

In vitro Plant Regeneration from Leaf Explants of *Artemisia vulgaris* L. – A Medicinal Herb

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Abstract

A reliable protocol for callus induction and regeneration were developed for leaf explants of *Artemisia vulgaris* L. Percent of callus induction and regeneration were higher in the young leaves. MS medium containing 1.0 mgL⁻¹ BAP and 3.0 mgL⁻¹ NAA is the optimum concentration for induction of callus. So produced callus was subcultured on Murashige – Skoog (MS) medium with 1.0 mgL⁻¹ 6-benzylaminopurine (BAP), 3.0 mgL⁻¹ gibberellic acid (GA₃) produced the highest mean number of shoots (35.85±0.81) per explant. Half strength of MS was found to be the best for rooting, however, addition of IAA (0.5-1.0 mgL⁻¹) was found essential to induce longer roots. More than 84% of the rooted plants were established in polycups after hardening.

Keywords: Callus induction, Plant regeneration, Leaf explants, *Artemisia vulgaris* L

1. Introduction

Artemisia vulgaris Linn, an important perennial medicinal herb belongs to the family *Asteraceae*. The plant is aromatic, shrubby 0.6-2.4m high and pubescent. The plant has a hot, sharp and pungent taste. It is considered to be a valuable stomachic, deobstruent and antispasmodic. The expressed juice is used in diseases of children. The leaves and shoot tips are administered in nervous and spasmodic affections connected with debility, in asthma and diseases of the brain. It shows antispasmodic and anthelmintic activity (Kirtiker and Basu, 1935).

Leaf explants have been employed for regeneration by many researchers observed in *Centaureum erythraea* (Baresova *et al.*, 1985); *Jatropha integerrima* (Sujatha and Dhingra, 1993); *Solanum pseudocapsicum* (Baburaj and Gunasekaran, 1994); *Guizotia abssinica* (Jadimath, 1998); *Jatropha curcus* (Sardana *et al.*, 2000). The present study describes an optimized regeneration system in *Artemisia vulgaris* callus derived from leaf explant cultures on a variety of medium composition.

2. Materials and methods

2.1 Plant material

Leaf explants from 6-month-old mature plant of *Artemisia vulgaris* L. were used to initiate *in vitro* cultures. The leaf segments were washed with 5% (w/v) bavistin, 10% (w/v) antibiotic and 5% (w/v) Tween 20 by continuous shaking for 20 min followed by washing in tap water, then rinsed three to five times with double distilled water.

2.2 Callus induction

To induce callus, the sterile leaf explants were inoculated on MS Medium, containing BAP (0.5-1.0 mgL⁻¹) and NAA (0.5-3.0 mgL⁻¹), 0.8% (w/v) agar as gelling agent and 30% (w/v) sucrose. The p^H of the medium was adjusted to 5.8 before autoclaving, a light intensity of 10 μmol m⁻² s⁻¹ (cool white fluorescent light) and a temperature of 25±2°C.

2.3 Shoot induction and elongation

Shoots were induced by transferring the leaf calli on MS medium containing different concentrations and combinations of BAP (0.5-1.0 mgL⁻¹) and GA₃ (0.5-3.0 mgL⁻¹). Elongation of shoots were also observed on the same medium.

2.4 Rooting and Acclimatization

Microshoots were excised from the parent cultures and transferred onto half strength MS medium supplemented with different concentrations and combinations of IBA, IAA and NAA for root induction. The rooted shoots were gently removed from the culture vessels, washed under running tap water and transferred to polycups containing sand:soil:vermiculite (1:1:1) in the greenhouse conditions for acclimatization.

3. Results and discussion

3.1 Callus induction

To induce callus from leaf explants, they were inoculated on MS medium containing 0.5-1.0 mgL⁻¹ BAP and 0.5-3.0 mgL⁻¹ NAA (Table 1 & Fig 1.A). Among the various concentrations and combinations highest (81%) callus was achieved on MS medium supplemented with BAP (1.0 mgL⁻¹) and NAA (3.0 mgL⁻¹). Young leaves have been proved to be callus in other plant species, such callus potential has been reported to vary from species to species and often differs in varieties of same species (Vasil, 1982). Similar observations have been reported in *Jatropha integerrima* (Sujatha and Dhingra, 1993); *Jatropha curcus* (Sujatha and Mukta, 1996); *Solanum tuberosum* (Jayasree *et al.*, 2001); *Solanum torvum* (Jaseela and Nair, 2004); *Rauwolfia micrantha* (Vishwanath *et al.*, 1997); *Rauwolfia serpentina* (Nishi Koshta *et al.*, and Anitha *et al.*, 2002). Combinations of BAP and NAA have been used for regeneration by many investigators.

