Scientific Research and Essays Vol. 7(14), pp. 1501-1507, 16 April, 2012
Available online at http://www.academicjournals.org/SRE
DOI: 10.5897/SRE11.1894
ISSN 1992-2248 ©2012 Academic Journals

Full Length Research Paper

Effect of growth regulators on *in vitro* germination of *Artemisia absinthium*

A. Mannan[1,2]*, T. N. Syed[1], M. A. Yameen[2], N. Ullah[1], T. Ismail[2], I. Hussain[2] and B. Mirza[1]

[1]Department of Biochemistry, Quaid-I-Azam University Islamabad 45320, Pakistan.
[2]Department of Pharmaceutical Sciences, COMSATS Institute of Information Technology, Abbottabad 22060, Pakistan.

Accepted 22 March, 2012

The present study was carried out to optimize the plant growth regulator conditions of callus formation, shoot regeneration and root induction, by using various explants of *Artemisia absinthium*. Leaf, hypocotyl and root explants of 1 to 3 weeks old *in vitro* grown seedlings were used for callogenesis and direct organogenesis at different concentrations and combinations of phytohormone added in B5 medium. The best callus formation and shoot regeneration response was observed from leaf explants obtained from 1 week old *in vitro* grown seedlings of *A. absinthium*. The leaf explants produced the best callus cultures when grown on B_5 medium and contains either 0.1 mg/L benzyl aminopurine (BAP) or 0.1 mg/L kinetin (Kin) while root explants produced the best callus culture when placed on B_5 medium containing 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) or 1 mg/L naphthaleneacetic acid (NAA). Callus cultures obtained from leaf explants when placed on 0.1 mg/L BAP were green and more compact led to shoot formation. Direct shoot regeneration response of leaf explants was the best when placed on B_5 medium with either 0.1 mg/L BAP or 0.1 mg/L Kin. The produced healthy shoot induced the good roots when placed on B_5 medium containing 0.025 mg/L NAA and 0.1 mg/L BAP.

Key words: *Artemisia absinthium*, plant growth regulators, callogenesis, organogenesis, Gamborg's B5 medium.

INTRODUCTION

Medicinal plants are being used successfully in the treatment of various ailments. *Artemisia absinthium* that is also called wormwood or vilayati afsanteen is also one of them that belong to the genus *Artemisia*. This is a large genus of herbs with more than 200 species that belongs to the family Asteraceae. *Artemisia* is a perennial herb growing wild in northern Pakistan (Mannan et al., 2010). The phytopharmacological evaluation of aerial parts of this genus showed the presence of anti-inflammatory (Ahmad et al., 1992), antipyretic (Ikram et al., 1987), antifertility (Rao et al., 1988), antibacterial (Kovats et al., 2010), anthelmintic (Tariq et al., 2009) and antimalarial activities (Cubukcu et al., 1990).

Plant tissue culture is an innovative technique for enhanced production of valuable drugs from medicinal plants (Thorpe, 1990). It helps in multiplying superior genotypes several times faster than normal one (George and Sherrington, 1984). Similarly, tissue culture of various *Artemisia* species has been done is many laboratories to multiply its growth and production (Clemente et al., 1991; Mannan et al., 2009; Paniego and Giulietti, 1994; Whipkey et al., 1992; Zia et al., 2007). The objective of the present study was to find out most appropriate tissue culture conditions for *A. absinthium*, a medicinally valuable species, by using different plant growth regulators as tissue culture of this plant is very difficult. The suitable tissue culture medium will solve this problem.

*Corresponding author. E-mail: abdulmannan_ka@yahoo.com

MATERIALS AND METHODS

Preparation of plant material

A. absinthium seeds were collected from Astoor (Gilgit-Baltistan, Pakistan) in August 2007 at the altitude of 11000 feet. Seeds were surface sterilized with 70% ethanol followed by 0.1% mercuric

Table 1. The CIM with Gamborg's B5 salts, vitamins and plant growth regulators.

