Induction of Callus in *Artemisia absinthium* L. -- A Valuable Medicinal Plant

Sumira Tyub, Azra N. Kamili, Hashim J. Chishti and A.M.Shah
Plant Tissue Culture Laboratory, Centre of Research for Development,
University of Kashmir, Srinagar 190 006, J&K India

ABSTRACT

Callus cultures were established from leaf and shoot tip explants of *Artemisia absinthium* L. MS medium containing different concentrations and combinations of BAP and NAA were used for callus induction. Different phytohormone concentrations resulted in different types and degrees of callus. Low BAP concentration produced low degree yellow coloured callus and higher BAP concentration produced intense green nodular callus in leaf explants. Combination of BAP and NAA produced loose but intense callus in leaf explants. Shoot tips were poorest to callus induction as compared to leaf explants. However, a combination of BAP (2.6 μM) and NAA (2.2μM) resulted in elongation of shoot apex in addition to callus formation at the base of the explants. No shoot multiplication was observed in shoot apices and callus raised in both the explants appeared non-regenerative.

Keywords: *Artemisia absinthium*, callus cultures, leaf, shoot tip

Abbreviations: MS- Murashige and Skoog; BAP- 6-Benzyl amino purine; NAA- Napthalene acetic acid; Kn- Kinetin; 2, 4-D- dicholorophenoxy acetic acid

INTRODUCTION

Artemisia absinthium L. commonly called worm wood is a medicinally and economically important plant belonging to family Asteraceae. It is a strong smelling biennial herb having bitter taste. It is traditionally used as medicine because of its anti helminthic properties. Chemicals derived from this plant are of growing interest due to their potential utility as antibacterial, antipyretic, cytostatic and antimalarial agents (Kaul, 1997; Abivardi and Benz, 1984; Chemosova *et al.*, 1987; Rao *et al.*, 1997; Zafar *et al.*, 1990). *Artemisia absinthium* is the main source of drug "Afsantheen" used in India in chronic fevers, swellings and inflammation of livers. The dried herb is used as home remedy against round worms. Crushed leaves are used as insect repellent and dried herb is kept in costly garments to avoid insect damage. The overexploitation by mankind for its medicinal properties, excessive

collection and habitat destruction leads to regular eradication of this plant species from its natural habitat which in turn results the threatened status of the plant.

Callus cultures raised from a bit of medicinally important plant tissue can be used for extraction of secondary metabolites or drugs for commercial use without sacrificing the whole plant (Bhosle and Paratkar, 2005). So the alternative technique of plant tissue culture helps the conservation of medicinal plants in nature by continuously recovering secondary metabolites from suspension cultures which are obtained from callus cultures. Hence considering the medicinal and economic importance of plant, its *invitro* culture was established. In this regard callus cultures were initiated using leaf and shoot tip explants of *Artemisia absinthium* on MS medium supplemented with various concentrations and combination of BAP and NAA.

MATERIAL AND METHODS

The plant material was collected from Pulwama district of J & K state. The explants were surface sterilized using 3-7% sodium hypochlorite for 10-15min after lab detergent labolene was used. The rest of the procedure was same as communicated in earlier papers (Bashir *et al.*, 2005; Tyub *et al.*, 2005). The medium used was Murashige and Skoog's (MS) (1962) supplemented with various concentrations and combinations of BAP and NAA. The cultures were incubated at 25±3°C and 16/ 8 hr. light/ dark photoperiod.

