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In vitro Mass Propagation of Artemisia (*Artemisia annua* L.) cv: Anamed

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Abstract

An efficient *in vitro* propagation protocol was developed for anamed (A-3) cultivar of *Artemisia annua*. Two and 1.5% concentration of NaOCl treatment for 10 and 20 min were found to be optimum for sterilization of shoot tip and nodal explants, respectively. Maximum percentage (98.75 ± 2.50) shoot induction was observed from nodal explants cultured on MS supplemented with 0.8 mg/l BAP + 0.1 mg/l IBA followed by 82.50 ± 2.88% from shoot tip explants on the same medium with 0.8 mg/l TDZ for shoot tip explants. The highest number of shoots (8.05 ± 0.66/explant) was regenerated on MS + 1 mg/l BAP + 0.1 mg/l IBA. Best rooting with mean values of 18.25 ± 0.95/explant root number and root length (6.35 ± 0.10 cm) was recorded on 1/2 MS + 0.5 mg/l IBA. Up on acclimation and transplanting, 80% survival efficiency was observed on the soil mix ratio of 2 : 1 : 1 (decomposed coffee husk, forest soil and sand, respectively). The developed regeneration protocol enables a large scale commercial production and a possible system towards the genetic improvement of this crop.

Introduction

Artemisia (*Artemisia annua* L.) is a medicinal herb (Asteraceae). It is a crop for the production of artemisinin, a sesquiterpene lactone with anti-malarial effects against susceptible and multi-drug resistant *Plasmodium* species, originated in China (Tiruneh et al. 2010). Now, *A. annua* grows throughout the temperate regions of the world (Ferreira et al. 2005). In Africa, Artemisia is currently cultivated in Cameron, Ethiopia, Kenya, Mozambique, Tanzania, Uganda and Zambia - all in high-altitude regions and/or regions with a pronounced cool period (Ferreira and Janick 2002, EABL 2005).

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Artemisia species are one of the many traditional medicinal plants used for the treatment of infectious and non-infectious health problems. *A. annua* L. is one of the plant species listed the WHO monographs. The selection of medicinal plants for inclusion in the WHO monographs is based on their worldwide use (WHO 2003). At present, Artemisia is processed by pharmaceutical firms for the production of artemisin for Artemisin based combination therapies (ACTs) in the treatment of malaria (Ferreira and Janick 1995, WAC 2007). Three native *Artemisia* species (*A. absinthium*, *A. abyssinica*, and *A. afra*) are found in Ethiopia which was validated for the treatment of protozoal infections (Nibret and Wink 2010). *A. annua* was introduced to Ethiopia in 2001 in the form of traditional tea to cure malaria, asthma, hemorrhoid etc. The conventional propagation of Artemisia by seeds is a constraint for its production due to its tiny seeds that require a symbiotic association within its own microflora for germination. Micropropagation enables rapid rate of clonal multiplication of an elite plant species, allowing production of disease-free, genetically stable and uniform progenies (Hu and Wang 1983). Micropropagation of different *Artemisia* species such as *A. scorpia* has been previously established and reported by various authors (Aslam et al. 2006), *A. vulgaris* (Sujata and Kumari 2007, Govindaraj et al. 2008). There are a few reports on micropropagation of *A. annua* cultivars other than *anamed* using shoot explants. Most reports suggest a strong influence of genotype on the proliferation process (Elhag et al. 2006). Almaarri and Yu Xie (2010). To develop an efficient protocol that enables rapid *in vitro* multiplication of *A. annua* anamed (A-3) from shoot tip and nodal explants.

Materials and Methods

Healthy and vigorously growing young plants of *A. annua* A-3 cultivar raised from stem cuttings were obtained from Wondo-Genet Agricultural Research Center (WARC), then transplanted in pots, grown in the lath-house, and used as a source of explants (Fig. 1). Shoot tip explants (1 - 1.5 cm long) and nodal explants (1.5 - 2 cm long) from middle lateral branches were excised and washed three times with tap water and commercial liquid detergent using a piece of sponge. The cleaned explants were then transferred to the laminar flow hood, immersed in 70% (v/v) ethanol for 30 sec, rinsed in sterile distilled water three times and surface sterilized further using NaOCl disinfectant solutions for the specified time, as per the treatment combinations. Finally, the explants were rinsed four times with double distilled water.

