# *In vitro* Plant Regeneration from Stem Derived Callus of *Artemisia Vulgaris* L.

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**Abstract:** The present study was undertaken to evaluate the most suitable concentration of growth regulators for callus and subsequent organogenesis from stem explants of *Artemisia vulgaris* L. 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 and Skoog (MS) medium with 1.0$mgl^{-1}$ 6-benzylaminopurine (BAP), 3.0$mgl^{-1}$ gibberrelic acid ($GA_3$) produced the highest mean number of shoots (31.50± 0.51) per explants. Callus derived shoots rooted, in MS medium containing 0.5 $mgl^{-1}$ IAA. The rooted plants were hardened and transferred to the garden soil, showed 95 % survival rate.

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**Key Word:** *Artemisia vulgaris* L., Stem explants, Callus induction, Regeneration.

## Introduction

*Artemisia vulgaris* Linn, is 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 stem & leaves juice is used in curing the convulsions and measles in 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. The boiled leaves are used as a poultice in headache; dried and cut into small fragments they are used to cauterize wounds (Kirtikar and Basu, 1935).

In the culture of stem segments *Torenia*, Tanimoto and Harada (1982) observed that cytokinins had a profound influencive effects on shoot bud organogenesis. Similarly, the morphogenic potentialities of the stem explants in vitro have been analysed by various investigators like Michiyokata (1985) in *Camellia sinensis*; Lakshmi sita (1986) in *Dalbergia latifolia*; Sharma and Thorpe (1990) in *Morus alba*; Sarasan et al. (1994) in *Hemidesmus indicus*.

The present study describes an optimized regeneration system *in Artemisia vulgaris* from callus derived from stem explants cultures on a variety of medium composition.

## Materials and Methods

Healthy *Artemisia vulgaris* L. plants were obtained from Bhoodevi nursery, Mysore and were raised in pots containing soil and fram yard manure (1:1) under greenhouse condition at Dos in Botany, university of Mysore, Mysore- India. Stem explant was collected from potted plants and processed for aseptic condition. Stem explants washed with 5% (w/v) bavistin, 10 % (w/v) amphicilin, 0.1 % (v/v) Tween 20 for 15 min and again washed thoroughly under running tap water for 20 min. The washed explants were finally treated with sodium hypochloride (0.4 %) for three-four min under aseptic conditions and washed five times with sterile distilled water to remove traces of sodium hypochloride. After surface sterilization, the stem explants were cut into small pieces (1×1 cm length) and inoculated in MS supplemented with BAP ( 0.5 and 1.0 $mgl^{-1}$) and NAA (0.5, 1.0, 1.5, 2.0, 2.5 and 3.0 $mgl^{-1}$) in different concentrations and combinations for production of callus.

For shoot multiplication, 20-25 old callus was transferred to MS supplemented with different concentrations and combinations of BAP (0.5 and 1.0 $mgl^{-1}$) and $GA_3$ (0.5-3.0 $mgl^{-1}$). After 30 days, the fully developed shoot lets were transferred to half strength of MS basal medium supplemented with IAA (0.5 and 1.0 $mgl^{-1}$) for root development. Further 25 days, culture vessels containing the rooted plantlets were transferred to polycups containing sterile soil and vermiculate (1:1) and kept in greenhouse 65-70 % humidity. After 20 days, the fully acclimatized planlets were transferred to the field.

For all the above studies, MS basal medium containing 3% w/v sucrose, 0.9 % w/v agar and various concentrations and combinations of BAP, $GA_3$ and IAA for *in vitro* plant regeneration. The $p^H$ of the media was adjusted 5.8 before autoclaving at 15 $lbs/cm^2$ at $121^0C$ for 20 minutes. Cultures after

inoculation were incubated at 25±2$^0$C and 65-70% relative humidity with photoperiod of 16/8h at 3000 lux intensity by florescent tubes.

