JJBS

Volume 11, Number 3,July 2018
ISSN 1995-6673
Pages 265 - 271

Jordan Journal of Biological Sciences

In Vitro Multiplication of the White Wormwood, *Artemisia herba-alba asso*

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Received October 24, 2017; Revised November 27, 2017; Accepted December 15, 2017

Abstract

Artemisia herba-alba is a wild aromatic medicinal shrub which grows in the arid areas of North Africa and the Middle East. It is heavily subjected to loss and threats due to heavy grazing, cultivation and collection by the people who used it in folk medicine. *In vitro* propagation was experimented with different combinations of plant growth regulators to develop a suitable protocol to propagate this plant. *In vitro* microshoots of *A. herba-alba* were initiated from seeds. A maximum germination of seeds (94 %) was obtained using Paper-Bridge inserted in half Murashige and Skoog (MS) media and supplemented with 15 g/L of sucrose and 1.0 mg/L Gibberellic Acid (GA_3). Proliferation of the *in vitro* plant was experimented at different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mg/L) of 6-Benzylaminopurine (BAP), kinetin, or 6-(gamma, gamma- Dimethylallylamino) purine (2iP). Rooting was experimented at different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mg/L) of, Indole-3-acetic acid (IAA), Indole-3-butyric acid (IBA) or alpha-Naphthalene acetic acid (NAA). The maximum proliferation of the *A. herba-alba* (23.6) microshoots was obtained when MS medium was supplemented with 1.0 mg/l 2iP and 1.5 mg/L GA_3. The maximum root number (18.8) and length (14.08 cm) was achieved using 0.5 mg/L IBA. Both IAA and NAA failed to promote root induction. The rooted plants acclimatized successfully with 50 % of survival and were grown in the greenhouse. These results indicate the enormous potential of *A. herba-alba* for a large-scale multiplication, and it represents the first step to conserve its germplasm.

Keywords: *Artemisia herba-alba*, White wormwood, Shih, *In vitro* culture, Micropropagation, Seeds germination

1. Introduction

Artemisia herba-alba belongs to the Asteraceae family. It is a perennial dwarf shrub that grows in arid and semi-arid climates. It is an attribute of the steppes and deserts of the Middle East, North Africa, Spain, extending into the north western Himalayas (Haouari and Ferchichi, 2009; Hedi *et al*., 2010). *A. herba-alba* is a native plant of Jordan (locally known as Shih). This plant is used locally in folk medicine due to its high content of essential oils, sesquiterpene lactones, and other chemical compounds (Aburjai *et al*., 2007; Mohamed *et al.*, 2010; Sharaf *et al*, 2012).

Due to the increased demand and consumption of *A. herba-alba* for medicinal purposes, the spreading out of urbanization and overgrazing, there is a need to conserve this valuable genetic resource from extinction through mass production. Plant tissue culture techniques are considered the most effective methods for propagating a high number of plant species (George, *et al*., 2008). Stem cuttings and seed multiplication methods are not always suitable to all conditions depending highly on the species (Gurib-Fakim, 2014). Plant tissue culture enables the mass propagation of uniform plants, and helps overcome the problems of propagation. Hundreds or even thousands of rooted plants will be successfully acclimatized from a few plant materials in a short time. Different levels of various plant growth regulators (PGRs) were used in the previous studies to induce proliferation and rooting *in vitro* for some medicinal plants (Al- Qudah, *et al*., 2011; Evenor and Reuveni, 2004; Mostafa, *et al*., 2010; Musallam, *et al*., 2011; Owies, *et al*., 2009). The application of the micropropagation technique is to multiply plants rapidly in a short time. This technique produces plants that are more likely to maintain genotypic and phenotypic fidelity to the original clone (George, *et al*., 2008; Hofman, *et al*., 2002; Machakova, *et al*., 2008; Shatnawi, *et al*., 2007; Staden, *et al*., 2008).

