Some Factors Affecting Micropropagation of Some Wild Medicinal Plants *2-Achillea fragrantissima* (Forssk.) SCH. BIP., Plant

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Abstract: *Culturing leaf explants of Achillea fragrantissima on Murashige and Skoog (MS) medium supplemented with 2, 4-dichlorophenoxy acetic acid (2, 4-D) at 1.0 or 2.0 mg/l resulted in high callus fresh weight after 30 and 60 days without significant differences between the two concentrations. MS medium strength at full or $^1/_2$ strength was not significantly effective on shoots number, shoots length and number of leaves/shoot, where benzyl adenine (BA) concentration at 0.5 mg/l was enough to enhance shoots number, shoot length and leaves number/shoot. The best rooting for shoots and root length was obtained from MS $^1/_4$ strength combined with 0.1 mg/l indole-3-acetic acid (IAA). The acclimatization process of plantlets were successful on sand: peat moss medium 1: 1 v/v with a percentage of 90% survival plants.*
Key words: *Achillea, Murashige and Skoog medium (MS), 2, 4-dichlorophenoxy acetic acid (2, 4-D), benzyl adenine (BA) and indole-3-acetic acid (IAA).*

INTRODUCTION

Medicinal plants have a great position in agricultural and industrial production. They are considered as the main source for natural medicine or the essential source for the effective substances which are raw material in some chemical constitutes.

Achillea fragrantissima (Forssk.) Sch. Bip., (Fam. Asteraceae) plants are white-wooly, with erect stems which attain up to 1m high, leaves are small, estipulate thick white to grayish-green and oblong, serrate with undivided lamina. Flower heads terminal discoid composed of numerous tubular florets with golden-yellow colors. Odour is aromatic and the taste is bitter (Tackholm, 1974). The part which used is the fresh or dry whole plant, the fresh herb contains volatile oil that reaches about 1.0% which consists of 59 components of which α-pinene, β-pinene, limonene, cineole, linalool, carvacrol, eugenol artemisia ketone, palustrol sabonene hydrate, α and β-thujones, santolina alcohol and α-terpineol. The plant also contains tannin, flavonoids, fatty acids and bitter substances (Mustafa, *et al.*, 1995).

Achillea fragrantissima was used by bedouin for the treatment of caught, aromatic bitter stomachic and anthelmintic. As well as the volatile oil prepared from the flowering tops showed a broad spectrum activity against various microbes, 13-0-desacetyl 1-β-hydroxy iso afragloucolide caused inhibition of phasic contraction and of the tone of rat isolated ileum, uterus and aorta. It increased the phasic contraction of isolated urinary bladder cirsiliol caused relaxation of contracted rats proximal aorta, trachea, urinary bladder and uterus (Aboutable, *et al.*, 1986).

Some species of medicinal and aromatic plants are disappeared gradually and consequently from Sinai natural environment. So, a critical importance should be done to face this problem by using tissue culture technique.

Tissue culture has become one of the most ways to reproduce crops that are difficult to propagate by conventional methods such as by seeds or cutting. Micro propagation allows the production of large number of plants in a relatively small growing area and in a relatively shorter time. Also, micro propagation produces rapidly and large quantities of the plants identical to their parents. The speed of tissue culture technology development have been accelerated by its particle commercialization for many crops (Nizar, 2001).

The main objectives of these studies were to establish callus formation for *Achillea fragrantissima* (Forssk.) Sch. Bip. by tissue culture technique and to test capability of the plant to regenerate through somatic embryogenesis as well as examine micro propagation technique of the plant.

MATERIALS AND METHODS

This work was carried out in the Plant Tissue Culture Laboratory in Faculty of Environmental Agricultural Sciences, El-Arish, Suez Canal University during the period from 2002 to 2005.

Wild plants of *Achillea fragrantissima* (Forssk.) Sch. Bip., plants were collected from El-Hasana region, North Sinai.

Explants preparation and sterilization:

Leaves (leaf blade) of *Achillea fragrantissima* were rinsed with a small amount of soap for 1-2 minutes and rinsed again under running tap water for 15 min. to remove all the remaining detergent and washed with sterilized distilled water. The explants were soaked for 3 minutes in chlorox (10%) then washed again with sterilized distilled water for 3-4 times to remove all traces of the disinfection. All steps of the sterilization had been done under aseptic conditions inside the culture cabinet (Laminar air flow hood) and by using sterilized instruments. The purpose

of this procedure was to disinfect the plant tissue from fungi, bacteria, and other contamination, without harming the regenerative capacity of the explant.

