African Journal of Biotechnology Vol. 6 (16), pp. 1874-1878, 20 August 2007
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2007 Academic Journals

Full Length Research Paper

Hormonal regulation for callogenesis and organgenesis of *Artemisia absinthium* L

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Accepted 14 June, 2007

Callus cultures were induced from leaf and stem explants of *Artemisia absinthium*, at different auxin and cytokinin concentrations. Moderate concentrations of growth regulators either in combination or in single in MS medium produced friable, light green and non-embryogenic callus from both explants. These totipotent cells gave rise to shoots when transferred to same or different growth regulator containing medium as second subculture. Complete rooting was achieved on full and half strength basal MS medium supplemented with different auxin concentrations. Synergetic effect of plant growth regulator plays an important role in callus induction and cell differentiation.

Key words: *Artemisia absinthium*, callogenesis, organogenesis, plant growth regulators.

INTRODUCTION

Medicinal plants are source of important therapeutic aid for alleviating human ailments (Dev, 1997). *Artemisia absinthium* L. commonly known as wormwood or "vilayati afsanteen" is a perennial herb growing in the northern hilly areas of Pakistan (Haq, 1983). *A. absinthium* L. is traditionally used because of its antiheliminthic, insecticidal (Smith and Secoy, 1981), antiseptic and febrifuge properties (Nadkarni, 1976). Oil of *A. absinthium* has been found to repel the flies and fleas (Erichsen-Brown, 1979) and mosquitoes (Morton, 1981) and to kill house flies (Kaul et al., 1978).

Micropropagation of *A. absinthium* has been previously established by using shoot tips (Nin et al., 1996), as an alternative, the culture of callus tissue provides an important tool that can be preliminary step in the regeneration of whole plants.

In the present study effect of plant growth regulators on callogenesis and organogenesis (shoot and root induction) was investigated on *A. absinthium* L.

MATERIALS AND METHODS

*A. absi*nthium seeds, collected from northern areas of Pakistan, were surface sterilized with 0.1% mercuric chloride and washed thoroughly with distilled autoclaved water under aseptic conditions. The seeds were germinated on plane agar medium containing 3% sucrose. After two weeks of germination, leaves and stems were excised at average size 2 – 3 cm and placed on pre-autoclaved Murashige and Skoog (MS, 1962) basal medium supplemented with different growth regulators. The cultures were kept in a cooled incubator with 16 h light cycle in every 24 h with temperature at 25 ± 1°C. Primary callus was transferred on regeneration medium for shoot induction after four weeks of callus initiation. Shoots emerged from callus were separated, callus was removed and planted again on full and half MS medium containing different concentrations of auxins for root initiation. Rooted plants were washed with distilled water and planted in soil and peat moss (3:1) under high moisture content. After one week, these plants were transferred to green house.

The experiments were entirely randomized with six replicates for each growth regulator(s) concentration(s). Statistical analyses were carried out by the ANOVA and Dunkens Multiple Test, at a 0.5% probability level.

RESULTS AND DISCUSSION

Callogenic response

The callogenic response form leaf and stem explants was observed at different growth regulators concentrations either singly or in combination (Table 1). Callogenic response from all explants started at the margins or from injuries. Plant growth regulator (PGR)-free basal MS medium also induced callogenic response from both explants where leave explant showed 75% callogenic res-

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Abbrevations: 2,4-D, 2,4-dichloro phenoxy acetic acid; NAA, nephthalene acetic acid; IBA, indol butyric acid; IAA, indol acetic acid; BAP, benzyl amino purine; Kin, Kinetin.

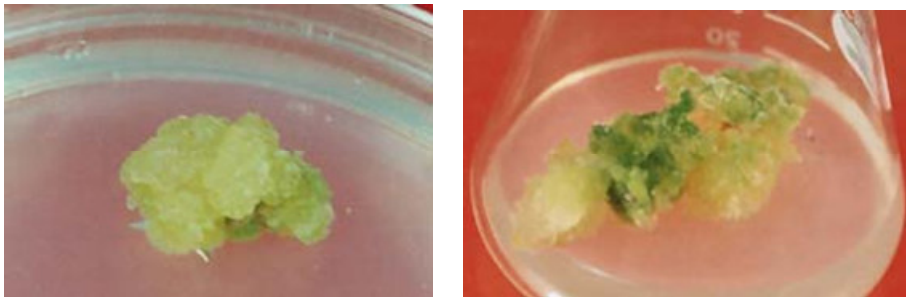
Table 1. Callogenic response from leaf and stem explant at different growth regulators[Z].