Different *Artemisia* species were *in vitro* propagated using micropropagation or organogenesis, such as *A. scorpia* (Aslam *et al*., 2006); *A. vulgaris* (Govindaraj *et al.*, 2008); *A. mutellina* (Mazzetti and Donato, 1998); *A. annua* (AL Maarri and Xie, 2010). According to the Jordan Plant Red List (Taifour and El-Oqlah, 2014), *A. herba-alba* was among the LC (least concern) endangered

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species. However, without the efforts and the interests in its multiplication, *A. herba-alba* may enter the endangered species list.

This study is aimed at utilizing tissue culture techniques for the sake of rapid multiplication of the *A. herba-alba* by developing a protocol for *in vitro* multiplication, rooting and *ex vitro* acclimatization of *A. herba-alba.*

2. Materials and Methods

2.1. Seeds Germination

Seeds of *A. herba-alba* were obtained from the National Center for Agricultural Research and Extension (NCARE). The seeds were surface-sterilized by washing thoroughly under running tap water for fifteen minutes with a few drops of mild detergent, antibacterial soap. They were then dipped in an antiseptic solution of 0.6 % sodium hypochlorite for five minutes, followed by washing under running tap water for fifteen minutes. After that the seeds were transferred into ethanol 70 % (v/v) for thirty seconds, and were rinsed with sterile distilled water three times (fifteen minutes each) under laminar airflow cabinet. The following media treatments were used to test the germination percentages:

MT1: Control: Agar media: the seeds of *A. herba-alba were* sowed on water media with 8 g/L agar in an aseptic plastic Petri dish.

MT2: Paper-Bridge on MS liquid media: The seeds were sowed on the top of a filter paper bridge inserted in MS (Murshige and Skoog, 1962) liquid medium for germination.

MT3: Paper-Bridge on half MS liquid medium: The seeds were sowed on the top of a filter paper bridge inserted in half MS liquid medium.

MT4: Paper-Bridge on half liquid MS with Gibberellic Acid (GA_3) media: The seeds were sowed on the top of a filter paper bridge inserted in half MS liquid medium.

MT5: Gibberellic Acid (GA_3) water media: The seeds were sowed on water media supplemented with 1 mg/L GA_3 and solidified with 8 g/L agar dispensed in a small aseptic plastic Petri dish.

MT6: Gibberellic Acid (GA_3) with solid full MS media: The seeds were sowed on MS media with 1 mg/L GA_3 and solidified with 8 g/L agar, dispensed in a small aseptic plastic Petri dish.

MT7: Gibberellic Acid (GA_3) with half MS media: The seeds were sowed on half MS media with 1 mg/L GA_3 and solidified with 8 g/L agar dispensed in a small aseptic plastic Petri dish.

Data on the percentage of seed germination were recorded after six weeks.

For further growth, i*n vitro* seedlings were transferred to the growth room under conditions of sixteen hours of light / eight hours of dark according to the photoperiod regime, photosynthetic photon flux density (PPFD) = 40-45 µ mol. /m^2 /sec), and a temperature of 24 ± 1 °C). Afterwards, the aseptically-grown cultures were directly sub-cultured to MS media without growth regulators for further growth. In order to establish mother stock plants, microshoots (5-7 mm) from seedlings were subcultured on MS medium with 1.0 mg/L Benzylamino purine (BAP), and 1.0 mg/L GA_3. Subculturing was performed by transferring the microshoots (5-7 mm) to a fresh medium every four weeks.

