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CALLUS GROWTH PHASE STUDIES OF *ROYLEA ELEGANS* WALL.- AN IMPORTANT MEDICINAL PLANT OF HIMALAYAS

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ABSTRACT

Roylea elegans Wall. (Lamiaceae) is an important aromatic plant. Therefore a protocol was set for its *in vitro* culture. Callus biomass initiated from leaf tip, leaf base and petiole explants using 0.5 to 2.0 mgl^{-1} Kn and 1. 0 to 3.0 mgl^{-1} 2,4-D in different combinations was measured. 2.0 : 2.0 $mgl^{-1}$of Kn and 2,4-D combination in MS medium was found to be most appropriate for obtaining maximum biomass in leaf tip and petiole explants while 0.5 : 2.0 $mgl^{-1}$of the same gave maximum biomass in leaf base explant.

Keywords : *Callus growth phase, Roylea elegans*

INTRODUCTION

It is well known that due to habitat destruction, over-grazing and legal or illegal exploitation of medicinal and aromatic plants there is a serious threat to the survival of many species due to which a large number of plants have been declared endangered in the Himalayan region. Plant tissue culture represents one way of possible recovery of some endangered and over harvested medicinal plants as well as a method for providing plant materials for extraction of medicinally important compounds. Considerable attention has now been given to the conservation and multiplication of Himalayan threatened species of medicinal importance (Arumugam and Bhojwani; 1990; Giri *et. al.*,1993; Mathur 1993; Sulaiman 1994).

Roylea elgans Wall. (family: Lamiaceae), also known as Karui, Titpatti is an important aromatic shrub found in the Himalayas upto 5,000 ft. Decoction of the leaves of Karui is bitter in taste and used in malarial fever (Gaur, 1999), as a febrifuge by the Jaunsaris. The drug is used as blood purifier and in curing pimples and tonsils etc. It possesses acute- inflammatory activity. The leaf extract has analgesic activity, reduces motor activity and has relaxant activities (Kumar *et.al.*, 1981). The active principles of *R. elegans* are triterpene (moronic acid), hentriacontane, triacontane, pentacosane, octacosanol, friedelin, beta-amyrin, beta-sitosterol,k betulonic acid, and anthraquinone glycoside.

Micropropagation and callus biomass studies of *R. elegans* has not, to our knowledge, been previously reported. Due to high medicinal importance, the plant population is declining as a

result of its over-exploitation.Therefore, the development of a protocol for obtaining the maximum biomass is pre-requisite for micropropagation which is being tried in the present investigation via callusing.

MATERIAL AND METHODS

Young top shoot cuttings having 3-4 nodes (each node with 2-3 leaves) are collected from healthy plants of *Roylea elegans* from the forest where they grow as wild plants. These explants are washed thoroughly with tap water followed by a wash with 1% (v/v) Labolene detergent for 15 minutes and then in running tap water. Plant parts viz., leaf and petiole are surface sterilized with 70 to 90 % ethanol for 30 seconds, followed by 0.1 % (w/v) $HgCl_2$ with two drops of Tween 80 per 100 ml solution for 1 minute. The explants are then rinsed several times with sterilized double distilled water. The petiole explants are cultured separately in the medium. The leaf cut vertically into two parts making leaf tip and leaf base are also placed dorsally or ventrally in the medium. Explants are inoculated in test tubes/conical flasks containing MS basal medium(Murashige and Skoog, 1962) supplemented with 3 % sucrose and 0.8% agar. The medium is fortified with auxin (2,4-D) and cytokinin (Kn), in varying concentrations and combinations as indicated in the results.

The pH of all the media is adjusted to 5.8 ± 0.1 using 0.1 NaOH or 0.1 N HCl. The medium is autoclaved at 1.06 kg cm^{-2} at 121°C for 25 to 30 minutes. All the cultures are incubated at 24°C±2°C temperature and 60% relative humidity with 16:8 hours light : dark photoperiod.

The fresh callus is weighed and then dried in an oven at 40°C for one week and then the dried callus also weighed to obtain dry weight values. Initially fresh weight (FW) and dry weight (DW) values were measured after 30 days and then at the interval of 15 days onwards upto 60 days after inoculation. Three replicates are tried for each combination and the data are tested statistically.

RESULTS AND DISCUSSION

In the leaf tip explant it is evident from the Table 1 that initial fresh weight (FW) in 1.0: 1.0 mgl^{-1} combination of Kn and 2,4-D was found to be 524.0 ± 7.0 mg which increased to 1147.5 ± 10.5 mg at the end of the study period. Dry weight values reflected 63.83 % to 72.02 % loss of moisture at successive stages of observations. 637.5 ± 4.5 mg of callus was obtained initially when higher concentration of 2,4-D (2.0 mgl^{-1}) was used which increased to 1227.0 ± 11.0 mg at