S/No.	CIM	Growth regulators (mg/L)
1	CIM1	BAP (0.1)
2	CIM2	BAP (0.5), NAA (0.5)
3	CIM3	2,4-D (0.5)
4	CIM4	2,4-D (1.0)
5	CIM5	Kin (0.1)
6	CIM6	NAA (1.0)
7	CIM7	NAA (0.5), 2,4-D (0.5), BAP (0.1)
8	CIM8	NAA (0.5), 2,4-D (0.5)

BAP, Benzyl aminopurine; 2,4-D, 2,4-dichlorophenoxyacetic acid; Kin, kinetin; NAA, naphthaleneacetic acid.

Table 2. The SRM with Gamborg's B5 salts, vitamins and plant growth regulators.

S/No.	SRM	Growth regulators (mg/L)
1	SRM1	2-4-D (0.5), kin (0.5)
2	SRM2	BAP (0.1), Kin (0.5)
3	SRM3	BAP (0.1), IAA (0.3)
4	SRM4	BAP (0.1)
5	SRM5	Kin (0.1)

chloride ($HgCl_2$), washed several times with sterilized distilled water and germinated on ½MS medium (Murashige and Skoog, 1962) of pH 5.7. Half MS medium contain 3% sucrose, solidified with 0.8% agar in petriplates. Then seeds were kept at 4°C in dark for 4 days and transferred to growth room in cool white fluorescent light of intensity 2000 lux of 16:8 h photoperiod at 25°C. After a few days, seeds started germination in growth room.

Callogenesis

Leaf, hypocotyl and root explants of 1 to 3 weeks old *in vitro* grown seedlings were inoculated on callus inducing media (CIM) by following the methodology of Nin et al. (1996) with some modification according to the experiment. Each CIM contained Gamborg's B5 salts, vitamins (Gamborg et al., 1968) and different type of hormones. The medium was solidified with 0.8% agar and sterilized by using autoclave. Hormones were filter sterilized and added to the already sterilized media (Table 1).

Leaves were cut transversely into two parts and placed on the medium with upside-down. Stems were cut crosswise into small pieces of about 1 cm length and placed separately, while roots were stacked together and cut into segments of about 1 cm length and placed in the packets of 5 to 10 segments. These explants were placed on CIM of different composition present in erlenmeyer flasks as shown in Table 1. Erlenmeyer flasks were placed in growth room under the controlled environment (cool white fluorescent light of intensity 2000 lux of 16:8 h photoperiod at 25°C). An average of 100 explants was raised for each treatment and each experiment was repeated thrice. After a few weeks explants started formation of callus, explants and calli of each treatment were transferred to fresh medium, weekly, during the first month. Afterwards, subcultures were made after every 2 weeks. Larger parts of callus were separated from the rest of the explants and cultivated separately on the medium for further growth.

The calli obtained from all type of explants were used for shoot regeneration. For this purpose, green healthy calli were cut into small pieces and cultured on B5 containing 0.5 mg/L 2,4-dichlorophenoxyacetic acid (2,4-D) or 0.1 mg/L benzyl aminopurine (BAP).

Shoot regeneration from explants

The method of direct organogenesis was applied for shoot regeneration with some modification (Nin et al., 1996). For this, leaf, hypocotyl and root explants of 2 to 3 weeks old seedlings were excised and placed on different shoot regeneration media (SRM) placed in erlenmeyer flasks for shooting response. Each shoot regeneration medium contained Gamborg's B5 salts, vitamins and different hormones combination as shown in Table 2. These flasks were kept in growth room in cool white fluorescent light of intensity 2000 lux of 16:8 h photoperiod at 25°C. An average of 100 explants was raised for each treatment and each experiment was conducted thrice. On the basis of visual observation, percentage of explants showing response for shoot regeneration was taken.