2.2. In vitro Multiplication

Shoot tips (STs) of the microshoot (5-7 mm) were subcultured in Erlenmeyer flasks (250 mL) with 100 mL of solid MS medium containing 3 % (w/v) sucrose, 0.8 % (w/v) agar supplemented with different concentrations (0.0, 0.5, 1.0, 1.5, and 2.0 mg/L), of BAP, Kinetin (6-Furfurylaminopurin) or 6-(Dimethylallylamino)-purine (2iP) with 0.1 mg/L 1-nephthaleneacitic acid (NAA), for a multiple shoot induction. In another experiment the microshoots (5-7 mm) were subcultured on MS medium supplemented with different concentrations of GA_3 (0.0, 0.5, 1.0, 1.5, 2.0 and 3.0 mg/L) to study the effect of gibberellic Acid (GA_3) on the microshoot proliferation. Also, GA_3 at 1.0 mg/L was used with different concentrations (0.0, 0.5, 1.0, 1.5 and 2.0 mg/L) of BAP, 2iP or Zeatin for their effect on multiplication. Data were recorded after six weeks including the number of proliferated microshoots, the microshoot height, callus formation and root formation.

2.3. In vitro Rooting

The microshoots were grown on hormone-free MS medium for one week to eliminate any carry over effect of any hormones that might affect the rooting. To induce rooting, individual microshoots (5-7 mm long) were isolated and transferred to 25×150 mm culture tube containing 12 mL agar-gelled MS rooting medium supplemented with different concentrations (0.5, 1.0, 1.5, and 2.0 mg/L) of IBA(Indol-3-butric acid), NAA or IAA (Indol-3-acetic acid). One set of cultures in which the seeds were sowed on basal MS medium without the addition of auxins and were kept as control. Six weeks later, the cultures were evaluated on the basis of the number and length of the roots as well as the shoots height.

2.4. Ex vitro Acclimatization

The plantlets with well-developed roots were removed from the culture medium. After washing the roots gently under running tap water (to remove the adhering medium), the plantlets were transferred to plastic cups (10-cm diameter) containing a growing medium (1 peat: 1 perlite) mixture. Each cup was covered with a perforated plastic bag to reduce evaporation, and was irrigated with distilled water every two days for three weeks followed by tap water for two weeks. After that, the survival percentage was recorded, and the acclimatized plantlets were maintained for four more weeks in greenhouse conditions at a temperature of 33 ±1°C.

2.5. Experimental Design and Statistical Analysis

The experiments were arranged in complete randomized design (CRD) with ten replicates for each treatment. The data were analyzed using SAS program and analysis of variance (ANOVA). Means were separated using the Least Significance Difference (LSD) at 0.05 probability level.

3. Results and Discussion

3.1. Seed Germination

Germination started after one week in all culture media, and the radical emergence was evaluated as a main indicator for the seed germination percentage. Full MS medium decreased the germination percentage of *A. herba-alba* seeds (Table 1). The high concentration of MS salt affected the germination percentage of *A. herba-alba* negatively; this is may be attributed to the fact that an increase in the osmotic potential prevents the imbibition of water and germination (Kaufman, 1969). The results showed that the highest germination (94 %) was obtained using paper bridge with half MS liquid media containing 1 mg/L GA_3, followed by (90 %) germination on half MS solid media containing 1 mg/L GA_3, (86 %) germination on solid MS media, and (82 %) germination on water and GA_3 media.

In vitro germination of most seeds was achieved by the use of Murashige and Skoog, (1962) medium. In this experiment, the paper bridge with half MS liquid and Gibberellic Acid (GA_3) medium yielded the highest germination percentage (94 %) as shown in Figure 1. Supplementing GA_3 in the germination medium enhanced the germination percentage of *A. herba-alba* seeds in both MS and ½ MS media, indicating that GA_3 promotes the seed germination. Iglesias and Babiano (1997) reported that Gibberellic acid (GA_3) affected the primary dormancy by inducing germination. Similar results were achieved when supplementing the medium with 2.0 mg/L Gibberellic Acid (GA_3) for Persian oregano "*Origanum vulgare* L." and Arabian oregano "*Origanum syriacum* L." (Arafeh *et al.*, 2006).