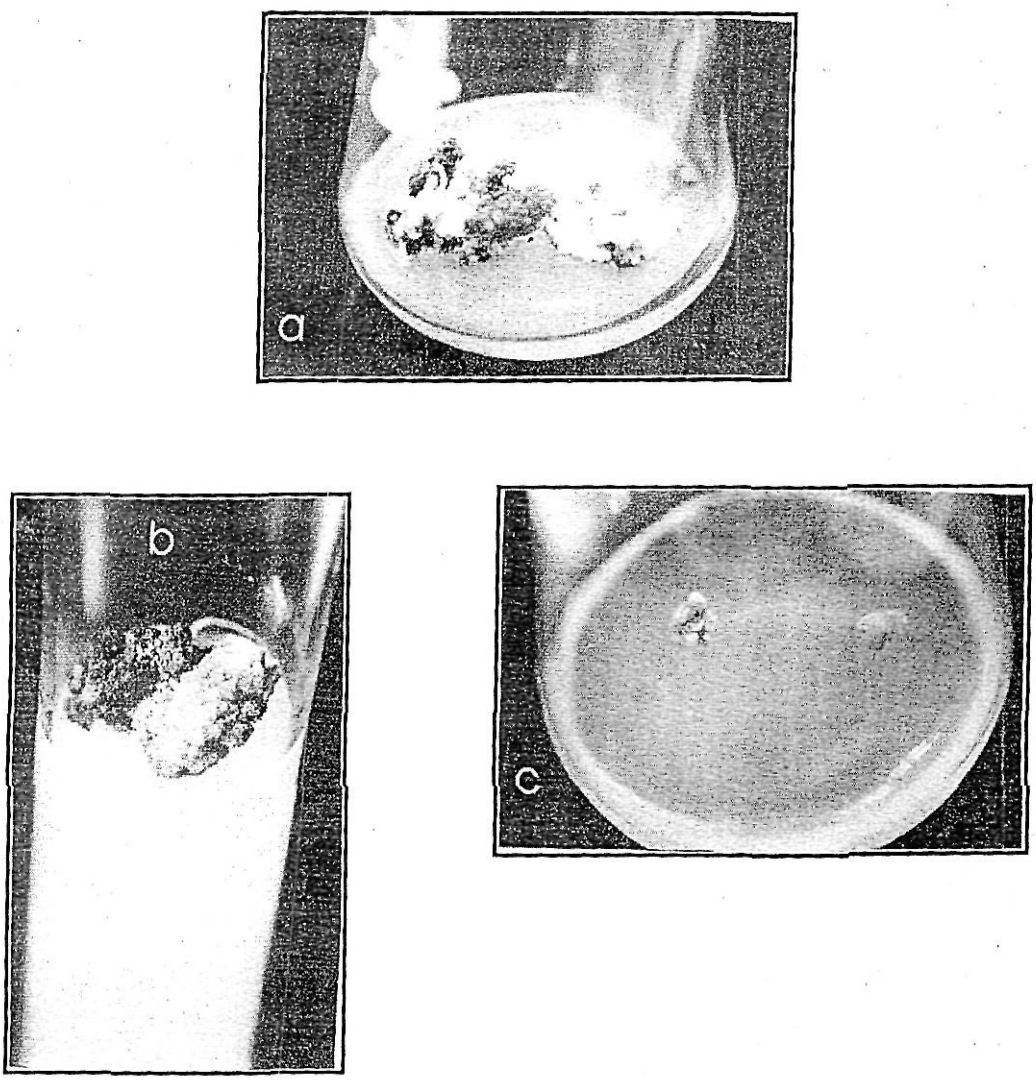

Fig.1 : In vitro callusing in *R.elegans.*

a. Callusing in leaf tip explant (2.0:1.0 Kn and 2,4-D).

b. Callusing in leaf base explant (0.5:2.0 Kn and 2,4-D).

c. Callusing in petiole explant (0.5:2.0 Kn and 2,4-D).

In the present study callus was induced from young parts of the plant of Karui as physiological age of the explant is an important factor in determining the morphogenetic response (Ammirato, 1996). Callus biomass measured on fresh and dry weight basis at the interval of 15 days up to 60 days in different explants exhibited that 2.0 : 2.0 mgl^{-1} of Kn and 2,4-D gave best

callus mass and thus maximum FW values in the leaf tip and petiole explants (Table1 &2). Higher concentrations of kn and 2,4-D reflected similar results in *Centella asiatical* (Bisht, 2002). In leaf base explants 0.5 : 2.0 mgl^{-1} concentration of Kn and 2,4-D reflected maximum FW at the end of the study period. Similar results have been depicted in *Psoralea corylifolia* leaves and stem explants (Saxena *et. al.* 1997) This reflects that higher concentration of 2,4-D (2.0mgl^{-1}) has higher capacity of callus induction resulting to maximum biomass values when combined with appropriate concentration of Kn. Callus production occurred with 2.0 mgl^{-1} 2,4-D alone in *Dalbergia latifolia* Roxb. (Sudhadevi and Nataraja, 1987) which is substantiating our findings. De Jong *et al* (1993) observed that MS media enriched with 2,4-D alter cell polarity and promotes subsequent cell division. This may be the reason for best callus mass initiation with higher concentration of 2.4-D in the present study.

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