Growth regulator	Concentration (mg/l)	Leaves Explant		Stem Explant	
		Callus formation (%)[X]	Response	Callus formation (%)[X]	Response
2,4-D	0.1	75.0	+ + +	87.5	+ + +
	0.25	100.0	+ + + +	87.5	+ + + +
	0.5	100.0	+ + + +	100.0	+ + + +
	0.75	100.0	+ + + +	75.0	+ + +
	1.0	87.5	+ + +	87.5	+ + +
	1.25	50.0	+ +	37.5	+ +
	1.5	50.0	+	37.5	+
IAA	0.1	87.5	+ + +	37.5	+ + +
	0.25	100.0	+ + + +	75.0	+ + + +
	0.5	100.0	+ + + +	100.0	+ + + +
	0.75	100.0	+ + +	50.0	+ + +
	1.0	87.5	+ +	12.5	+ +
	1.25	75.0	+	37.5	+
	1.5	75.0	+	12.5	+
NAA	0.1	100.0	+ + +	87.5	+ + +
	0.25	100.0	+ + + +	100.0	+ + + +
	0.5	100.0	+ + + +	100.0	+ + + +
	0.75	100.0	+ + +	100.0	+ + +
	1.0	87.5	+ +	37.5	+ +
	1.25	87.5	+ +	37.5	+ +
	1.5	37.5	+	12.5	+
IBA	0.1	75.0	+ +	75.0	+ +
	0.25	100.0	+ + + +	100.0	+ + + +
	0.5	100.0	+ + + +	100.0	+ + + +
	0.75	100.0	+ + +	100.0	+ + +
	1.0	87.5	+ +	50.0	+ +
	1.25	87.5	+	75.0	+
	1.5	50.0	+	37.5	+
BAP	0.1	100.0	+ +	75.0	+ +
	0.25	100.0	+ + + +	87.5	+ + + +
	0.5	100.0	+ + +	87.5	+ + +
	0.75	50.0	+ +	50.0	+ +
	1.0	12.5	+ +	12.5	+ +
	1.25	00.0	+	12.5	+
	1.5	12.5	+	12.5	+
Kin	0.1	75.0	+ + + +	100.0	+ + + +
	0.25	75.0	+ + +	100.0	+ + +
	0.5	12.5	+ +	37.5	+ +
	0.75	12.5	+ +	37.5	+ +
	1.0	-	+	-	+
	1.25	-	+	-	-
	1.5	-	-	-	-
BAP/IBA	0.5/0.05	87.5	+ + +	75.0	+ + +
	0.5/0.1	100.0	+ + + +	100.0	+ + + +
	0.5/0.25	100.0	+ + +	100.0	+ + +
BAP/NAA	0.5/0.05	100.0	+ + +	100.0	+ + +
	0.5/0.1	100.0	+ + + +	100.0	+ + + +
	0.5/0.25	100.0	+ + +	100.0	+ + +

Table 1. Contd.

BAP/2,4-D	0.5/0.1	100.0	+ + + +	100.0	+ + + +
	0.5/0.25	100.0	+ + + +	100.0	+ + + +
	0.5/0.5	100.0	+ + + +	100.0	+ + + +
Kin/IBA	0.5/0.05	87.5	+ +	87.5	+ +
	0.5/0.1	100.0	+ + +	100.0	+ + +
	0.5/0.5	75.0	+ +	87.5	+ +
Kin/NAA	0.5/0.05	100.0	+ +	75.0	+ +
	0.5/0.1	100.0	+ + +	100.0	+ + +
	0.5/0.5	87.5	+ +	100.0	+ +
Control	-	75.0	+ + +	50.0	+ +

[X] %age response of 6 replicates.
[Z] Rated after 30 days of culture: + = Low, ++ = good, +++ = very good, ++++ = excellent, - = nil.



