of leaves was significantly increased from 7.33 leaves of the control treatment (the significantly lowest value) to 16.00 leaves when using BA at 2.5 ppm. At 5 ppm BA, this record was significantly

increased to the highest value (18.67 leaves). However, further increase in BA concentration to 10 ppm negatively and significantly affected this parameter, reducing it to 12.33 leaves.

NAA

NAA significantly affected number of leaves . Applying NAA either at 0.01 or 0.05 ppm gave the same number of leaves (15.25 leaves), which was significantly higher than the corresponding value of (10.25 leaves) of the untreated control plants.

Interaction between BA and NAA

The interaction between BA and NAA significantly affected number of leaves. The highest significantly number (25.00 leaves) was a result of applying BA at 5 ppm and NAA at 0.01 ppm. Shoots treated with BA at 5 ppm and NAA at 0.05 ppm occupied significantly the second position in this concern, as they had 20 leaves. The significantly lowest value (6 leaves) was produced by the control untreated shoots, preceded without a significant difference by those treated with only NAA at 0.05 ppm as they had 7 leaves.

Table 6. Effect of BA and NAA on number of leaves of *Salvadora persica*

BA conc. (ppm)	NAA conc. (ppm) 0.0	0.01	0.05	Mean
0.0	6.00	9.00	7.00	7.33
2.5	15.00	15.00	18.00	16.00
5.0	11.00	25.00	20.00	18.67
10.0	9.00	12.00	16.00	12.33
Mean	10.25	15.25	15.25	
L.S.D. BA at 5%	0.99			
L.S.D. NAA at 5%	1.14			
L.S.D. BA x NAA at 5%	1.97			

In this connectionVenkateshwarlu (2008) stated that MS medium fortified with 1.0 mg benzyladenine /litre induced shoot buds on leaf segments of *Coccinia indica* (Fam. Cucurbitaceae). Number of shoots ranged from 1 to 3.

Rooting stage

Experiment 4: Effect of medium substrate and auxin type on *Salvadora persica*:

Effect of medium substrate and auxin type on shoot length Table (7)

Medium substrate

The effect of medium substrate on shoot length proved to be significant. Shoots grown on cotton were significantly taller (1.67 cm) than those grown on either agar (1.27 cm) or sand (1.15 cm). The last two records were not significantly different.

Auxin type

Auxin type had a non significant effect on shoot length. However, shoots grown on media supplied with NAA (1.38 cm) were taller than the correspondents on media supplied with IBA (1.34 cm).

Interaction between medium substrate and auxin type

The interaction between medium substrate and auxin type did not exert a significant influence on shoot length. Nevertheless, shoots grown on cotton and treated with IBA were the longest (1.70 cm), while those grown on sand and treated with the same auxin were the shortest (0.97 cm).

Table 7. Effect of auxin type and medium substrate on shoot length (cm) of *Salvadora persica*

Medium substrate	Auxin type IBA	NAA	Mean
Agar	1.37	1.17	1.27
Cotton	1.70	1.63	1.67
Sand	0.97	1.33	1.15
Mean	1.34	1.38	
L.S.D. substrate at 5%	0.37		
L.S.D. auxin at 5%	N.S.		
L.S.D. explant x MS at 5%	N.S.		

Effect of medium substrate and auxin type on fresh weight of shoots (g) Table 8

Medium substrate

The effect of medium substrate on fresh weight of shoots was significant. The significantly heaviest shoots were those grown on

cotton (0.29 g), followed without significant differences by those grown on agar (0.23 g). The significantly lightest shoots were those grown on sand (0.09 g).

Auxin type

Auxin type had a significant effect on fresh weight of shoots. Shoots grown on media fortified with NAA were significantly heavier (0.30 g) than those grown on media supplemented with IBA (0.10 g).

Interaction between medium substrate and auxin type

The interaction between medium substrate and auxin type was significant. Shoots grown on cotton augmented with NAA were significantly the heaviest (0.49 g), while those grown on sand supplemented with IBA were significantly the lightest (0.05 g).

Table 8. Effect of auxin type and medium substrate on fresh weight of shoots (g) of *Salvadora persica*

Medium substrate	Auxin type		Mean
	IBA	NAA	
Agar	0.16	0.30	0.23
Cotton	0.08	0.49	0.29
sand	0.05	0.12	0.09
Mean	0.10	0.30	
L.S.D. substrate at 5%	0.13		
L.S.D. auxin at 5%	0.11		
L.S.D. explant x MS at 5%	0.18		

Effect of medium substrate and auxin type on number of leaves Table (9)

Medium substrate

The effect of medium substrate on number of leaves was significant. The significantly highest record in this concern (7.33 leaves) was a result of growing *Salvadora* plantlets on cotton, followed with a significant difference with the corresponding record of plantlets grown on agar (3.33 leaves). The significantly lowest number of leaves was produced by plantlets grown on sand (1.83 leaves).