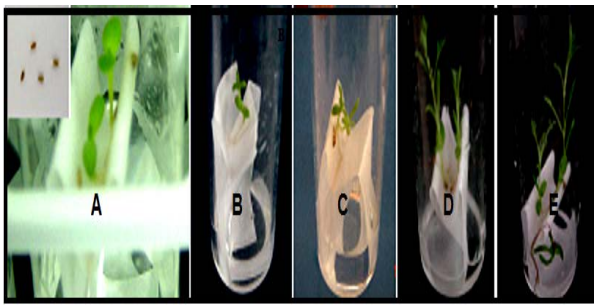

Figure 1. Seedlings of *Artemisia herba-alba* germinated on paper bridge with half MS liquid and Gibberellic Acid (GA_3) medium. (A-E): Seedlings after 1-5 weeks; respectively.

Table 1. Effect of different media on seed germination percentages of *Artemisia herba-alba*.

Germination Medium	Seed germination %
MT1: Control: Water media solid medium	48 c*
MT2: Paper-Bridge + MS liquid medium	28 d
MT3: Paper-Bridge + half MS liquid medium	54 c
MT4: Paper-Bridge + half MS liquid medium	94 a
MT5: Gibberellic Acid (GA_3) water media	82 ab
MT6: Gibberellic Acid (GA_3) MS solid medium	86 ab
MT7: Gibberellic Acid (GA_3) half MS media	90 ab

*Means within column followed by the same letter (s) are not significantly different using LSD at 0.05.

A. herba-alba responded significantly to the increased BAP concentrations up to 1.0 mg/L (Table 2). The shoot length and the number of microshoots, produced from the cultures on MS medium supplemented with (0.0, 0.5, 1.0, 1.5 and 2.0) mg/L BAP, were significantly different. The maximum shoot height (2.5 cm) was obtained from control treatment. Formation of callus occurred at the basis of treatments (including the control), but the largest occurred at 1.5 $mg.L^{-1}$ BAP. The best concentration of BAP which produced the highest shoot number (16.4 microshoots per explants) was 1.0 mg/L BAP. BA has been used to induce multiple shoots in *A. pallens* (Sharief and Chandra, 1991), and *A. vulgaris L.* (Sujatha and Kumari, 2007). Results of Al-Qudah *et al.*, (2011) also showed successful *in vitro* proliferation (8.8 shoot per explants) of *Teucrium polium* L. by using 2.0 mg/L of BAP combined with 0.1 mg/L NAA. Moreover, (Shibli *et al*., 2006) reported that higher BAP concentrations (1.5-2 mg/L) increased the number of microshoots, but it decreased the shoot length of *In vitro* propagated apple-root stock (MM.106). Similarly, *O. vulgare* explants were reported to give the highest shoot number on MS medium supplemented with high levels (1.6 and 2.0 mg/L) of BAP (Arafeh *et al.*, 2006).

Significant variations were obtained among concentrations of kinetin on *in vitro* shoot multiplication of *A. herba-alba* (Table 2). A low concentration of kinetin (0.5 mg/L) gave the highest number of shoots (8.20) of *A. herba-alba*, however, increasing the concentration of kinetin to 1.5 and 2.0 mg/L significantly reduced the number of shoots per explant. The highest shoot length (2.5 cm) was obtained at the control. Adding kinetin to the media negatively affected the shoot length of *A. herba-alba*. In addition to the control treatment, callus formation was only found in the medium supplemented with 0.5 mg /L kinetin indicating that higher kinetin in the culture medium of *A. herba-alba*, suppressed the callus formation. In contrast, Musallam *et al.*, (2011) reported highest callus percentage 90 % in the explants of *Capparis spinosa* on medium supplemented with a high concentration (2.0 mg/L) of kinetin. The highest shoot length (2.9 cm) of *Teucrium polium* L. was also obtained at a low level (0.4 mg/L) of kinetin (Al-Qudah *et al.*, (2011). However, the high concentration of kinetin oppositely affected the proliferation of some plants. For example, Bouhouche and Ksiksi, (2007) reported that, the highest *in vitro* proliferation rate of *Teucrium stocksianum* Boiss was achieved on medium containing 3 mg/l kinetin and 0.5 mg/L IAA. A maximum shoot induction and the number of microshoots /explants were reported for *Pandorea jasminodes* when using 1 mg/L kinetin (Kancherla and Bhalla, 2001). The highest number (3.9) of shoots, shoot length (5.5 cm), and number of leaves/explants (26.2) of *O. vulgare* were obtained when the media were supplemented with 2.0 mg/L of kinetin (Arafeh *et al.*, 2006).