**Results and Discussion**

Callus induction from stem explants was observed at different concentrations and combinations of BAP (0.5 and 1.0mgl$^{-1}$) and NAA (0.5- 3.0 mgl$^{-1}$), callus was visible seven days after inoculation in stem. The calli derived from stem explants were soft, whitish to creamish and friable. Among several combinations of growth regulators used, percent callus induction (69%) was found to be higher on MS supplemented with, BAP (1.0 mgl$^{-1}$) and NAA (3.0 mgl$^{-1}$) than in others tested for stem explants (Figure1).The best callogenic response in MS medium supplemented with BAP and NAA in stem explants of *Artemisia absinthium* (Nin et al., 1996) and *Sapium sebiferum* (Siril & Dher, 1996).

**Shoot Induction**

Shoots from stem derived callus was observed at different concentrations and combinations of BAP (0.5 and 1.0 mgl$^{-1}$) and $GA_3$ (0.5-3.0mgl$^{-1}$).The best shoot induction 31.50±0.51 per explants for stem callus was observed on 1.0 mgl$^{-1}$ BAP in combination with 3.0mgl$^{-1}$ $GA_3$ (Figure 2). The elongation were also achieved on the same parental medium (Figure 3).

Combination of BAP and $GA_3$, the effect of $GA_3$ 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 observed in *A. vulgaris*. There are many reports of $GA_3$ enhancing somatic embryogenesis and shoots from the calli in *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).

**Root Initiation and Elongation**

Roots were not induced during callus induction, shoot formation and shoot multiplication in the cytokinin and auxin regime. Individual shoots when implanted in half strength MS medium free from growth regulators, poor and few numbers of roots were elicited with low frequency. MS medium supplemented with IAA enhanced the frequency of rhizogenesis. Of the different concentrations of IAA tested, 0.5 mgl-$^1$ IAA induced the highest number of roots per shoot compared to other concentrations of tested (Table 3 & Figure 4). Root formed in IAA were thick and long role of IAA as an effective root inducing auxin have been reported in *Centaurium erythraea* (Piatczak and Wysokinska, 2003) and in *Amygdalus communis* L. (Akbas et al., 2009).

**Hardening and Field Transfer**

Rooted plantlets were transfered to polycups containing sterile soil and vermiculate (1:1) and kept in green house 65-70% humidity (Figure 5). Later they were transferred to the field with 95% survival rate. In conclusion, the above protocol describes rapid callus induction from stem 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.

**Table 1. Callogenesis response from stem explants at different concentrations and combinations of BAP and NAA.**

| Growth regulators in mgl$^{-1}$ | | % of Response | % of forming callus |
|---|---|---|---|
| BAP | NAA | | |
| 0.5 | 0.5 | 31 | 5.15±0.74 |
| 0.5 | 1.0 | 38 | 5.40±0.50 |
| 0.5 | 1.5 | 39 | 6.55±0.51 |
| 0.5 | 2.0 | 44 | 7.50±0.51 |
| 0.5 | 2.5 | 56 | 9.45±0.51 |
| 0.5 | 3.0 | 63 | 11.50±0.51 |
| 1.0 | 0.5 | 44 | 9.52±0.51 |
| 1.0 | 1.0 | 45 | 7.53±0.51 |
| 1.0 | 1.5 | 50 | 8.50±0.51 |
| 1.0 | 2.0 | 56 | 9.40±0.50 |
| 1.0 | 2.5 | 63 | 10.55±0.51 |
| 1.0 | 3.0 | 69 | 11.10±0.852 |

Values reperesent Means ± SE for 12 replicates per treatment in three repeated experiments.