The sterilized shoot tip and nodal explants were further cut down to 5 to 15 mm size by trimming all the dead and chlorine affected tissues prior to culture. They were then cultured in a plant growth regulator free medium of half

strength MS supplemented with 15 g/l sucrose, and solidified with 7 g/l agar-agar for 72 hrs. Explants that have shown early contamination and tissue death in the media were recorded and discarded.

For culture initiation, agar solidified (0.7% agar-agar) full strength MS added with 3% sucrose was supplemented with different concentrations of BAP and TDZ. The experiments were carried out with a treatment combination of four different concentrations of BAP (0, 0.4, 0.8, and 1.2 mg/l) and four different concentrations of TDZ (0, 0.4, 0.8, and 1.2 mg/l) alone and in combination at each concentration with 0.1 mg/l IBA. Experiments were carried out using CRD design in 4 × 4 × 2 × 2 factorial combinations.

Responsive shoot buds were transferred singly in a shoot multiplication medium. The shoot multiplication media used were composed of MS fortified with 30 g/l sucrose and different concentrations of BAP (0, 0.25, 0.5, 0.75, 1.0, 1.25 and 1.5 mg/l) each alone and interacting at each concentration with 0.5 mg/l Kn or 0.1 mg/l IBA. After three-four weeks, cultures proliferating shoot clumps were divided and subcultured on to a fresh medium.

Well developed microcuttings were transferred for rooting. For shoot elongation and rooting on agar solidified (0.7% agar-agar) half and full strength MS added with 3% sucrose was supplemented with different concentrations of IBA and NAA. Elongated plantlets (shoots with well-developed roots) were taken for acclimation. Individual *A. annua* plantlets were removed from the flasks and washed carefully under warm water to facilitate the removal of adhering agar from the root surface, rinsed in a fungicidal solution of 3% Kocide-101, and transferred to a sterilized potting mix of forest soil : sand : well decomposed coffee husk at a ratio (v/v) of 1 : 1 : 2 and kept under a plastic tunnel of high humidity (80 - 90%) to prevent desiccation for ten days. After the tenth day, plantlets were transferred to a 70% shade net, where they were kept for another month. Later, they were transferred to a 30% shade net where they were maintained for another month, prior to field transplanting.

The average of the data collected from two repetitions for each experiment were independently subjected to statistical analysis using the SAS statistical software (version 9.2) and ANOVA was constructed, followed by mean separation using appropriate procedures (REGWQ). When ANOVA indicated significant treatment effects (5, 1 or 0.1%) based on the F-test, probability level of 0.05 ($p \leq 0.05$) was considered to determine which treatments were statistically different from the remainder.

Results and Discussion

The effect of different concentrations of sodium hypochlorite solutions and different time durations of explants exposure to the sterilants were evaluated for determining the most effective treatment combination on sterilization of shoot tip and nodal explants. The analysis of variance showed that the concentration of sodium hypochlorite solution, time duration of explants exposure to the sterilants and interaction of concentrations to time duration had highly significant effect ($p < 0.0001$) on both of contamination and tissue death of shoot tips and nodal explants. The highly significant difference was also revealed between the two types of explants (treatment: explants = $p < 0.0001$) indicating that the level of contamination and tissue death was influenced by explants type and the mean average value for contamination and tissue death of shoot tips exceeded that of node explants.

The highest rate of contaminant free culture (48.33 ± 1.34) was obtained from treatment combinations of 1.5 and 2% concentration of NaOCl and 20 and 25 min exposure duration respectively for shoot tip explants. For nodal explants 1.5% concentration and 20 min exposure duration were found to be the most effective treatment combination with mean average result of 81.66 ± 1.34% contaminant free lively cultures (Table 1).