The best multiplication parameters and growth performance of *A. herba-alba* were obtained using 1.0 mg/L 2iP (Table 2, Figure 2), where the maximum number of the microshoots per explants was 22.9, and the longest shoots were obtained (2.61 cm) (Table 2). Callus formation appeared at all 2iP treatments, but increasing 2iP up to 2 mg /L reduced the callus size. This may be attributed to the activity of the plant hormone (2ip,

cytokinin) that exhibits either synergistic or antagonistic interactions at the cellular level (Danova *et al.*, 2017). Accordingly, this study suggests that the genotypic factor seems to be operating in response to particular concentrations of growth regulators (Lomin *et al.*, 2015). Higher concentrations of cytokinins more than 1.0 mg/L reduced the shoot numbers as well as the shoot length. It is well-known that exogenously supplied plant-growth regulators; strongly affect patterns of the plant growth and development in vitro. Logically, plant-growth regulator treatments at low or high concentrations have an impact on endogenous phytohormone homeostasis in the plant through multiple mechanisms (Danova *et al*., 2017). Cytokinin binding at specific concentration effects the plant growth depending on many factors such as the cytokinin receptors and the ligand specificity of receptors, and media conditions. Therefore, a special mechanism is required to control the cytokinin-responsive genes and the plant growth at specific concentration (Lomin *et al*., 2015). Sujatha and Kumari, (2007) reported that increasing the concentrations of cytokinin resulted in reducing the number of the microshoots of *A. vulgaris*. Catapan *et al.* (2000) reported that an average of 21 - 23 shoots could be induced from each nodal segment of *Phyllanthus caroliniensis* using MS medium supplemented with 0.5 – 1.0 mg/L 2iP. While, the lower concentration (0.25 mg/L) of 2iP failed to induce multiple shoots of *Psoralea corylifolia* L. in solid MS medium (Baskaran and Jayabalan, 2008).

Table 2. Effect of different cytokinins concentrations with 0.1 mg L^{-1} NAA, on the number of shoots, shoot length, callus width and length of *in vitro* grown *Artemisia herba-alba*

Cytokinin concentration mg/L	Shoot No.	Shoot Length (cm)	Callus Width (cm)	Callus length (cm)
		BA		
0.0	7.30 c*	2.50 a	1.30 b	1.20 c
0.5	10.50 b	1.71 b	1.49 b	1.56 b
1.0	16.4 a	1.70 b	1.92 a	1.30 c
1.5	7.50 c	1.39 c	1.87 a	1.90 a
2.0	11.6 b	1.34 c	0.88 c	0.81d
		Kinetin		
0.0	7.30 b*	2.50 a	1.30 b	1.20 a
0.5	8.20 a	1.19 bc	1.40 a	0.62 b
1.0	3.10 c	1.08 c	0.00 c	0.00 c
1.5	2.10 d	1.18 bc	0.00 c	0.00 c
2.0	3.50 c	1.29 b	0.00 c	0.00 c
		2 iP		
0.0	7.30 b*	2.50 a	1.30 c	1.20 c
0.5	8.80 b	2.02 b	2.08 a	1.86 a
1.0	22.9 a	2.61 a	1.45 bc	1.14 c
1.5	6.20 d	1.51 c	1.52 b	1.48 b
2.0	8.20 bc	1.57 c	0.46 d	0.52 d

* Means within columns for each cytokinin followed by the same letter (s) are not significantly different using LSD at 0.05.