Root induction from regenerated shoots

The rooting of *in vitro* grown shoots of *A. absinthium* was carried out according to the method of Nin et al. (1996). Rooting was induced in 100 ml erlenmeyer flasks containing 50 ml root inducing medium (RIM) of Gamborg's B5 salts, vitamins and different compositions of hormones as shown in Table 3. In this experiment, developed shoots were cultured on RIM and were kept in growth

Table 3. The RIM with Gamborg's B5 salts, vitamins and plant growth regulators.

S/No.	RIM	Growth regulators (mg/L)
1	RIM1	NAA (1.0)
2	RIM2	2,4-D (0.5)
3	RIM3	NAA (0.01), BAP (0.1)
4	RIM4	NAA (0.025), BAP (0.1)
5	RIM5	NAA (0.05), BAP (0.1)

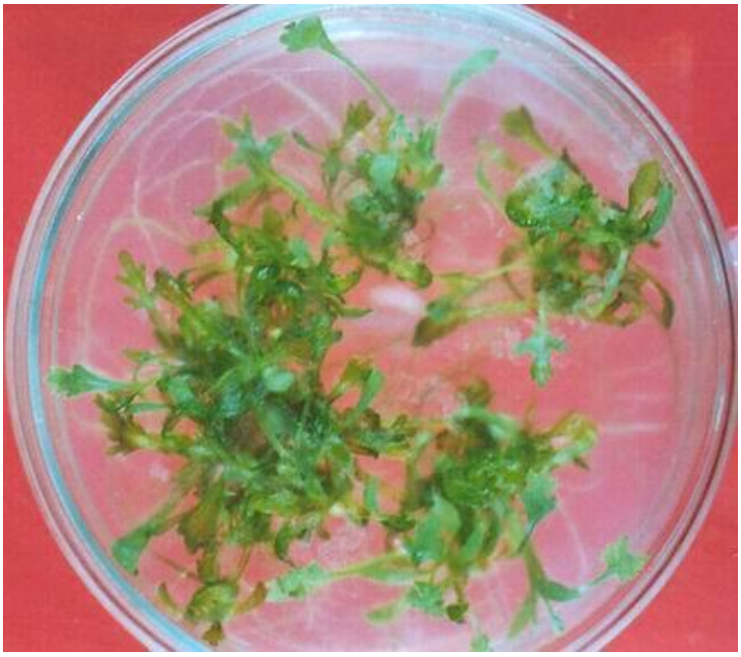

Figure 1. Seedling of *A. absinthium*.

room under said conditions to determine rooting potential of regenerated shoots in different media.

RESULTS AND DISCUSSION

When seeds of *A. absinthium* are grown on ½MS medium in the growth room with a cool white fluorescent light having an intensity of 2000 lux for 16:8 h photoperiod at 25°C, they started germination after 1 week of inoculation. The germinated seedling is shown in Figure 1.

Callogenesis

In callus culture experiment, callus was initiated on the cut ends of leaves, hypocotyl and roots explants after 2 weeks. The CIM1 and CIM5 revealed the best results for callus formation from leaf explants as shown in Figure 2 and Table 4. CIM3 and CIM6 produced best results for callus formation of root explants as shown in Table 4. Hypocotyl explants were found to be less potent in producing callus. These results are in agreement with the report of Vergauwe et al. (1996).

Callus cultures established from root and hypocotyl explants, by the use of 2,4-D were friable, white or yellowish and did not lead to formation of shoot, while the green and more compact callus cultures obtained from leaf explants by use of 0.1 mg/L BAP led to shoot formation. These results are in agreement with the findings of Paniego et al. (1993). This shows that there is a relationship between the color of callus and the chlorophyll content of the callus and its capacity to regenerate.

It was favorable to culture the callus under 16:8 photoperiods, since complete darkness led easily to browning of the callus, similar finding was previously reported by Paniego et al. (1993) and Nair et al. (1986). The CIM7 led to the formation of roots after 2 weeks.