(a) (b)

Figure 1. Callus induction from leaf axplant of *Artemisia absinthium* on MS medium; (a) BA 0.5 + NAA 0.1 mg/l and (b) BA 0.5 mg/l + 2,4-D 0.25 mg/l.

ponse while 50% stem explants produced calli. Nin et al. (1996) reported no callogenic response from leaf explant on PGR-free medium and explant died after few days.

2,4-D as callus inducing hormone produced light green, soft, friable and compact callus from leaf and stem explant. But at all concentration of 2,4-D organogenic response was not observed within observation time. Nin et al. (1996) stated that low concentration of 2,4-D stimulated adventitious root development from 86% of all explant of *A. absinthium*.

At all concentrations of BAP and Kin, the callogenic response was poor. Very low callus was developed which was green, soft and compact. Small and few numbers of leaves also emerged, when the callus remained on the same medium for six weeks or the callus turned to hard and embryogenic.

Callus produced at different concentrations of IAA and IBA was yellowish, soft and friable and callogenic response was 100% at lower concentration of both hormones. Nin et al. (1996) reported that callogenesis occurred in 100% of explants, independent of the cytokinins/auxin ratio. But at different concentrations of NAA, light green, soft and friable callus was observed. At low concentrations of NAA, small shoots emerged while at higher concentrations callus turned hard and compact.

The result shows that media supplemented with BAP either with NAA, 2,4-D or IBA produced 100% callogenic response from both explants. 0.5 mg/l BAP and 0.05-0.25 mg/l NAA in combination produced green, soft and friable callus from both explants (Figure 1). Nin et al. (1996) reported best callogenic response with BAP and NAA in the medium for *A. absinthium* whereas Benjamin et al. (1991) observed callus induction from shoot buds using BAP plus IAA for *Artemisia pallens*. 2,4-D at varying concentration (0.05 - 0.25 mg/l) in combination with BAP (0.5 mg/l) also produced light green and soft callus when supplemented in MS medium. When IBA and NAA were combined with Kin, the callogenic response was also low and callus was not good in texture. Xu and Jia (1996) observed best callus result in the presence of 2,4-D with Kin for *Artemisia sphaerocephala*.

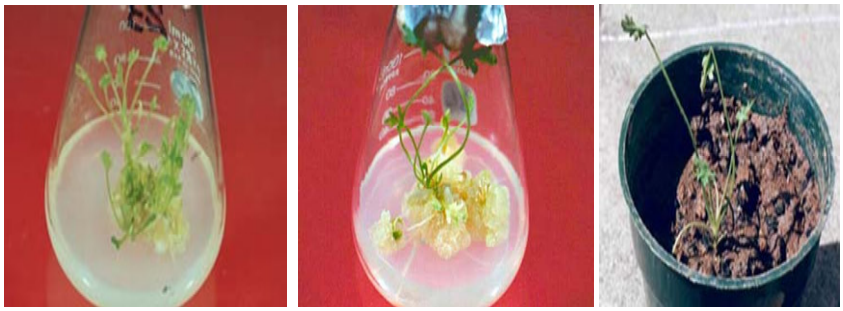
Shoot induction

Shoots from callus was observed at different concentration of BAP and Kin, alone and in combination with NAA (Table 2). At 0.5 mg/l BAP, 2.83 ± 0.87 shoot emerged while at 1.0 mg/l no shoot induction was observed Nin et al. (1996) also did not observe any shoot induction at low

Table 2. Effect of growth regulators on *in vitro* shoot induction of *Artemisia absinthium* from callus on MS medium[Z].