RESULTS AND DISCUSSION

Since several biochemical arrays can be performed from the callus cultures and looking into the importance of this plant present study was carried out on leaf and shoot tip explants of *A. absinthium.*

In leaf explants various BAP concentrations (2.5,5, 7.5, 10μM) helped in inducing low to intense degrees of callus formation (Fig 1&2), whereas combined influence of BAP (10μM) + NAA (10μM) was found highly effective in callus growth being intense as in BAP (10μM) (Fig 3 &Table 1). BAP (5μM) + NAA (5μM) was less effective than the former combination used (Fig4).This observation is not in agreement with that of Nin *et al.* (1996) who obtained multiple shoots from leaves of *A. absinthium* on combination of NAA and BAP but is in conformity with that of Kamili *et al.* (2001) who reported callus formation from leaf explants of *A. annua* on NAA although the callus showed rhizogenesis also. These results are again in line with Benjamin *et al.* (1990) who also reported callus formation in nodal segments of *A. pallens* when medium was adjuvanted with BAP and 2, 4-D instead of NAA. Our studies are also in agreement with Kamili *et al.* (2004) who observed formation of green nodular callus on BAP from petiole explants of *Artemisia annua.*

Different BAP concentrations used in shoot tip explants failed to induce callus de-differentiation while as only NAA (10μM) induced low callus formation at basal end (Fig 5 & Table 2). Combination of BAP (2.6μM) and NAA (2.2μM) also favoured low callus formation but this response was accompanied by the elongation of the shoot tips (Fig 6). Callus was either friable or nodular. Callus, even if in many cases was nodular depicted no re-differentiation potential hence remained non-regenerative in all the cultures during the study period and showed no shoot multiplication. In contrast, multiple shoots in etiolated hypocotyls and seedlings of *A. judaica* in presence of thidiazuron were registered by Liu *et al.* (2003). Such results were also observed by Usha and Swamy (1994) by the use of Kn in *A. pallens* and by Whipkey *et al.* (1992) in *A. annua* from leaves and nodal segments using BAP alone.

Hence, it is evident from this study that high BAP concentration (10μM) supports very high callus growth in leaf explants which reveals that endogenous level of cytokinin in explants might be low and hence need high exogenous level of BAP for getting high callus growth. NAA seems to have no additional effect on the growth of these explants. Moreover, leaf explants are better for the induction of callus than shoot apices. Hence for secondary metabolite production callus can be produced from leaf explants, for its maintenance, in *Artemisia absinthium*-- a valuable medicinal plant.

Table 1. Response of leaf explants on various concentrations of phytohormones

Medium	Response	Degree of callus formation	Percentage response
MS basal	No response	-	-
MS + BAP(2.5μM)	Slight yellow friable callus formed which turned brown after some time	+	70
MS + BAP(5μM)	Moderate yellow coloured callus formed	++	80
MS + BAP(7.5μM)	Moderate green nodular callus formed	++	80
MS + BAP(10μM)	Intense green nodular callus was formed	++++	90
MS + BAP(5μM)+ NAA(5μM)	Moderate yellow, friable callus formed	++	90
MS + BAP(10μM)+ NAA(10μM)	Intense greenish yellow friable callus formed	++++	90

+ low; ++ moderate; ++++ intense

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Fig. 1: Callus formation on BAP 5 µM from leaf explants of *A. absinthium.*
Fig. 2: Intense callus formation on BAP 10 µM from leaf explants of *A. absinthium.*
Fig. 3: Intense callus formation on BAP 10 µM + NAA 10 µM from leaf explants of *A. absinthium.*
Fig. 4: Callus formation on BAP 5 µM + NAA 5 µM from leaf explants of *A.absinthium.*
Fig. 5: Callus formation on NAA 10 µM from basal end of shoot tip explants of *A. absinthium.*
Fig.6: Callus formation at base of shoot tip explants of *A. absinthium* followed by elongation on BAP (2.6µM) and NAA (2.2µM)

Table 2. Response of shoot tip explants on various concentrations of phytohormones

Medium	Response	Degree of callus formation	Percentage response
MS basal	No response	-	-
S + BAP (2.5μM)	No response	-	-
MS + BAP (5μM)	No response	-	-
MS + BAP (7.5μM)	No response	-	-
MS + BAP (10μM)	No response	-	-
MS + NAA (5μM)	No response	-	-
MS + NAA (10μM)	Slight brown friable callus formed at basal end	+	70
MS + NAA (2.6μM) +BAP (2.2μM)	Slight brown friable callus formed at basal end followed by elongation of explant	+	70

+ low

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