Plant Regeneration from Callus Cultures of *Artemisia annua* Linn.

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ABSTRACT

Fresh leaves of *Artemisia annua* Linn., obtained from young healthy plants growing in the Botanical Garden of University of Kashmir, were cultured on MS medium (1962) adjuvanted with different phytohormones. Medium supplemented with different NAA concentrations favoured callus development where rhizogenesis occurred. Callus when grown on medium fortified with NAA – 5μM + Kn- 10μM, was stimulated to initiate multiple shoot buds which in turn developed into multiple shoots. Elongated shoots (3 – 4 cm) were separated and cultured on root inducing medium containing NAA–10μM. Full fledged plantlets were recovered in multiples.

Keywords : *Artemisia annua*, leaves, callus, shoot regeneration, plantlets.

Abbreviations: MS - Murashige and Skoog; NAA - Naphthalene acetic acid; Kn - Kinetin

INTRODUCTION

Artemisia annua belongs to family Asteraceae. It is an annual herb, introduced and cultivated in India, naturalized in Kashmir. The genus has medicinal importance. Chinese chemists isolated Quinghaosu from the plant which has been successfully used against malaria. The herb also yields essential oil of interest to perfumery, cosmetics and dermatology. The oil has specific fungicidal properties. Herbal medicines are in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects (Kamboj, 2000). Because of these uses the herb is continuously being exploited to meet the demands. The natural method of propagation is through seeds which remain dormant for two years. The propagation rate is much less than the rate at which the herb is exploited. Hence a need arises to switch over to faster propagation techniques. *In vitro* culture is nowadays considered to be a better technique for quick propagation of plants and has been applied to a number of medicinal herbs (Upadhyay *et. al.*, 1989; Arora and Bhojwani, 1989; Chaturvedi,

1975; Furmanowa *et.al.*, 1984; Furmanova and Guzowska 1989; Chandel and Sharma, 1996; Bhojwani and Arumugam, 1993; Sharma *et.al.*, 1995; Tandon and Rathore, 1992; Sen and Sharma, 1991). The present work reports a procedure for regeneration of multiple plantlets from leaf derived callus of *A.annua*.

MATERIAL AND METHODS

The young plants of *A.annua* Linn. were collected from medicinal plant unit, Tangmarg, Kashmir and immediately transplanted in the Botanical garden of University Campus. The fresh and healthy leaves were collected from the plants and used as explants. The leaves were thoroughly washed with Laboline (5% v/v) containing 1% wetting agent Tween-20 and then with running water. Surface sterilization of leaves was carried out with 0.1% $HgCl_2$ for 15 min. The explants were finally given 3 – 4 rinses with autoclaved double distilled water to remove the traces of sterilant. The leaves were cut into 1 cm segment in previously autoclaved petri dishes under laminar air flow and were aseptically inoculated on MS (1962) basal medium augmented with different phytohormones and 3% sucrose. The pH of the medium was adjusted to 5.8 before gelling the medium with 0.8% agar. This was followed by dispensing of the medium into culture vials which were plugged by non-absorbent cotton. The medium was sterilized by autoclaving at 1.06 kg/cm pressure and temperature 121°C, for 15 – 20 minutes before inoculations were performed. The cultures were grown under continuous fluorescent light of 2500 – 3000 lux at 25 ± 3 °C and relative humidity of 50 –60 %. For each treatment 10 replicates were used. Observations were taken after 4 weeks of incubation.

RESULTS

Callus Induction

The leaf explants cultured in the presence of different auxin concentrations induced callus formation and continued to proliferate but its degree of proliferation varied with different auxin concentrations (Table 1). The callus was invariably green, compact, nodular and shining (Fig.1). Root formation was observed in all the calli but profuse rooting occurred on MS + NAA– 0μM (Fig. 2). No callusing was observed in absence of growth regulators.

Regeneration from Callus

Callus growing on different NAA concentrations were subcultured on hoot inducing medium (MS+ NAA – 5μM + Kn – 10μM) at the end of 4 weeks