Increasing the sterilant concentration from 0.5 to 2% active chlorine maintaining 10 min exposure duration constant had reduced the rate of contamination from 86.66 ± 2.88% to zero and from 78.33 ± 2.88% to zero for shoot tips and nodal explants, respectively. However, only 48.33 ± 1.34% for shoot tips and 68.33 ± 2.88% for nodal explants survived to grow and develop. This effect was mainly attributed to the high level of tissue death i.e., 51.66 ± 2.88% for shoot tips and 31.66 ± 2.88% for nodal explants caused by the maximum concentration of the sterilant solution. Exposure duration of explants to the sterilant chemical also had significantly affected the effectiveness of the chemical in that it increased in time from 10 to 25 min at two per cent constant concentration had decreased the percentage of clean lively culture from 48.33 ± 1.34 to 36.66 ± 0.13 and from 68.33 ± 2.88 to 40.00 ± 0.00 for shoot tip and nodal culture, respectively increasing the rate of tissue death (Table 1). The rate of tissue death severely affected shoot tip explants than nodal explants as shoot tip tissues are relatively young and more susceptible to the chemical action.

Generally, occurrence of high contamination rate of culture at a relatively lower concentration and shorter exposure time treatment combinations was possibly due to the insufficiency of sterilant concentration and exposure duration to remove or kill the contaminant agents mainly fungi and bacteria. High

Table 1. Effects of different concentrations of NaOCl and time of exposure on level of contamination and tissue death of shoot tips and nodal cultures of *A. annua* cv. anamed.

Conc. (%)	Time (min)	Cont. (%) (shoot tips)	Tissue death (%) (shoot tips)	Clean culture (%) (shoot tips)	Cont. (%) (node)	Tissue death (%) (node)	% Clean culture (node)
		Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.	Mean ± S.D.
0	0	100.0a ± 0.00	0.00j ± 0.00	0.00i ± 0.00	100.0a ± 0.00	0.00g ± 0.00	0.0j ± 0.00
0.5	10	86.66b ± 2.88	0.00j ± 0.00	13.23h ± 0.13	78.33b ± 2.88	0.00g ± 0.00	21.6i ± 0.24
0.5	15	80.00c ± 5.00	0.00j ± 0.00	20.00g ± 0.00	75.00b ± 0.00	0.00g ± 0.00	25.0h ±0 .00
0.5	20	75.0c ± 5.00	0.00j ± 0.00	25.00f ± 0.00	66.66c ± 2.88	0.00g ± 0.00	33.3g ± 0.13
0.5	25	65.00d ± 0.00	3.33j ± 2.88	31.33e ± 0.13	60.00d ± 0.00	0.00g ± 0.00	40.0f ± 0.00
1.0	10	58.33e ± 2.88	5.00j ± 0.00	36.66d ± 0.13	56.66d ± 2.88	0.00g ± 0.00	43.3f ± 0.13
1.0	15	55.00e ± 0.00	11.66i ± 2.88	33.33 ± 0.13	50.00e ± 0.00	0.00g ± 0.00	50.0e ± 0.00
1.0	20	45.00f ± 0.00	16.66h ± 2.88	38.3cd ± 2.88	41.66f ± 2.88	0.00g ± 0.00	58.3d ± 0.13
1.0	25	35.00g ± 5.00	28.33g ± 2.88	36.66d ± 0.13	36.66g ± 2.88	3.33g ± 2.88	60.0d ± 0.00
1.5	10	25.00h ± 0.00	35.00f ± 0.00	40.00c ± 0.00	20.00h ± 0.00	11.66f ± 2.88	68.3c ± 1.34
1.5	15	13.33i ± 2.88	40.00e ± 0.00	46.66b ± 1.34	10.00i ± 0.00	13.33ef ± 2.88	76.6b ± 2.88
1.5	20	8.33ij ± 2.88	45.00d ± 0.00	46.66b ± 1.34	1.66j ± 2.88	16.66e ± 2.88	81.6a ± 1.34
1.5	25	3.33jk ± 2.88	48.33d ± 2.88	48.33a ± 1.34	0.00j ± 0.00	28.33d ± 2.88	71.6c ± 1.34
2.0	10	0.00k ± 0.00	51.66c ± 2.88	48.33a ± 1.34	0.00j ± 0.00	31.66d ± 2.88	68.3c ± 2.88
2.0	15	0.00k ± 0.00	55.00b ± 0.00	45.00b ± 0.00	0.00j ± 0.00	40.00c ± 0.00	60.0d ± 0.00
2.0	20	0.00k ± 0.00	60.00a ± 0.00	40.00c ± 0.00	0.00j ± 0.00	53.33b ± 2.88	46.6ef ± 1.34
2.0	25	0.00k ± 0.00	63.33a ± 2.88	36.66d ± 0.13	0.00j ± 0.00	60.00a ± 0.00	40.00f ± 0.0