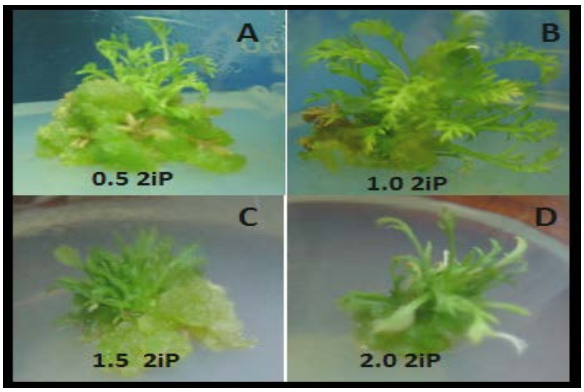

Figure 2. Effect of 2iP at different concentrations (A: 0.5, B: 1.0, C: 1.5 and D: 2.0 mg/L) on the microshoot proliferation of *A. herba-alba*.

Using GA_3 at 1.0 mg/L concentration gave the highest shoot numbers (14.2). While, the highest shoot length (3.41 cm) was obtained using GA_3 at a concentration of 1.5 mg/L (Table 3, Figure 3). Similar results were reported by Sujatha and Kumari (2007), who found that adding GA_3 to the media stimulated the shoot elongation of *A. vulgaris* L. The addition of GA_3 with BA or 6- (dimethyl-lallylamino)-purine (2iP) gave a similar response in Betula cultures (Jamison and Renfroe, 1998). GA_3 stimulates the elongation of shoots by inhibiting the action of auxins in meristematic regions (Taiz and Zeiger, 2014). Tian *et al.*, (2010), reported that GA_3 was beneficial for shoot and stem elongation.

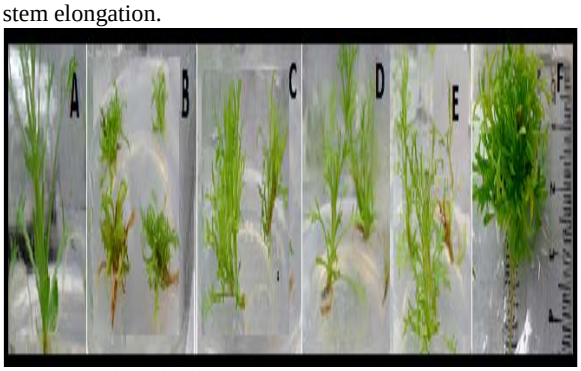

Figure 3. Effect of different GA_3 concentrations on *in vitro Artemisia herba-alba* grown on MS media (A): MS free medium, (B): 0.5 mg/L GA_3, (C): 1.0 mg/L GA_3, (D): 1.5 mg/L GA_3, (E): 2.0 mg/L GA3, (F): 3.0 mg/L GA_3.

Table 3. Effect of different GA_3 concentrations on the number of shoots, and shoot length of *in vitro* grown *Artemisia herba-alba*

GA_3 (mg/L)	Number of shoots	Shoot length (cm)
0.0	2.7 e*	1.45 e
0.5	4.0 d	1.98 d
1.0	14.2 a	2.50 c
1.5	7.5 b	3.41 a
2.0	7.0 b	2.50 c
3.0	5.8 c	2.90 b

* Means within each column followed by the same letter (s) are not significantly different using LSD at 0.05.

Adding GA_3 to the BA medium significantly increased the shoots length and number (Table 4). Combining GA_3 at (1.5 mg/L) with BA (0.5 mg/L) was effective in stimulating the shoot elongation (1.98 cm) of *A. herba-alba*. Similar results were obtained of the microshoots of *A. vulgaris L.* ; they were best elongated on MS medium containing 0.1 mg/L BA and 0.5 mg/L GA_3 (Sujatha and Kumari, 2007), and on Betula uber (Ashe) Fernald cultures (Jamison and Renfroe, 1998).