Growth Regulators	Conc. (mg/l)	Response (%)[Y]	Average No. of shoots[XT]	General description
BAP	0.1	33.4	0.5 ± 0.342 [d]	1-2 shoots, small green leaves
	0.25	50.0	0.833 ± 0.477 [cd]	1-3 shoots, small green leaves
	0.5	83.3	2.833 ± 0.703 [ab]	4-5 shoots, small green leaves
	0.75	50.0	0.833 ± 0.477 [cd]	Small light green leaves
	1.0	00.0	-	No response
Kin	0.1	33.4	0.5 ± 0.342 [d]	1-2 shoots greenish small leaves
	0.25	00.0	-	No response but embryogenic callus
	0.5	00.0	-	No response but embryogenic callus
	0.75	00.0	-	No response but embryogenic callus
	1.0	00.0	-	No response but embryogenic callus
NAA	0.1	66.7	0.83 ± 0.307 [cd]	1-2 shoots with green leaves
	0.5	00.0	-	No response
BAP/NAA	0.5/0.05	83.3	2.0 ± 0.447 [bc]	2-3 shoots small green leaves
	0.5/0.1	83.3	3.6 ± 0.615 [a]	4-5 shoots small green leaves
Kin/NAA	0.5/0.05	33.4	-	Very small green leaves and callus become hard
	0.5/0.1	50.0	-	Very small leaves but callus become hard
LSD			1.377	

[X]Mean ± standard error.
Interval of confidence 95%.
[Y]Data are mean of 6 replicates.
[T]Mean separation by LSD.
[Z] Rated after 30 days of culture.
Values with the different letters on the same column are significantly different.



(a) (b) (c)

Figure 2. Organogenesis of *Artemisia absinthium*. (a) Shoot induction from callus on MS medium with BAP 0.5 and NAA 0.1 mg/l. (b) Root induction on MS medium with IBA 0.5 mg/l. (c) After one week of acclimatization.

concentrations BAP in *A. absinthium*. However Le (2001) reported that new axillary shoots development was promoted in *Artemisia annua* by addition of BAP in MS medium.

The best shoot induction (3.6 ± 0.615) was observed on BAP (0.5 mg/l) in combination with NAA (1.0 mg/l) (Figure 2a). Geng et al. (2001) observed shoot cluster in *A. annua* L. on MS medium supplemented with BAP and NAA. Mackay and Kitto (1988) and Nam-cheol et al. (1992) also reported shoot induction on MS medium supplemented with BAP and NAA in different *Artemisia* species. Shoot induction was very low or absent at differ-

Table 3. Effect of growth regulators on *in vitro* rooting of *Artemisia absinthium*[Z].

Growth Regulators	Conc. (mg/l)	Full MS		½ MS	
		Response (%)[Y]	Average no. of roots [X]	Response (%)[Y]	Average no. of roots [X]
IAA	0.1	-	-	-	-
	0.25	66.7	1.5 ± 0.5	66.7	0.75 ± 0.75
	0.5	-	-	-	-
	1.0	66.7	1.5 ± 0.5	-	-
	2.0	-	-	-	-
IBA	0.1	-	-	-	-
	0.25	-	-	-	-
	0.5	33.4	0.333 ± 0.211	33.4	0.25 ± 0.25
	1.0	-	-	-	-
	2.0	-	-	-	-
NAA	0.1	-	-	-	-
	0.25	-	-	-	-
	0.5	-	-	-	-
	1.0	-	-	-	-
	2.0	-	-	-	-

[X]Mean ± standard error.
[Y] Data are mean of 6 replicates.
[Z] Rated after 30 days of culture.

rent concentrations of Kin alone or in combination with NAA. At 0.1 mg/l Kin, shoot induction was 33.4% (0.5 ± 0.342) and at higher concentrations response was absent while the callus turned hard, compact and embryogenic.

Root induction

Root induction was not good in the present study (Table 3). Five concentrations of auxins (IAA, IBA, NAA) were tested in full and half strength MS medium. The basic problem in rooting was development of callus at the base of shoots which inhibit root induction from shoots (Figure 2b). At 0.25 mg/l IAA at full MS and ½ MS, 1 - 2 roots were observed. Root induction was 66.7% on both. When these plants were transferred on the same medium, they did not produce further roots. Le (2001) reported that shoots developed profuse roots system on MS medium containing IBA (0.5 mg/l) within two weeks in *A. annua.*

Plants that produced roots were transferred to pots filled with soil and peat moss (3:1) under high humid condition till maturation of leaves (Figure 2c), and then transferred to green house.

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