Means with the same letter in a column are not significantly different using Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test at $\alpha = 0.05$.

concentration of NaOCl and long exposure duration of explants in the sterilant solution resulted better removal of microbes due to the powerful oxidant property of active chlorine that disintegrates the lipids in the cell wall of bacteria and fungi. The effect of the sterilant chemical could also alter or denature the shape and function of microbial enzymes (George et al. 2008). However, the increase in sterilant concentration and exposure time above certain optimum limit cause loss of explants because of the oxidant chemical ingredient killing the plant tissue as well. Hence, the optimum treatment combination (concentration and time) for effective sterilization of explants should be determined based on the two aspects of the observations, i.e. a relatively minimum level of contamination as well as tissue death that gives the maximum percentage of clean lively culture as indicated in Table 1.

The present result is partially in agreement with Nair et al. (1986) who reported the minimum level of contamination with 1.5% active chlorine in NaOCl solution for 15 min. Almaarri and Yu Xie (2010) similarly reported 1% chlorox (a commercial preparation of NaOCl with 5.25% active chlorine) for 15 min as effective treatment combination for sterilization of shoot tip leaf and nodal explants. Sujata and Kumari (2007) reported the use of 0.1% mercuric cloride for 3 min as effective treatment to remove microbes from shoot tip and nodal explants on initiation of aseptic culture *in vitro*. The use of mercuric chloride however, is highly discouraged as such chemicals have been known to have a serious harmful residual effect to both human and the environment. Thus, the trend is to try for the substitute with a relatively safe or less harmful chemical such as NaOCl solution as employed in this study.

The nodal explants gave greater response than shoot tip explants (Table 2). Although it requires further investigation to be carried out, this might be related to the variation in endogenous level of auxin in shoot tip- and nodal sections of the given genotype (Hopkins and Huner 2004).

The highest rate of shoot induction (98.75 ± 2.50%**)** was obtained on MS medium supplemented with 0.8 mg/l BAP and 0.1 mg/l IBA from nodal explants while shoot tip (82.50 ± 2.88%) on MS containing 0.8 mg/l TDZ (Table 2). For both shoot tip and nodal explants, MS supplemented with 0.8 mg/l BAP or 0.8 mg/l TDZ used alone or in combination with 0.1 mg/l IBA were found to be the optimum media for *in vitro* shoot initiation of *A. annua* (Table 2). From all treatments the minimum rate of shoot induction was observed on MS containing 0.4 mg/l TDZ and 0.1 mg/l IBA (1.25 ± 2.50% and 6.25 ± 4.78%) for shoot tip**-** and nodal explants, respectively.

Shoot bud development capacity of both shoot tip- and nodal cultures increased with the increase in concentration of BAP and TDZ from zero to 0.8

mg/l and reduced with further addition of both BAP and TDZ. This could be due to the exogenous application of cytokinins which release shoot buds from apical dominance. The addition of optimum amount, however, might reduce shoot induction rate by inhibiting the availability of the required endogenous amount of auxin for shoot initiation as cytokinins do not act alone unless combined with auxins.

Table 2 Effects of BAP, TDZ and IBA on *in vitro* shoot induction rate of *A. annu*a from shoot tip and nodal explants culture on MS.