Auxin type

The effect of auxin type on number of leaves was significant. Plantlets grown on amedium impregnated with NAA had significantly

greater number of leaves (6.89 leaves) compared to 1.44 leaves of those having IBA in their growing medium.

Interaction between medium substrate and auxin type

The effect of the interaction between medium substrate and auxin type on number of leaves was found to be significant. The significantly greatest number of leaves (12.67 leaves) belonged to plantlets grown on cotton impregnated with NAA, followed with a significant difference by number of leaves found on plantlets grown on agar supplemented with NAA (5.33 leaves). Plantlets grown on any type of media supplemented with IBA the record(1.33, 2.00 and 1.00 leaves for agar+IBA, cotton+IBA and sand+IBA, respectively, or on sand impregnated with any type of auxin 1.00 and 2.67 leaves for sand+IBA and sand+NAA, respectively as record which was significantly lowest number in the same concern.

Table 9. Effect of auxin type and medium substrate on number of leaves of *Salvadora persica*

Medium substrate	Auxin type		Mean
	IBA	NAA	
Agar	1.33	5.33	3.33
Cotton	2.00	12.67	7.33
Sand	1.00	2.67	1.83
Mean	1.44	6.89	
L.S.D. substrate at 5%	1.43		
L.S.D. auxin at 5%	1.17		
L.S.D. explant x MS at 5%	2.02		

Effect of medium substrate and auxin type on number of roots Table (10)

Medium substrate

The influence of medium substrate on the number of roots was found to be significant. The significant greatest number in this question was that of plantlets grown on cotton (1.17 roots), followed without significant differences by those of plantlets grown on agar (0.50 roots). Shoots grown on sand did not produce roots at all.

Auxin type

Auxin type effect on number of roots was significant. Plantlets treated with NAA had 1.11 roots, while shoots treated with IBA did not produce roots at all.

Interaction between medium substrate and auxin type

The interaction between medium substrate and auxin type was significant. Plantlets grown on cotton and treated with NAA had 2.33 roots, significantly greater than those grown on agar and treated with the same auxin (1.00 root).

Table 10. Effect of auxin type and medium substrate on number of roots of *Salvadora persica*

Medium substrate	Auxin type IBA	NAA	Mean
Agar	0.00	1.00	0.50
Cotton	0.00	2.33	1.17
Sand	0.00	0.00	0.00
Mean	0.00	1.11	
L.S.D. substrate at 5%	0.90		
L.S.D. auxin at 5%	0.73		
L.S.D. explant x MS at 5%	1.27		

In this respect, Shinde and Karale (2007) mentioned that NAA at 2.0 mg/litre resulted in maximum number of roots/shoot of *Tamarindus indica*.

Effect of medium substrate and auxin type on root length (cm) Table (11)

Medium substrate

Root length was significantly influenced by the medium substrate. Roots produced on cotton were significantly longer (0.35 cm) than those produced on agar (0.08 cm). No roots were produced on sand at all.

Uxin type

The effect of auxin type on root length was significant. Plantlets grown on media fortified with NAA had longer roots (0.29 cm), as shoots grown on media supplemented with IBA did not have only roots.

Interaction between medium substrate and auxin type

Plantlets produced on cotton supplemented with NAA were significantly longer (0.70 cm) than those produced on agar supplemented with the same auxin (0.17 cm). No roots were produced on at all on other combinations of media and auxins.

Table 11. Effect of auxin type and medium substrate on root length (cm) of *Salvadora persica*

Medium substrate	Auxin type		Mean
	IBA	NAA	
Agar	0.00	0.17	0.08
Cotton	0.00	0.70	0.35
Sand	0.00	0.00	0.00
Mean	0.00	0.29	
L.S.D. substrate at 5%	0.22		
L.S.D. auxin at 5%	0.18		
L.S.D. explant x MS at 5%	0.31		

Similarly Shinde and Karale (2007) reported that earliest response of *Tamarindus indica* to rooting with highest rooting and maximum length of root was observed with IBA 2 mg/litre.

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إكثار بعض أشجار الزينة النادرة بواسطة زراعة الأنسجة
Salvadora persica: السواك

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أجرى هذ البحث فى معمل قسم بحوث نباتات الزينة –مركز البحوث الزراعية- الدقى- جيزة خلال الفترة من 2004 -2007 وذلك بهدف عمل بروتوكول للاكثار السريع للنبات السواك.

أولا : مرحلة التأسيس :

لم يكن لمعاملات التعقيم تأثير معنوى على النسبة المئوية للبقاء لمنفصلات السواك. ومع ذلك فإن المعاملة المشتركة بكلوريد الزئبق + الكلوروكس أعطت أعلى نسبة مئوية للبقاء ، أما الكلوروكس فقد أعطى أقل قيمة.