Aseptic cultures of *Achillea fragrantissima* were established from leaf sections. After removal of the outside tissue, the remainder of each leaf was cut into pieces of (0.5×0.5 cm) and each leaf segment was embedded horizontally on the agar surface in glass jars (150 cm^3) containing 30 ml of MS (1962) basal medium.

Callus formation:

Media preparation:

The basal medium described by Murashige and Skoog (1962) was used and sterilized by autoclaving at 121°C for 20 min. The pH of the medium was adjusted at 5.7. The following treatments were conducted with the used medium.

Callus induction:

In order to induce callus formation on leaf explants MS medium was supplemented with 0.0, 1.0 or 2.0 mg/l of 2, 4-dichlorophenoxy acetic acid (2, 4-D) in full MS strength medium.

After 30 days, from represented samples the callus fresh weight was determined and again reculture on the same medium for another 30 days then the fresh weight was again determined.

Micro propagation:

Shoot formation:

Similar pieces of callus about (1 gm) weight were cultured in jars as mentioned before on full or $^1/_2$ strength MS medium supplemented with 0.0, 0.5, 1.0 or 1.5 mg/l BA. After six weeks the following data were recorded: The number of proliferated shoots, shoot length (cm) and leaves number/shoot.

Root formation:

For root formation, shoots from the obvious shooting formation were transferred to rooting medium contains $^1/_4$ or $^1/_2$ MS strength medium supplemented with indole-3-acetic acid (IAA) at 0, 0.1, 0.2 and 0.3 mg/l. The jars were incubated under the above mentioned conditions in room culture. After eight weeks the number of roots/shoot and root length were determined.

All cultures were kept in culture room and subjected to 3000 lux photoperiod for 16 hours fluorescent light and 8 hours dark. The temperature was 25±2°C.

The obtained plantlets after eight weeks were transferred to acclimatization in 1 peat moss : 1 sand medium covered with polyethylene bags in a shade place at 25±2°C, after two weeks the polyethylene cover was removed as the acclimatization process showed a success survival percentage of 90%.

Statistical analysis:

Data were statistically analyzed by using randomized complete block design in simple or factorial arrangement in ten replicates (Snedecor and Cochran, 1990). Mean separations were done by using MSTATC computer program V.4 (1986) and least significant differences (LSD) was computed for means separation of all parameters obtained in these studies.

RESULTS AND DISCUSSION

1-Effect of 2, 4-D concentrations on callus fresh weight of *Achillea fragrantissima* plant:

Data in Table (1) and photo (1) indicate that 2, 4-D at 1 or 2 mg/l significantly increased callus fresh weight comparing to control. There was no significant difference between the two concentrations of 2, 4- D up to 30 day's culture. Reculture of callus on the same concentrations up to 60 day resulted in more increase in callus weight.

In this regard, Song *et al.* (1991) working on leaf segments of *foeniculum vulgare* mentioned that callus embryogenesis induction was highest on MS medium supplemented with 0.01 mg/l 2, 4-D alone. Also, Finnie and Vanstaden (1989) induced callus formation using 2, 4-D on MS medium for *Sandersonia* and *Gloriosa* plants. An emberyogenic callus developed within 2 weeks from hypocotyls and primary leaf segments of *Cuminum cyminum* explants on MS medium containing 0.8 mg/l 2, 4- D alone or plus 0.4 or 0.8 mg/l kinetin (Azza and Noga, 2002).

2-Effect of BA concentrations and medium strength on shoots differentiation of *Achillea fragrantissima* plant:

Data in Table (2) and photo (2) show the effect of BA concentration and MS strength on shoots differentiated from callus culture.

It is clear that MS strength (full or $^1/_2$ strength) did not show significant differences on shoots number, shoot length or leaves number/shoot. On the other side a significant differences was found due to supplementing medium with BA up to 1.5 mg/l comparing to control (without BA). The addition of BA up to 1.5 mg/l resulted in significant increase for shoots number. All concentrations of BA resulted in similar response without significant differences for the all studied characters i.e. shoots number, shoot length and leaves number/shoot.

The interaction between BA concentrations and medium strength was insignificant. So using BA at 0.5 mg/l with $^1/_2$ MS medium strength is prefer to obtain suitable shoots number from explants leaves callus of *Achillea fragrantissima*.

Similar findings were reported by Azza and Noga (2001) on cumin hypocotyle and stem internodes explants of *Cuminum cyminum* since, they found that the best response towards shoot initiation on MS medium was obtained with 0.56 mg/l BA, while no shoot initiation could be obtained on medium lacking BA. Also, Lee *et al.* (1994) found that BA concentrations at 0.1 and 0.3 mg/l induced shoot formation on excised leaf sections of *Lance coreopsis*. Moreover, El-Sawy *et al.* (2000) showed that the type of cytokinins is an important factor for bud shoot development. BA was the most effective cytokinins for promoting shoot production of *Dracina marginata*.