Plant growth regulators			Shoot bud induction (%)	
BAP (mg/l)	TDZ (mg/l)	IBA (mg/l)	Shoot tip	Node
0	0	0	6.25h ± 4.78	0.00i ± 0.00
0.4	0	0	42.50cd ± 2.88	53.75c ± 2.5
0.8	0	0	81.25a ± 2.50	93.75a ± 4.78
1.2	0	0	30.00e ± 0.00	36.25e ± 2.50
0	0.4	0	40.00d ± 0.00	51.25c ± 2.50
0	0.8	0	82.50a ± 2.88	88.75a ± 2.50
0	1.2	0	26.25f ± 2.50	31.25f ± 2.50
0.4	0	0.1	22.50g ± 2.88	25.00g ± 0.00
0.8	0	0.1	78.75b ± 2.50	98.75a ± 2.50
1.2	0	0.1	46.25c ± 2.50	50.00c ± 2.50
0	0.4	0.1	1.25i ± 2.50	6.25h ± 4.78
0	0.8	0.1	67.50bc ±2.88	85.00a ± 0.00
0	1.2	0.1	40.00d ± 0.00	46.25d ± 2.50

Means with the same letter in a column are not significantly different as determined by Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test at $\alpha = 0.05$.

A low concentration of auxin together with a relatively high concentration of cytokinin is reported to be useful for shoot induction; although it has been repeatedly reported that exogenous auxin does not promote axillary shoot proliferation (George et al. 2008). Such an effect was observed in the present study that, the combination of BAP and IBA produced the best response when the low concentration of auxin (0.1 mg/l IBA) is combined with a relatively higher concentration of cytokinins (Table 2). The combination of 0.4 mg/l BAP and 0.1 mg/l IBA gave no or very low response of shoot bud induction rather than callus formation while the same amount of IBA with 0.8 mg/l BAP produced the highest shoot induction and even with the maximum 1.2 mg/l BAP better results were obtained (Table 2). This backs up the knowledge that, it is the ratio of auxin to cytoknin, not the absolute level of auxin that suppresses shoot bud growth (Hartmann et al. 2009).

The present result of shoot induction rate is in agreement with the report of Banyai et al. (2005) who considered 1 mg/l BAP with 0.1 mg/l NAA as the best supplemented medium for leaf-explants-derived shoot regeneration. Almaarri and Yu Xie (2010) reported 100 and 66.6% shoot induction in different genotypes of *A. annua* on MS fortified with TDZ (1 mg/l) and BAP (1 mg/l), respectively. Similar results have also been reported by Sujata and Kumari (2007).

Those shoot buds that performed well on the prevailing shoot induction medium were transferred into MS supplemented with BAP (0.25 - 1.5 mg/l) alone and in combination with 0.5 mg/l Kn or 0.1 mg/l IBA. Cultures were subcultured twice and the effect of hormones on *in vitro* shoot multiplication of *A. annua*, anamed cultivar was evaluated.

In this study, the significance of BAP and the interaction of BAP with Kn and IBA were considered. The ANOVA revealed that the concentration of BAP both alone and in combination with Kn or IBA had a highly significant effect ($p < 0.0001$) on shoot multiplication rate. Shoot buds raised from both shoot tip- and nodal explants responded almost similarly on shoot multiplication indicating the non-significant effect of explants at this stage. The proliferation rate had shown a progressive increase from the first sub-culture to the second by an average of 33.3%.

In this study, the best result (number) of shoot proliferation (10.20 ± 1.44) was obtained on MS containing 0.75 mg/l BAP and 0.5 mg/l Kn (Table 3). Nevertheless, the microshoots in this medium were less vigorous and bushy as compared to MS fortified with 1 mg/l BAP and 0.1 mg/l IBA (Fig. 1) resulting in the production of 8.05 ± 0.66 shoots (Table 3). In MS containing 0.75 mg/l BAP 9.25 ± 2.88 shoots with a good morphological appearance were observed. Shoot buds on MS fortified with 0.25 mg/l BAP and 0.1 mg/l IBA have totally changed into callus while at 0.5 mg/l and 0.75 mg/l BAP together with the same amount of IBA had given better and good shoot proliferation of adventitious origin. The swollen callus like tissue on these media was not friable that may be grouped as organized and semi-organized callus.