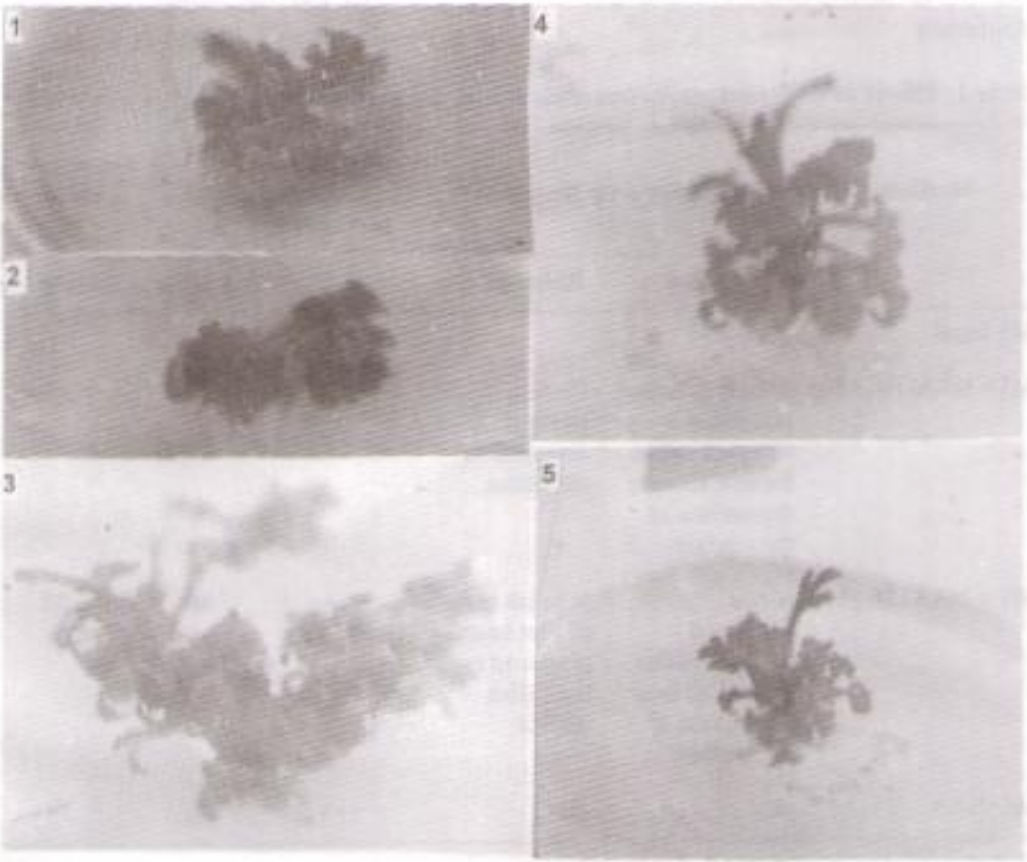

Fig. 1 - 5. In vitro response of *Artemisia annua* leaf to different phytohormones.

1 - Green and nodular leaf callus on MS+NAA-20µM (after 2 weeks);

2 - Multiple adventitious roots in leaf callus on MS+NAA-10µM (after 4 weeks);

3 - Multiple shoot formation in leaf callus on MS+NAA-5µM+Kn-10µM (after 4 weeks);

4 - Isolated shoots on rooting medium MS+NAA-10µM (after 1 week);

5 - Root formation in isolated shoots on MS+NAA-10µM (after 4 weeks).

culture period. The results obtained are given in Table 2. Multiple shoot buds were initiated in 60% cultures previously grown on NAA – 20μM. These shoot buds continued their elongation on the same medium. Multiple shoots of different sizes were produced after 4 weeks (Fig. 3.). Average number of shoots per culture was 30. In other cases no caulogenesis was observed but the callus continued to proliferate.

Table 1. Effect of different auxin concentrations on leaf explants of *Artemisia annua*

Medium	Morphogenetic Response*		Degree of Callus formation	% response	
	Callus	Rhizogenesis		Callus	Rhizogenesis
MS basal	-	-	-	-	-
MS+ NAA-10 μM	Cream coloured nodulated compact callus formed all over the surface of the explant	Profuse rooting occurred in the callus	++	100	40
MS + NAA- 20 μM	Green nodulated compact callus formed all over the surface of the explant	Whitish tufts of root hairs produced on the callus surface	++++	80	20
MS+NAA – 25 μM	-do-	-do-	+++	80	20

* the data scored at the end of 4 weeks culture period ; 10 explants / treatment
– = no growth , ++ = moderate, +++ = high, ++++ = intense.

Rooting of Shoots

Isolated shoots (approx. 2-3 cm) were cultured on rooting medium (MS + NAA – 10 μM) (Fig.4). The roots started coming out after Ist week of culture and continued their growth. The adventitious roots were short but thick and the number of roots was 3 –4 per shoot (Fig. 5). Rooting was successful in 70% cultures. The ..plete plantlets were allowed to grow for 4 – 6 weeks on the rooting medium. Further trials will be followed for hardening of these plantlets born *in vitro*.

Table 2. Response of subcultured leaf callus of *A.annua* on shooting medium

Primary Culture Medium	Shooting Medium	Response*	% response
MS+NAA-10μM	MS+NAA-5μM+Kn-10μM	Callus proliferation, no caulogenesis	100
MS+NAA-20 μM	-do-	Multiple shoot formation and their elongation.	60
MS+NAA-25μM	-do-	Callus poliferation, no caulogenesis	100

* Data scored after 4 weeks culture period; 10 explants / treatment

DISCUSSION

Tissue culture offers an attractive alternative for propagation of plants at a much faster rate. Attempts were made to culture the leaf explants of *A.annua* for its regeneration and multiplication. In general MS medium fortified with auxin NAA favoured callus formation which was compact and nodulated. Rhizogenesis occurred in all the concentrations of NAA. Benjamin *et al.* (1990) reported callus formation in nodal segments of *A. pallens* when medium was adjuvanted with BA and 2,4 –D but no rhizogenesis was recorded in that callus. However, in present studies NAA alone proved effective for callus formation and rhizogenesis in leaf explants. Nin *et al.* (1996) have also reported only callus formation from leaf explants of *A.absinthium* but in presence of BA, thus partly contradictory to our results.

Callus recovered from NAA –20 μM supplemented medium was subcultured under the influence of NAA – 5 μM + Kn – 10μM. Multiple shoots emerged out which elongated on the same medium. These findings come very close to the report of Nin *et al.*(1996) who also have used a combination of auxin (NAA) and cytokinin (BA) to get caulogenesis in the leaves of *A. absinthium.* Multiple shoot regeneration have also been reported by Whipkey *et al.* (1992) in *A. annua* from leaves and nodal segments but they used cytokinin BA alone, whereas Usha and Swamy (1994) reported the use of Kn for multiple shoot

formation in *A. pallens*. Isolated shoots developed roots when subcultured on NAA – 10 μM supplemented medium. Similar results were obtained by Usha and Swamy (1994) in *A. pallens* but on contrary Whipkey *et al.* (1992) achieved rooting in presence of IBA.. The results indicate that the cultured tissues of *A. annua* possess the potentiality to regenerate and produce plants in multiples.

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