3.2 Shoot induction and elongation

Shoots from callus was observed at different concentrations of 0.5-1.0 mgL⁻¹ BAP and 0.5-3.0 mgL⁻¹ GA₃ (Fig.1B). The best shoot induction (35.85±0.81) per explant for leaf was observed on BAP (1.0 mgL⁻¹) in combination with GA₃ (3.0mgL⁻¹). The elongation of shoots were achieved (4.11±0.85) on the same medium (Table 2 & Fig.1C).

Combination of BAP and GA₃

The effect of GA₃ on the embryogenic frequency became significant after two weeks in the induction medium and caused a noticeable rise in the intensity and number of embryos and shoots in *Artemisia vulgaris*. There are many reports that GA₃ enhances the number of somatic embryos and shoots from the calli of *Santalum album* L. (Lakshmi *et al.*, 1979); *Rumex acetosella* L. (Culafic *et al.*, 1987); *Mentha piperita* (Ghanti *et al.*, 2004) and *Phyllanthus amarus* (Chitra *et al.*, 2009),

3.3 Rooting and Acclimatization

The shoots formed in the calli derived from leaf segments were transferred onto half strength MS medium supplemented with different concentrations and combinations of IAA, NAA and IBA for root induction. Data were recorded for percentage of shoots forming roots, number of roots/shoots and root length. Root induction occurred in 10-12 days of culturing with highest root induction (87%) on MS medium containing 0.5 mgL⁻¹ (IAA) (Table3 & Fig.1D). Root formed in IAA were thick, long and white. The role of IAA as an effective root inducing auxin had also been in *Amygdalus communis* L. (Akbas *et al.*, 2009) and *Centaureum erythraea* (Piateczak and Wysokinska, 2003).

The plantlets having sufficient root and shoot system were taken out from the culture vessels and were washed under running tap water to remove the agar attached to shoots. The plantlets were transferred to polycups (Fig.1E) containing autoclaved sand: soil: vermiculite (1:1:1) mixture in the greenhouse condition. They were transferred to the field condition.

In conclusion, the above protocol describes rapid callus induction from leaf explants, which can ensure a stable supply of this medicinally oil yielding plant irrespective of any seasonal variation and may serve as a better source for biological active compounds.

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Table 1. Callogenesis from leaf explants of *Artemisia vulgaris* L. at different concentrations of BAP and NAA.

Growth regulator in mg l ⁻¹		% of response*	% of forming callus
BAP	NAA		
0.5	0.5	25	4.00±0.00
0.5	1.0	26	4.00±0.00
0.5	1.5	31	5.00±0.00
0.5	2.0	44	7.00±0.00
0.5	2.5	50	8.00±0.00
0.5	3.0	56	8.85±0.00
1.0	0.5	35	6.00±0.00
1.0	1.0	44	7.00±0.001
1.0	1.5	56	9.00±0.26
1.0	2.0	68	10.90±0.58
1.0	2.5	75	12.00±0.61
1.0	3.0	81	13.00±0.31

*Mean of 12 replicates per treatment in three repeated experiments

Table 2. Effect of growth regulator on *in vitro* shoot induction from leaf explants of *Artemisia vulgaris* L.