An increase in number of shoots per shoot bud culture with increased concentration of BAP from zero up to 1 mg/l might be due to the effect of BAP in releasing primordia of lateral buds from dormancy or breaking apical dominance by inhibiting the level of endogenous auxins. Cultures on a higher level of above 1 mg/l BAP had developed into bushy and ill defined shoot buds and they did not respond when subcultured on the same medium.

The present results are in agreement with those of Banyai et al. (2005) who reported best proliferation rate on MS + 1 mg/l BAP + 0.1 mg/l NAA. On the other hand, Elhag et al. (1991) reported the development of 14 - 28 microshoots

per shoot bud for different genotypes other than cv. anamed of *A. annua* from shoot tip and lateral buds cultured on both full and half MS fortified with 3 mg/l BAP. In this study however, cultures on MS containing above 1 mg/l BAP performed poorly in morphological appearance as well as in number.

Table 3. Effect of BAP, Kn and IBA on *in vitro* shoot multiplication of *A.annua* cv. anamed.

Plant growth regulators			Mean number of
BAP (mg/l)	Kn (mg/l)	IBA (mg/l)	shoots per shoot bud
0.25	0.00	0.00	2.50n ± 0.66
0.50	0.00	0.00	5.65i ± 0.05
0.75	0.00	0.00	9.25b ± 2.88
1.00	0.00	0.00	8.40c ± 1.44
1.25	0.00	0.00	4.45l ± 1.44
1.50	0.00	0.00	3.60m ± 0.66
0.25	0.50	0.00	5.00k ± 0.00
0.50	0.50	0.00	7.75e ± 2.88
0.75	0.50	0.00	10.20a ± 1.44
1.00	0.50	0.00	8.20cd ± 1.44
1.25	0.50	0.00	6.40h ± 1.44
1.50	0.50	0.00	3.55m ± 0.05
0.25	0.00	0.10	0.00o ± 0.00
0.50	0.00	0.10	3.45m ± 0.66
0.75	0.00	0.10	6.75g ± 0.66
1.00	0.00	0.10	8.05d ± 0.66
1.25	0.00	0.10	7.40f ± 1.44
1.50	0.00	0.10	5.30j ± 1.44
0.00	0.00	0.00	0.00n ± 1.44

Means with the same letter in a column are not significantly different as determined by Ryan-Einot-Gabriel-Welsch (REGWQ) multiple range test at $\alpha = 0.05$.

In this study both IBA and NAA were found to be effective in promoting root induction as well as shoot elongation at half strength MS basal medium. More than 90% of microcuttings were rooted after three weeks of culture. The highest number of roots (18.25 ± 0.95), root length (6.35 ± 0.10) and shoot height (6.65 ± 0.05) was recorded from half MS containing 0.5 mg/l IBA followed by half MS containing 0.5 mg/l NAA resulting (13.75 ± 0.50) root number, (5.30 ± 0.00) root length and (5.70 ± 0.00) shoot height (Table 4). The roots formed in half strength MS were normal - long and thick with many thin branches than on full MS.