كان لتركيز بيئة موراشيج وسكوج MS تأثير معنوى على العدد الكلى للأوراق فقط. ونتجت أعلى القيم لهذه الصفة على التركيز العادى لهذه البيئة ، بينما أدى إستعمال البيئة بضعف التركيز 2MS الى الحصول على أدنى قيمة فى هذا الصدد. وبالرغم من أن تأثير تركيز بيئة MS على طول الفرع ، الوزن الرطب للأفرع وعدد الأفرع لم يكن معنويا ، فإن أعلى القيم لهذه الصفات قد نتجت من إستعمال التركيز العادى لبيئة MS. كما نتجت أدنى القيم لصفة طول الفرع بإستعمال بيئة ½MS ، ولصفة عدد الأفرع بإستعمال بيئة 1½MS .

ثانيا : مرحلة الإكثار :

كان لتركيز البنزايل ادنين (BA) تأثيرا معنويا على الوزن الطازج للأفرع ، عدد الأفرع ، وعدد الأوراق بينما كان التأثير ،غير معنوى على طول الفرع. وفى غياب BA كانت قيم صفات الوزن الطازج للأفرع ، عدد الأفرع ، وعدد الأوراق هى الأدنى. أما فى وجود BA فقد نتجت القيم الأعلى لصفة الوزن الطازج للأفرع بغض النظر عن تركيز BA. وبالنسبة لعدد الأفرع ، فقد نتجت أعلى قيمة عند إستعمال BA بتركيز 2.5 أو 5 جزء فى المليون ،

بينما فى حالة طول الفرع وعدد الأوراق فإن أعلى القيم لها لم تنتج إلا بإستعمال BA بتركيز 5 جزء فى المليون.

أثر نفتالين حمض الخليك (NAA) معنويا على طول الفرع ، الوزن الرطب للأفرع ، وعدد الأوراق ، بينما لم يكن التأثير معنويا على عدد الأفرع. وظهرت أعلى القيم لصفات طول الفرع ، الوزن الطازج للأفرع ، وعدد الأوراق نتيجة لإستعمال NAA بتركيز 0.05 جزء فى المليون. ومع ذلك ، فقد بلغت قيم صفات طول الفرع ، وعدد الأوراق أعلى مرتبة عند إستعمال NAA بتركيز 0.01 جزء فى المليون.بينما نتجت أقل القيم للصفات المذكورة عند عدم إستعمال NAA بالمرة.

وقد أثر التفاعل بين BA و NAA على عدد الأوراق تأثيرا معنويا ، بينما لم يكن هذا التأثير معنويا على طول الفرع ، الوزن الطازج للأفرع ، وعدد الأفرع. ونتج أكبر عدد للأوراق من إستعمال BA بتركيز 5 جزء فى المليون و NAA بتركيز 0.01 جزء فى المليون ، بينما نتج العدد الأدنى للأوراق على الأفرع الغير معاملة (الكنترول) ، وتلك المزروعة فى بيئة تحتوى على NAA بتركيز 0.05 جزء فى المليونز

ثالثا : مرحلة التجذير :

كان لمهد البيئة تأثيرا معنويا على طول الفرع ، الوزن الطازج للأفرع ، عدد الأوراق ، عدد الجذور ، وطول الجذر حيث. تحققت القيم الأعلى لهذه الصفات نتيجة لإستعمال القطن مهدا للزراعة. وفى حين ادى إستعمال الرمل الى الحصول على أدنى القيم لصفات طول الفرع ، الوزن الطازج للأفرع ، وعدد الأوراق ، فإن الآجار تسبب فى الحصول على أدنى القيم لصفات عدد الجذور وطول الجذر.

وكان لنوع الأكسين تأثيرا معنويا على قيم صفات الوزن الطازج للأفرع ، عدد الأوراق ، عدد الجذور ، وطول الجذر بينما كان التأثير غير معنوى على طول الفرع. وبصرف النظر عما إذا كان التأثير معنويا ام لا ، فإن قيم الصفات المذكورة كانت أعلى عند إستعمال NAA وأقل عند إستعمال اندول حمض البيوتريك (IBA) .

كان للتفاعل بين مهد البيئة ونوع الأكسين أثرا معنويا على الوزن الطازج للأفرع ، عدد الأوراق ، عدد الجذور ، وطول الجذر ، وأثرا غير معنوى على طول الفرع. وقد شجع إستعمال القطن المحتوى على NAA إنتاج أعلى القيم لهذه الصفات جميعها ، بإستثناء طول الفرع حيث أعطى القطن المحتوى على IBA نتيجة أفضل.

أعطت النبيتات النامية على أى بيئة محتوية على IBA ، أو على الرمل المحتوى على أى نوع من الأكسينات ، العدد الأقل معنويا من الأوراق. وأعطت بيئة الآجار المحتوية على NAA القيم الأدنى لصفات عدد الجذور ، وطول الجذر ، وكان للرمل المحتوى على IBA نفس التأثير على طول الفرع والوزن الطازج للأفرع.هذا وقد فشلت جميع الأفرع النامية على أى نوع من البيئة المحتوية على IBA فى إنتاج جذور. أما عند إستعمال NAA فإن الأفرع النامية على الرمل هى التى فشلت فى التجذير.