3-Effect of MS medium strength and IAA concentrations on rooting of *Achillea fragrantissima* plant

Data in Table (3) show that $^1/_4$ strength of MS medium was significantly effective in increasing roots number and root length of *Achillea fragrantissima* shoots. The effect of IAA on root number and root length indicate that using IAA at 0.1 or 0.2 mg/l were the more effective treatments in significantly enhancing those parameters comparing to control or the high concentration 0.3 mg/l.

The results of interaction between medium strength and IAA were significant for both parameters, where $^1/_4$ MS strength when combined with any concentration of IAA was significantly effective than $^1/_2$ MS strength. However it appears that $^1/_4$ MS strength combined with 0.1 mg/l IAA was satisfy to obtained good rooting process of Achillea shoots.

In this regard Rout *et al.* (2000) induced rooting of *Psoralea corylifolia* L. shoots within 6-8 days of culture on half strength of MS medium supplemented with IAA at 0.005- 0.01 mg/l and 2% sucrose. Rooting was drastically reduced and friable callus formed when the medium supplemented with a higher concentration of auxin. Also, Ebrahimie *et al.* (2003) found that the combination of 1/4 MS and 0.2 mg/ IAA gave the highest number of roots and root length of *Cuminum cyminum* plantlets.

The acclimatization:

The plantlets were successfully acclimatized on peat moss: sand (1:1) medium with 90% survival percentage of plants, photo (3).

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Table (1): Effect of 2, 4-D concentrations on callus fresh weight (gm) of *Achillea fragrantissima* plant

2,4-D concentrations (mg/l)	Culture age	
	30 days	60 days
0.0	1.00	1.50
1.0	1.68	5.78
2.0	1.76	6.14
L.S.D. at 0.5%	0.59	1.18
L.S.D. at 0.1%	N.S.	1.71

Table (2): Effect of BA concentrations and medium strength on shoot number, shoot length and leaves number/shoot of *Achillea fragrantissima* plant

Medium strength	BA Concentrations(mg/l)				Medium average
	0.0	0.5	1.0	1.5	
			No. of shoots		
Full MS	0.20	2.20	2.60	2.60	1.90
$^1/_2$ MS	0.00	3.00	2.20	3.00	2.05
BA average	0.10	2.60	2.40	2.80	----
L.S.D. Medium at 0.5% N.S.		L.S.D. BA at 0.5%	0.58	L.S.D. Interaction at 0.5%N.S.	
L.S.D. Medium at 0.1% N.S.		L.S.D. BA at 0.1%	0.76	L.S.D. Interaction at 0.1%N.S.	
			Shoot length (cm)		
Full MS	0.60	2.40	2.60	3.20	2.20
$^1/_2$ MS	0.00	2.80	2.40	2.80	2.00
BA average	0.30	2.60	2.50	3.00	----
L.S.D. Medium at 0.5% N.S.		L.S.D. BA at 0.5%	0.82	L.S.D. Interaction at 0.5% N.S.	
L.S.D. Medium at 0.1% N.S.		L.S.D. BA at 0.1%	1.06	L.S.D. Interaction at 0.1% N.S.	
			Number of leaves/shoot		
Full MS	0.20	2.60	2.00	2.60	1.85
$^1/_2$ MS	0.00	2.40	2.40	2.40	1.80
BA average	0.10	2.50	2.20	2.50	----
L.S.D. Medium at 0.5% N.S.		L.S.D. BA at 0.5%	0.68	L.S.D. Interaction at 0.5% N.S.	
L.S.D. Medium at 0.1% N.S.		L.S.D. BA at 0.1%	0.89	L.S.D. Interaction at 0.1% N.S.	