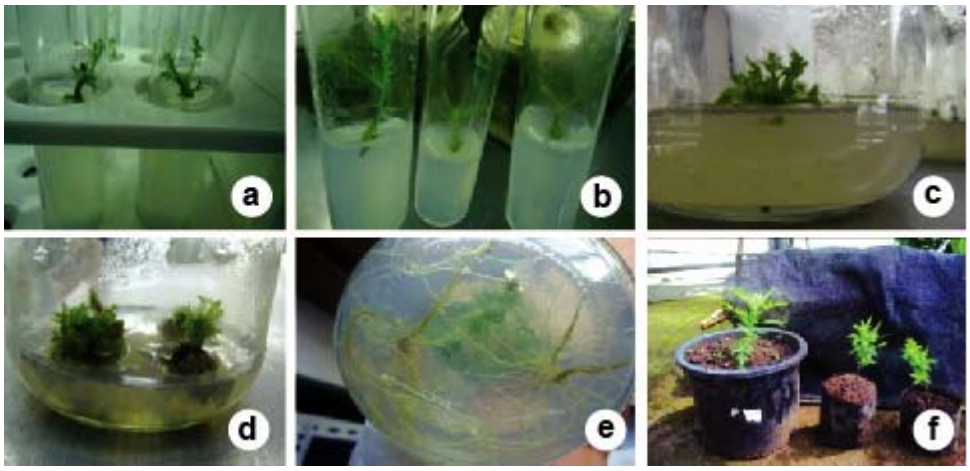

Fig. 1. Axillary shoot buds developed from (a) shoot tip explants on 0.8 mg/l TDZ after three weeks of culture, (b) nodal explants on 0.8 mg/l BAP + 0.1 mg/l IBA after three weeks of culture, (c) multiple shoots *in vitro* on MS + 1 mg/l BAP + 0.1 mg/l IBA from shoot tip explants, d) multiple shoots *in vitro* on MS + 0.75 mg/l BAP + 0.5 mg/l Kn c. MS + 1 mg/l BAP from nodal explants (e) *in vitro* rooted shoots in half MS + 0.5 mg/l IBA b: Full MS + 0.5 mg/l IBA, (f) transplanted and established plantlets in pot/polytene tube (after seven weeks).

Table 4. Effect of MS strength, IBA and NAA on *in vitro* root initiation and shoot elongation of *A. annua* cv. anamed.

MS level	IBA (mg/l)	NAA (mg/l)	Number of roots	Root length (cm) mean ± S. D.	Shoot height (cm) mean ± S.D.
1/2	0	0	7.50e ± 5.94	2.74ef ± 2.26	4.22cd ± 1.40
1/2	0.5	0	**18.25a ± 0.95**	**6.35a ± 0.10**	**6.65a ± 0.05**
1/2	1	0	8.75d ± 0.50	4.17c ± 0.09	4.75c ± 0.10
MS	0	0	4.25h ± 3.25	1.60h ± 1.19	2.68f ± 0.71
MS	0.5	0	11.75c ± 0.50	3.67d ± 0.05	4.22cd ± 0.05
MS	1	0	6.25f ± 0.50	2.45f ± 0.10	2.57f ± 0.05
1/2	0	0.5	**13.75b ± 0.50**	**5.30b ± 0.00**	**5.70b ± 0.00**
1/2	0	1	8.75d ± 0.50	2.92e ± 0.05	4.52c ± 0.12
MS	0	0.5	7.25e ± 0.50	2.62ef ± 0.05	3.47e ± 0.15
MS	0	1	5.50g ± 0.57	2.17g ± 0.05	2.75f ± 0.10

Means with the same letter in a column are not significantly different as determined by REGWQ at α=0.05.

These results are in agreement with those reported by Nair *et al.* (1986) and Elhag et al. (1991) concerning the better performance of half strength MS for *in vitro* rooting of *A. annua* in terms of rooting i.e., > 75 and 100% rooting in three weeks with the addition of 0.5 mg/l of either IBA, NAA or IAA and 0.3 mg/l NAA, respectively. Almaarri and Yu Xie (2010) also noted the same results in

half MS fortified with 1 mg/l IBA. Said medium yielded optimum results in that it gave 80% rooting after 20 days. The present results however showed reduction in rooting affected by increased concentration of both IBA and NAA from 0.5 to 1 mg/l. This may be due to the high concentration of auxin in the range that normally stimulates elongation of shoots (10^{-5} to 10^{-6} M), with concomitant significant inhibition of root growth. The inhibition of root growth and development might partly be due to the ethylene production which is triggered by high auxin concentration (Hopkins and Huner 2004, Hartman et al. (2009). Jamaleddine et al. (2011) reported MS + 0.1 mg/l Kn + 0.01 mg/l NAA as optimum for shoot elongation. In the present study however best shoot heights were observed from cultures on half strength MS than full MS fortified with 0.5 mg/l IBA or NAA (Table 4)

In the present study, employing the acclimation procedure indicated in the methodology, the plantlets showed 80% survival efficiency. There were no observable variations with respect to morphological and growth characteristics between *ex vitro* grown parent plants and *in vitro* raised potted plants. The transplanted plants established well in pots (Fig. 1)

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