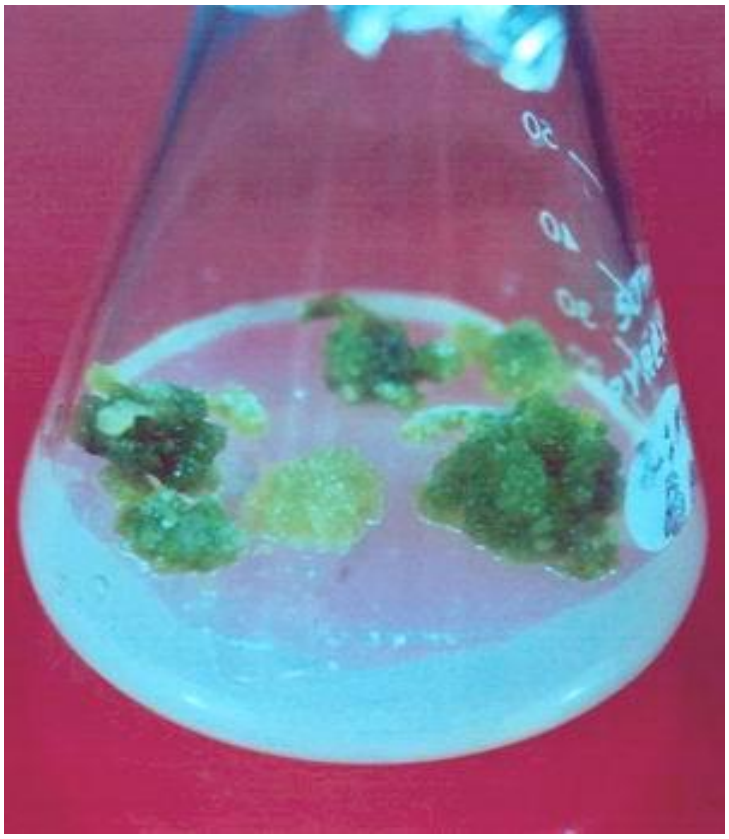

Figure 2. The best callus cultures.

Table 4. The callus inducing capacity of CIM.

S/No.	Media	Leaf	Hypocotyl	Root
1	CIM1	+ + +	+	-
2	CIM2	+	+	+
3	CIM3	-	-	+ + +
4	CIM4	+ +	+	-
5	CIM5	+ + +	+	-
6	CIM6	-	+	+ + +
7	CIM7	+	+ +	+
8	CIM8	+	+	+

Note: +, 10 to 20%; ++, 20 to 80%; +++, 80 to 100%; –, no callus.

Root induction at such an early stage was undesirable, but this medium was tested further for its root inducing capacity on induced shoots.

Age of explants also affected its callus inducing capacity. It was observed that 1 week old explants were more responsive (80 to 100%) for callus formation than 2 weeks (40 to 95%) or 3 weeks (30 to 80%) old explants (Table 5). Nin et al. (1996) reported that age of explants is very important in the various tissue culture experiments.

Direct shoot regeneration from various explants

Direct shoot regeneration response from leaf explants when placed on SRM4 and SRM5 were 80 to 100% as shown in Figure 3 and Table 6. Shoot regeneration response of hypocotyl explants was not good (10 to 20%) while root explants did not show any response. These results clearly indicated that leaf explants were more potent for direct shoot regeneration and the explants from 1 week old seedlings were more responsive for shoot

Table 5. Effect of age of explants on callus induction.

S/No.	Age of explants	Leaf (%)	Hypocotyl (%)	Root (%)
1	1 week	100	80	100
2	2 weeks	95	40	90
3	3 weeks	80	30	70