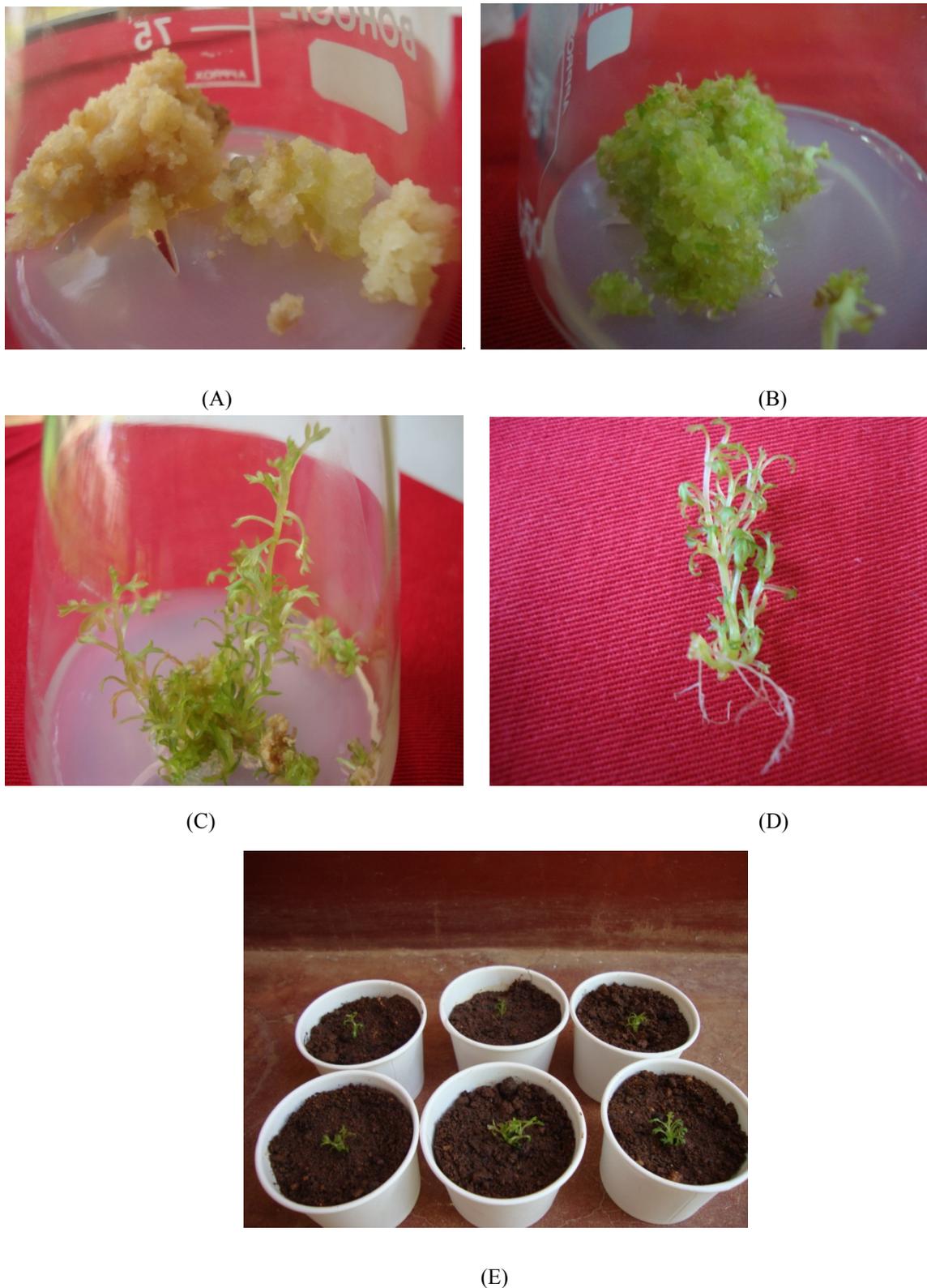
Growth regulator in mg ^l ⁻¹		% of response	No. of shoots/explant*	Shoot length (cm)*
BAP	GA ₃			
0.5	0.5	44	7.50±0.51	1.74±0.15
0.5	1.0	50	10.40±0.50	1.80±0.07
0.5	1.5	63	13.90±0.85	2.14±0.12
0.5	2.0	68	18.45±0.51	2.42±0.16
0.5	2.5	81	21.50±0.51	3.03±0.10
0.5	3.0	88	25.50±0.51	3.05±0.10
1.0	0.5	56	9.55±0.51	3.12±0.69
1.0	1.0	63	12.50±0.51	3.19±0.26
1.0	1.5	75	19.63±1.11	3.24±0.05
1.0	2.0	81	24.38±0.92	3.50±0.08
1.0	2.5	93	29.45±2.39	3.58±0.08
1.0	3.0	100	35.85±0.81	4.11±0.85

*Mean of 12 replicates per treatment in three repeated experiments

Table 3. Effect of different concentrations of IAA on rooting of *in vitro* shoots of *Artemisia vulgaris* L.

IAA in mg ^l ⁻¹	% of response*	No. of roots/shoot*	Root length (cm)*
0.5	87	7.45±0.59	4.40±0.26
1.0	80	3.65±0.50	3.51±0.08
1.5	78	4.58±0.58	2.74±0.34
2.0	72	3.50±0.51	2.71±0.81

*Mean of 12 replicates per treatment in three repeated experiments



(A) Callus induction from leaf explants of *Artemisia vulgaris* Linn. on MS medium containing BAP (1.0 mg/l) +NAA (3.0 mg/l). (B) and (C) Sub-culturing of callus on MS medium supplemented with BAP (1.0 mg/l) and GA₃ (3.0mg l⁻¹), differentiated into multiple shoots and shoot elongation respectively. (D) Rooting from regenerated shoot on MS half strength medium containing IAA (0.5mg l⁻¹). (E) Hardened plantlets.