**Table 2: Effect of Growth regulator on *in vitro* shoot induction from stem explants *A. vulgaris* L.**

| Growth regulators in $mgl^{-1}$ | | % of response | No. of shoots/explant | Shoot length (cm) |
|---|---|---|---|---|
| BAP | $GA_3$ | | | |
| 0.5 | 0.5 | 50 | 8.85±0.81 | 1.78±0.08 |
| 0.5 | 1.0 | 56 | 13.10±0.85 | 1.82±0.07 |
| 0.5 | 1.5 | 63 | 14.90±0.78 | 1.89±0.49 |
| 0.5 | 2.0 | 64 | 18.90±0.78 | 2.01±0.07 |
| 0.5 | 2.5 | 75 | 24.00±0.79 | 2.19±0.09 |
| 0.5 | 3.0 | 81 | 27.85±0.81 | 2.50±0.18 |
| 1.0 | 0.5 | 63 | 11.52±0.51 | 2.58±0.34 |
| 1.0 | 1.0 | 64 | 13.16±0.834 | 2.71±0.07 |
| 1.0 | 1.5 | 75 | 18.75±1.02 | 2.86±0.23 |
| 1.0 | 2.0 | 81 | 22.95±0.826 | 3.31±0.70 |
| 1.0 | 2.5 | 94 | 28.85±0.988 | 3.33±0.19 |
| 1.0 | 3.0 | 100 | 31.50±0.51 | 4.10±0.32 |

Values reperesent Means ± SE for 12 replicates per treatment in three repeated experiments.

**Table 3. Effect of different concentrations of IAA on *in vitro* rooting from stem explants of *A. vulgaris* L.**

| IAA in $mgl^{-1}$ | % of response | No. of roots/ shoot | Root length (cm) |
|---|---|---|---|
| 0.5 | 85 | 6.58±0.59 | 3.51±0.08 |
| 1.0 | 78 | 4.58±0.58 | 3.74±0.34 |
| 1.5 | 72 | 3.50±0.51 | 2.71±0.18 |
| 2.0 | 67 | 1.95±0.75 | 3.01±0.96 |

Values reperesent Means ± SE for 12 replicates per treatment in three repeated experiments.

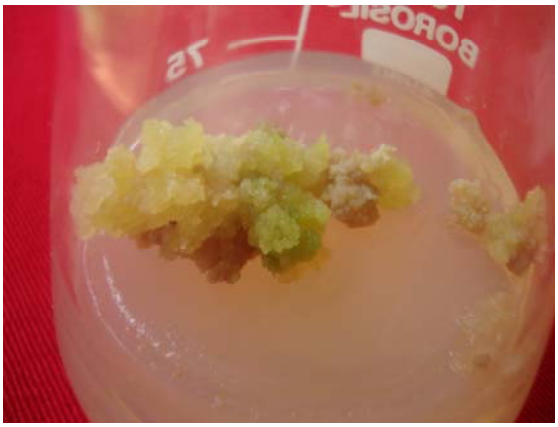

Figure 1

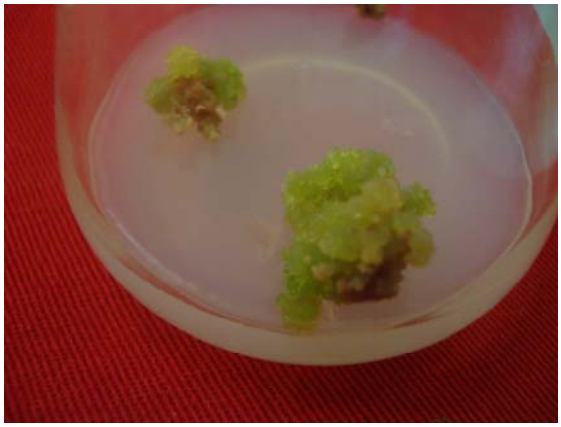

Figure 2

Figure 3

Figure 4

Figure 5

**Figure 1:** :Callus induction from stem explants of *Artemisia vulgaris* on MS medium containing BAP (1.0 $mgl^{-1}$) +NAA (3.0 $mgl^{-1}$).

**Figure 2 & 3:** Induction of multiple shoot and elongation on MS medium containing BAP (1.0$mgl^{-1}$) with $GA_3$ (3.0$mgl^{-1}$), respectively.

**Figure 4:** Direct rooting from regenerated shoot on MS mediu containing IAA (0.5 $mgl^{-1}$).

**Figure 5:** Hardened plantlets.

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