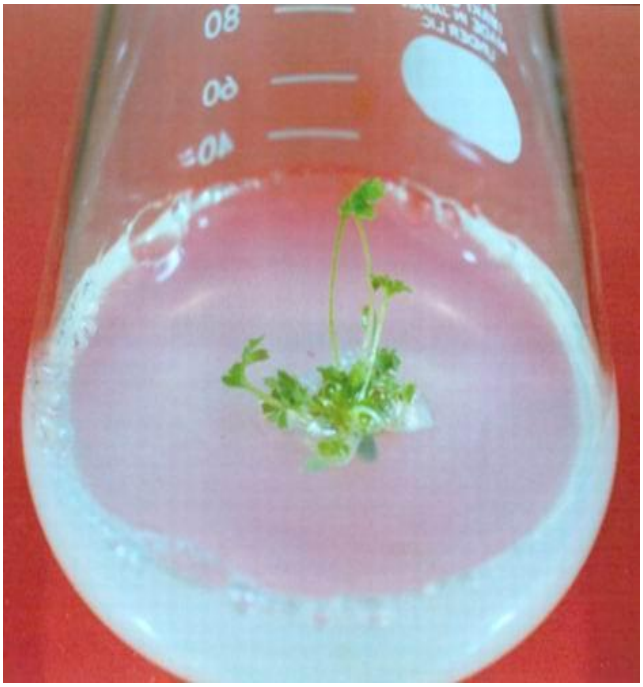

Figure 3. Shoot of leaf explants.

Table 6. The shoot regeneration capacity of SRM.

S/No.	Media	Leaf	Hypocotyl	Root
1	SRM1	+	-	-
2	SRM2	++	-	-
3	SRM3	+	-	-
4	SRM4	+++	+	-
5	SRM5	+++	+	-

Note: +, 10 to 20%; ++, 20 to 80%; +++, 80 to 100%; -, no shoots.

formation than 2 or 3 weeks old ones. The shoot regeneration continued and explants led to the formation of shoot clusters. The results of current study are similar to findings of Vergauwe et al. (1996) while working on shooting media.

Root induction from regenerated shoots

For rooting, the developed healthy shoots were transferred to RIM. Among all the hormonal combinations investigated, RIM4 was found to be the best as shown in Figure 4 and Table 7. The results of current study are in accordance with the views of Akin-Idowu (2009).

Acclimatization

A few rooted plantlets were transferred to pots for acclimatization and placed in the growth chamber for 2 to

Figure 4. The rooted shoots of *A. absinthium*.

Table 7. Effect of various root induction media.

S/No.	Media	Response
1	RIM1	-
2	RIM2	-
3	RIM3	++
4	RIM4	+++
5	RIM5	+

Note: +, 10 to 20%; ++, 20 to 80%; +++, 80 to 100%; -, no rooting.

Figure 5. Acclimatized plant of *A. absinthium*.

3 weeks and finally they were exposed to the environment as shown in Figure 5.

Plant tissue culture is well known and well established technique for enhanced production of medicinal plants

(Thorpe, 1990). Plant regeneration ability depends on plant species and genotype. Tissue culture of *A. absinthium* is a very difficult job. Improvement in organogenesis of this plant may be carried out by growing its various explants on different phytohormone combinations (Nin et al., 1996). The reproducible methodology and the best hormone combinations described in present study, however, represents a successful regeneration of *A. absinthium* plantlet as shown in Figure 5. Further work can be done in this direction for improving the concentration of various secondary metabolites of this plant that shows very important antimalarial, antimicrobial, etc. properties.

ACKNOWLEDGEMENT

Authors are thankful to Higher Education Commission of Pakistan for providing the necessary financial sport of conducting the experiment.

REFERENCES

Ahmad F, Khan RA, Rasheed S (1992). Study of analgesic and anti-Inflammatory activity from plant extracts of *Lactuca scariola* and *Artemisia Absinthium*. J. Islam. Acad. Sci., 5(2): 111-114.

Akin-Idowu PE, Ibitoye DO, Ademoyegun O (2009). Tissue culture as a plant production technique for horticultural crops. Afr. J. Biotechnol., 8(16): 3782-3788.

Clemente M, Contreras P, Susin J, Piiego-Alfaro F (1991). Tissue cultures of *Artemisia pallens*: Organogenesis, terpenoid production. Plant Cell Tiss. Org. Cult., 21: 159-164.