In the current study, supplementing the medium of GA_3 (1.5 mg/L) with different concentrations of 2iP increased the shooting length and number. The addition of (1.5 mg/L) GA_3 with (1.0 mg/L) 2iP effectively increased the shoot elongation (3.28 cm), and at the same time retained the highest number of shoots (23.6); (Table 4 and Figure 4). Similar results were reported for *Betula uber* (Ashe) Fernald, (Jamison and Renfroe, 1998).

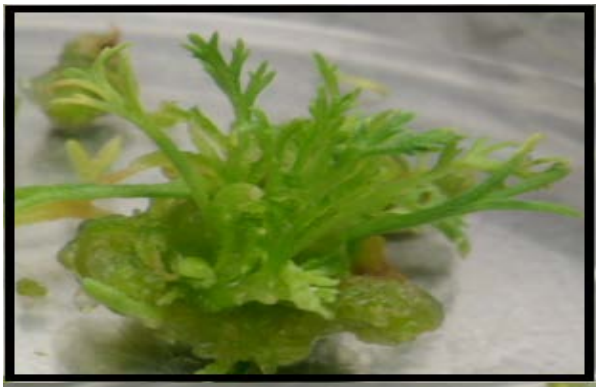

Figure 4. Effect of 1.5 mg/L GA_3 with 1.0 mg/L 2iP on *in vitro* grown *Artemisia herba-alba*.

Table 4. Effect of different cytokinins with concentrations plus 1.5 mg/L GA_3, on the number of shoots, shoot length and callus diameter of *in vitro* grown *Artemisia herba-alba*

Cytokinin (mg/L)	Number of shoots	Shoot length (cm)	Callus Diameter (cm)
BA			
0.0	8.1 d*	1.67 b	0.0 c
0.5	19.1 a	1.98 a	0.1 c
1.0	17.3 b	1.68 b	0.5 b
1.5	12.1 c	1.18 c	0.0 c
2.0	7.0 d	1.10 c	0.9 a
2ip			
0.0	8.1 d*	1.67c	0.0 c
0.5	16.6 b	2.90 b	0.0 c
1.0	23.6 a	3.28 a	0.0 c
1.5	15.2 bc	1.83 c	0.30 b
2.0	14.5 c	1.75 c	1.10 a
Zeatin			
0.0	8.1 d*	1.67 c	0.0 d
0.5	11.6 d	2.12 a	1.0 c
1.0	18.3 c	1.86 b	1.4 b
1.5	25.1 b	1.65 c	2.2 a
2.0	29.4 a	1.43 d	2.5 a

* Means within each column for each cytokinen followed by the same letter (s) are not significantly different using LSD at 0.05.

In the presence of GA_3, increasing the Zeatin concentration increased both the shoots' number and callus formation but with varied responses to shoot length (Table 4). Zeatin at 0.5 mg/L with 1.5 mg/L GA_3 gave the longest shoot (2.12 cm) with (11.6) shoot number and with less formation of callus. Among the different types of cytokinin tested, Pena-Ramirez *et al*., (2010), found that Zeatin was the most effective. However, a comparison of the relative effectiveness of different cytokinins for multiple shoot formation, Sharief and Chandra, (1991) reported that the most effective cytokinins in the order of their efficiency are: BA, Kinetin, Zeatin, and finally Adenine.

Among the different types of cytokinins (zeatin, BA, and 2iP) tested in this study, BA was the best. The explants cultured on BA medium performed healthy and strong plants. While using zeatin and 2iP resulted in some changes in the shape of leaves and the condensing of shoots (clusters).