Table (3): Effect of MS medium strength and IAA concentrations on roots number/plantlet and root length of *Achillea fragrantissima* plant

MS Medium strength	Roots number/shoot					Root length (cm)				
	IAA Conc.(mg/l)				MS Medium average	IAA Conc.(mg/l)				MS Medium average
	0.0	0.1	0.2	0.3		0.0	0.1	0.2	0.3	
$^1/_2$ MS.	1.00	1.80	2.80	0.00	1.40	1.20	1.80	2.60	0.00	1.40
$^1/_4$ MS.	1.00	2.80	2.60	2.60	2.25	1.60	2.40	2.20	3.00	2.30
IAA average	1.00	2.30	2.70	1.30	----	1.40	2.10	2.40	1.50	----
L.S.D. Medium at 0.5%				**0.36**					**0.04**	
L.S.D. Medium at 0.1%				**0.47**					**0.05**	
L.S.D. IAA at 0.5%				**0.51**					**0.06**	
L.S.D.IAA at 0.1%				**0.67**					**0.08**	
L.S.D. Interaction at 0.5%				**0.72**					**0.09**	
L.S.D. Interaction at 0.1%				**0.95**					**0.11**	

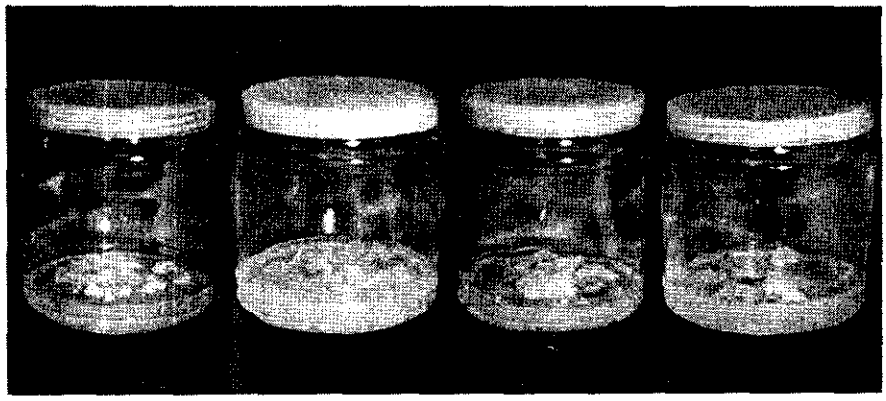

Photo. (1): Effect of 2, 4-D on callus fresh weight of *Achillea fragrantissima*

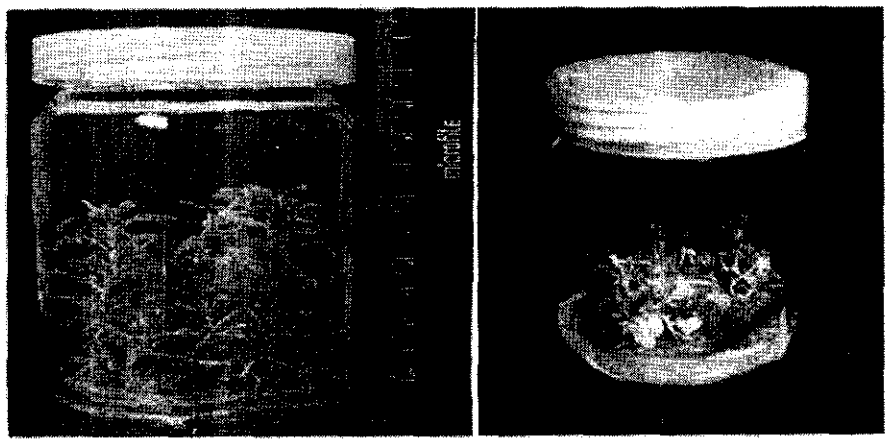

Photo. (2): Effect of BA and medium strength on shoots differentiation of *Achillea fragrantissima*

Photo. (3): Seedling of *Achillea fragrantissima* **after 60 days from** acclimatization

بعض العوامل المؤثرة على الإكثار الدقيق لبعض النباتات الطبية البرية
2- نبات الاشيليا

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زراعة أجزاء أوراق نبات الاشيليا في بيئة موراشيج وسكوج مضافا إليها ٢، ٤- ثنائي كلورو فينوكسى حمض ألخليك بتركيز ١ أو ٢ مليجرام/لتر، أعطت أعلى وزن طازج للكالس بعد ٣٠ و٦٠ يوم بدون فروق معنوية بين التركيزين.

بيئة موراشيج وسكوج كاملة أو نصف قوة أعطت فروق غير معنوية في عدد الفروع وطولها وعدد الأوراق/فرع، بينما تركيز ٠,٥ مليجرام/لتر بنزيل أدنين كان كافي لزيادة طول وعدد الفروع وعدد الأوراق/فرع.

أفضل تجذير وطول للجذور تم الحصول علية عند بيئة قوتها ٤/١ موراشيج وسكوج متحدة مع ٠,١ مليجرام/لتر أندول-٣-حمض ألخليك.

تم إجراء عملية أقلمة للبادرات الناتجة في بيئة بيت موس: رمل بنسبة (١:١) حيث كانت نسبة النباتات الحية ٩٠ %.