Cubukcu B, Bray DH, Warhurst DC, Mericli AH, Ozhatay N, Sariyar G (1990). *In vitro* antimalarial activity of crude extracts and compounds from *Artemisia abrotanum* L. Phytother. Res., 4(5): 203-204.

Gamborg OL, Miller RA, Ojima K (1968). Nutrients requirements of suspensions cultures of soybean root cells. Exp. Cell Res., 50: 148-151.

George EF, Sherrington PD (1984). Plant propagation by tissue culture. Exegetics LTD, Westbury.

Ikram M, Shafi N, Mir I, Do MN, Naguyen P, Le-Quesne PW (1987). 24-Zeta-Ethylcholesta-7, 22-diene-3beta-ol; a possibly antipyretic constituent of *Artemisia absinthium*. Planta Med., 53: 389.

Kovats N, Goloncser F, Acs A, Refaey M (2010). Quantification of the antibacterial properties of *Artemisia absinthium*, *Artemisia vulgaris*, *Chrysanthemum leucanthemum* and *Achillea millefolium* using the *Vibrio fischeri* bacterial bioassay. Act. Bot. Hungar., 52(1): 137-144.

Mannan A, Ahmed I, Arshad W, Asim MF, Qureshi RA, Hussain I, Mirza B (2010). Survey of artemisinin production by diverse *Artemisia* species in northern Pakistan. Malaria J., 9: 310.

Mannan A, Syed TN, Mirza B (2009). Factors affecting *Agrobacterium tumefaciens* mediated transformation of *Artemisia absinthium* L. Pak. J. Bot., 41(6): 3239-3246.

Murashige T, Skoog F (1962). A revised medium for rapid growth and bioassay with tobacco tissue culture. Physiol. Plant., 15: 472-493.

Nair MS, Acton N, Klayman DL (1986). Production of artemisinin in tissue cultures of *Artemisia annua* L. J. Nat. Prod., 49: 504-507.

Nin S, Morosi E, Schiff S, Bennici A (1996). Callus cultures of *Artemisia absinthium* L.: initiation, growth optimization and organogenesis. Plant Cell Tiss. Org. Cult., 45: 67-72.

Paniego NB, Giulietti AM (1994). *Artemisia annua* L.: dedifferentiated and differentiated cultures. Plant Cell Tiss. Org. Cult., 36: 163-168.

Paniego NB, Maligne AE, Giuletti AM (1993). Biotechnology in Agriculture and Forestry, In: Medicinal and Aromatic Plants: (Bajaj. Y.P.S. ED.) V.Springer-Verlag. Heidelberg. 24: 64-78.

Rao VSN, Menezes AMS, Gadelha MDT (1988). Antifertility screening of some indigenous plants of Brazil. *Fitoterapia*, 59: 17-20.

Tariq KA, Chishti MZ, Ahmad F, Shawl AS (2009). Anthelmintic activity of extracts of *Artemisia absinthium* against ovine nematodes. Vet. Parasitol., 160: 83-88.

Thorpe TA (1990). The current status of plant tissue culture. In: Plant tissue culture, applications and limitations. (Bhojwani, S.S. Ed.). Amsterdam Elsevier, pp. 1-3.

Vergauwe A, Cammaert C, Vandenberghe D, Genetello C, Inze D, Montagu MV, Den EeckhoutEV (1996). *Agrobacterium tumefaciens* mediated transformation of *Artemisia annua* L. plants and the regeneration of transgenic plants. Plant Cell Rep., 15: 929-933.

Whipkey A, Simon JE, Charles DJ, Janick J (1992). *In vitro* production of artemisinin from *Artemisia annua* L. J. Herbs Spices Medic. Plants, 1: 15-25.

Zia M, Mannan A, Chaudhary MF (2007). Effect of growth regulators and amino acids on Artemisinin production in the callus of *Artemisia absinthium*. Pak. J. Bot., 39(3): 799-805.