Plant regeneration from callus cultures of *Valeriana wallichii* DC.

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ABSTRACT

Petiole explants of *Valeriana wallichii* DC., a threatened medicinal plant, were used for inducing callus. Optimum callus formation was observed on Murashige and Skoogs' (1962) medium supplemented with 3.0 mg/l NAA and 0.25 mg/l Kn. Shoot regeneration was achieved upon transferring the callus to medium containing 1.0 mg/l Kn and 0.25 mg/l NAA. Shoot regeneration was achieved upon transferring the callus to medium containing 1.0 mg/l Kn and 0.25 mg/l NAA. Complete plantlets were obtained on the same medium or upon transfer of the regenerated shoot buds to medium containing 5.0 mg/l Kn and 1.0 mg/l IAA. Nearly a thousand callus regenerated plants were successfully transferred to the field following previously standardized hardening procedures.

ABBREVIATIONS

BAP - 6 - Benzylaminopurine; 2,4-D - 2,4-dichlorophenoxyacetic acid; 2iP - 2 - isopentenyladenine; IAA - indole - 3 - acetic acid; IBA - indole - 3 - butyric acid; Kn - Kinetin; MS - Murashige and Skoogs' medium (1962); NAA - α - napthalene acetic acid; Z - Zeatin.

INTRODUCTION

*Valeriana wallichii* DC. is found as ground vegetation in deciduous - coniferous mixed forests across the Himalayan ranges between 1500 and 3000 m (Kapoor and Sarin, 1987). The sedative and tranquilizing properties of the plant are due to the presence of iridoid esters called valepotriates (Thies and Funke, 1966). *V. wallichii* is, thus, used in more than 50 prescriptions in the 'Ayurvedic' and 'Yunani' systems of medicine and is a principal source of standardized preparations like Valmane, Baldrisedon and Harmonicum (Becket and Chavadej, 1988).

However, this important medicinal plant is not cultivated anywhere in India and all demands for its domestic and foreign trade are met from its wild populations. Over the years, its indiscriminate collection has led to its large scale depletion in the wild and has necessitated its replenishment and cultivation (Husain, 1983). In this context, a previous report by Mathur et al. (1988) dealt with the successful establishment of a protocol for rapid propagation of the plant using apical and axillary shoot cuttings. An alternative approach for obtaining plants of *V. wallichii* would involve regeneration from callus cultures. Though the establishment of callus cultures and studies related to valepotriate production from callus have been reported earlier by Becker et al. (1977, 1980) the regeneration of plantlets from callus cultures of *V. wallichii* had not been achieved. The present communication describes a procedure for the regeneration of plantlets from petiole derived callus cultures of *V. wallichii*, as a prerequisite for further work on the improvement of the plant using in vitro techniques.

MATERIALS AND METHODS

MS medium (1962), supplemented with 3.0% sucrose, 100 mg/l myo-inositol and 0.7% (w/v) agar was used for all in vitro experiments. In each case the pH of the medium was adjusted to 5.8±0.1 prior to autoclaving. Cultures were maintained at 25±2°C with 16 h photoperiod using cool, fluorescent light (3000 lux). For each treatment at least 15 replicates were used and each experiment was repeated twice. Observations were taken after 6 wk of incubation.

For callus induction, petiole explants, (ca 1.0-1.5 cm in length) were obtained from plantlets growing in vitro on MS medium containing 5.0 mg/l Kn and 1.0 mg/l IAA (Mathur et al. 1988). The explants were placed on different levels of IAA, IBA, NAA (1.0-4.0 mg/l) and 2,4-D (1.0-3.0 mg/l) in combination with 0.0-2.0 mg/l of Kn.

Experiments on regeneration from callus cultures were conducted using callus maintained on medium containing NAA and Kn for 20 wk. Approximately 1.0 g of callus was placed in each culture tube containing different levels and combinations of cytokinins (Kn, BAP, 2iP, Z) and auxins (IAA, IBA, NAA) as detailed in the results. Following regeneration of shoots, complete plantlets were obtained on the growth and development medium (5.0 mg/l Kn and 1.0 mg/l IAA). These were subsequently transferred to pots filled with a mixture of soil : vermiculite : farm yard manure (2:1:1). They were maintained under high humid conditions (95-100% relative humidity) for the initial 3-15 days and transferred to field following a total hardening period of 20-30 days.

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in the glasshouse (Mathur et al. 1988). For histological studies the regenerating tissue was embedded in paraffin wax following standard procedure (Johansen 1940) and sectioned at 10 μm. Aniline blue and safranin stains were used.

RESULTS AND DISCUSSION

Callus induction and maintenance

The responses of the petiole explants on media containing different levels and combinations of auxins and kinetin are summarized in Fig. 1.

No callusing was observed in the absence of growth regulators. However, when a portion of the leaf lamina remained attached to the petiole explant, roots were produced from the cut end even on the basal medium. Similarly on media containing Kn alone at 0.5-1.0 mg/l concentration, no callusing was observed but roots were formed. On higher Kn levels root formation was inhibited.

Amongst the auxins, both IAA and IBA were found unsuitable for callus induction. In both cases only slight callusing was observed at the 4.0 mg/l level either alone or in combination with 0.2-0.5 mg/l of Kn. On low auxin levels and in combinations with 0.2-2.0 mg/l Kn, rhizogenesis was the predominant response. However, as reported earlier by Becker and Schrall (1980), callusing was observed on all levels of NAA ranging from a moderate response on 1.0 and 2.0 mg/l to an extensive response on 3.0 and 4.0 mg/l. A slight degree of root formation took place on media containing low NAA : Kn ratios (viz. 1.0 mg/l NAA with 2.0 mg/l Kn). On media with high (3.0-4.0 mg/l) NAA and low Kn (0.2-0.5 mg/l) a fast growing, friable, light green callus was obtained. However, on 4.0 mg/l level of NAA both with and without Kn, rapid browning of the callus took place within 25-30 days. Moderate callusing was also observed on media containing 1.0 mg/l of 2,4-D either alone or in combination with 0.2-1.0 mg/l of Kn and on 2.0 mg/l level of 2,4-D with 2.0 mg/l of Kn. In general, further increase in 2,4-D levels led to browning and death of the explants within 20 days.

On the basis of the above mentioned observations a callus stock was generated on 3.0 mg/l NAA with 0.25 mg/l Kn over a 20 wk duration with subcultures every 5 wk, for experiments on regeneration from callus.

Regeneration from callus

The results for experiments carried out for regeneration from callus are summarized in Fig. 2.