3.2. In vitro Rooting

IBA gave the best root formation (root number and length) of *A. herba-alba in vitro* propagated plants (Table 5, Figure 5). *A. herba-alba* plants propagated at media supplemented with either IAA or NAA did not give any root. The maximum number of roots (18.8) resulted from the medium supplemented with 0.5 mg/L of IBA (Table 5). Root induction in *Teucrium stocksianum* Boiss was achieved on half-strength MS medium containing IBA (Bouhouche and Ksiksi, 2007). Similarly, *Coffee Arabica* microshoots were rooted on half MS medium supplemented with 3 mg/L of IBA (Ebrahim *et al.,* 2007). Also in *Talinum portulacifolium* L., roots development was facilitated by an MS medium supplemented with both 0.8 mg/L IBA and 0.2 mg/L NAA (Thangavel *et al.,* 2008). For *in vitro* grown *Origanium vulgare*, the highest number of roots was obtained at 1.6 mg/L IBA (Arafeh *et al*, 2006). Also in *Sealvia fruticosa* Mill, rooting was optimized at 0.6 mg/L IBA or 0.5 mg/L IAA (Arikat, 2004).

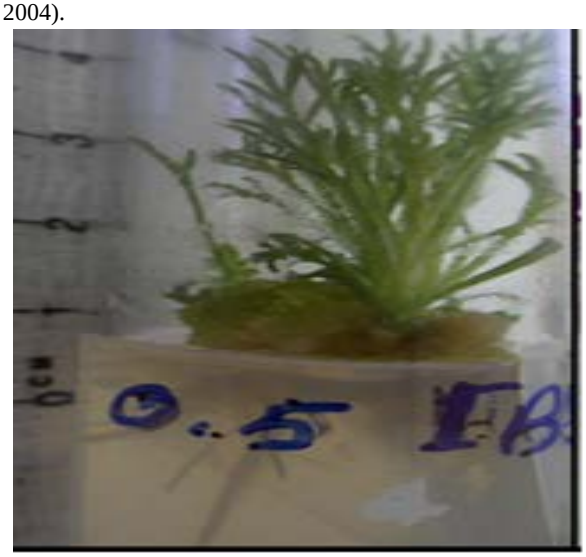

Figure 5. Effect of IBA at 0.5 mg/L on *in vitro* rooting of *Artemisia herba-alba* after 4 weeks.

Table 5. Effect of different IBA concentrations on shoot height, number of roots, root length and callus width of *in vitro* grown *Artemisia herba-alba*

IBA (mg/L)	Shoot length (cm)	No. of Roots	Root Length (cm)	Callus width (cm)
0.0	13.27 a*	12.70 b	8.22 b	1.00 c
0.5	11.57 b	18.8 a	14.08 a	2.80 a
1.0	1.72 cd	0.20 c	0.05 c	2.60 a
1.5	2.02 c	0.00 c	0.00 d	2.20 b
2.0	2.02 c	0.00 c	0.00 d	2.00 b

* Means within columns for each cytokinin followed by the same letter (s) are not significantly different using LSD at 0.05.

The current study concludes that the complexity and nonlinearity of spatiotemporal interactions between both cytokinin and auxins hormones and gene expression in root and shoot development, need modelling plant hormone gradients with a system approach in which experimental data and modelling analysis are closely combined.

3.3. Acclimatization

In vitro rooted plants of *A. herba-alba* showed a survival percentage of 50 %. Acclimatized plants appeared normal and did not exhibit any morphological abnormalities (Figure 6). The survival rate obtained in this study is lower than other micropropagated medicinal plants like *Origanium vulgare* 71 %, (Arafeh *et al.*, 2006), or *Capparis spinosa* 63 %, (Mussallam *et al.*, 2011). Some factors should be evaluated to improve the acclimatization stage for getting high plantlet survival rates.

Figure 6. Acclimatization of *Artemisia herba-alba*.

4. Conclusions

It can be concluded from this study that the seeds of *A. herba-alba* are considered a good starting material for establishing *in vitro* cultures due to the high percentage of *in vitro* germination. MS medium supplemented with plant growth regulators (0.5 mg/l BA + 1.0 mg/L GA_3) is considered suitable for mother stock multiplication. While 2iP 1.0 mg/L and BAP 1.0 mg/L gave higher shoot proliferation, satisfactory rooting was achieved only with IBA at 0.5 mg/L. Acclimatization of *A. herba-alba* resulted in (50 %) of acclimatized plants that remained healthy and showed normal growth in the greenhouse.

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