RESEARCH ARTICLE | International Journal of Plant Sciences, (July to December, 2009) Vol. 4 Issue 2 : 354-356

Caulogenesis in *Heracleum candicans* Wall.

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Accepted : February, 2009

SUMMARY

Callus was initiated from petiole explants on MS medium supplemented with 0.5 mgl^{-1} 2,4-D and 0.5mgl^{-1} BAP. The best combination of growth regulator for maximum callus growth was obtained upon subculture of callus to a medium supplemented with 1mgl^{-1} 2,4-D, 0.25 mgl^{-1} Kn, 1% sucrose and 825 mgl^{-1} NH_4NO_3.

Key words : Callus, *Heracleum candicans*, Tissue culture, Xanthotoxin.

Heracleum candicans (Apiaceae) is an important medicinal herb grows wild in open grasslands, sloppy wetland and rock crevices of northwest Himalayas whose roots contain xanthotoxin is used to treat leucoderma and in the suntan lotion formulations (Davies and Dale, 1979; Duncan, 1955). The objective of the present study is to perfect the conditions for proliferic callus formation in this species. There is no report on caulogenesis of this species. It is reported here for the first time a protocol for callus formation. The results obtained can be useful in studies related to the xanthotoxin production from callus cultures.

MATERIALS AND METHODS

Petiole excised from mature plants growing wild in Budhal (4300m altitude), Jammu, India were used as explants. They were surfaced sterilized in 70% ethanol for 30 sec. followed by 0.1% $HgCl_2$ for 2 min and rinsed 4-5 times with sterilized distilled water. MS medium fortified with varying concentrations of 2,4-D, IBA, NAA, BAP, Kn either singly or in combination were employed in this study (Eriksson, 1965).

Callus initiation was assessed visually using scale of 1-4 (smallest to largest). Scale '0' was given when no callus was formed. Callus index was calculated as

$$\text{Callus index } Z \frac{(n \times G)}{N} \times 100$$

where n- total number of explants forming callus, G- average callus rating on explants and N- total number of explants cultured.

Callus growth was determined by measuring fresh weight after 4 weeks of culture. The growth rate was expressed as the ratio of the increase in fresh weight (FW) to the initial FW (400 mg per callus piece). MS medium containing varying concentrations of 2,4-D and Kn, carbohydrates, NH_4NO_3 and KNO_3 were used for assessing callus growth. Five callus pieces (400 mg FW each) per treatment were used.

The pH of all medium was adjusted to 5.8 prior to solidifying with 0.8% agar and autoclaved at 15 psi for 15 min. All cultures were maintained at a temperature of 25±2^{O}C and 50±5 % relative humidity under 16h illumination of 30µE m^{-2} s^{-1} provides by Bajaj fluorescent tubes (40W). Each experiment was repeated at least once. Mean were analyzed by analysis of variance (ANOVA) and compared with Duncan's new multiple range test (Duncan, 1955).

RESULTS AND DISCUSSION

Callus was formed from the cut ends of the explants within 2-3 weeks of culture. The type and concentration of auxin significantly affected callus initiation (Table 1). Optimal callus formation occurred on MS medium containing 0.5mgl^{-1} 2,4-D. Superiority of 2,4-D for callus initiation attributed to its stable nature in the medium (George, 1993). 2,4-D when used in combination with BAP

Table 1 : Effect of growth regulators on callus initiation from different explants of *Heracleum candicans* after 4 weeks of culture

Growth regulators[1] (mgl^{-1})	Explant type[2]		
	Petiole	Root	Leaf lamina
Basal medium	-	-	-
2, 4-D (0.5)	328	280	155
NAA (0.5)	289	253	100
IBA (0.5)	80	20	-
IAA (0.5)	70	22	-
2, 4-D (0.5) + Kn (0.1)	290	162	10
2, 4-D (0.5) + Kn (0.5)	212	152	20
2, 4-D (0.5) + BAP (0.1)	310	160	22
2, 4-D (0.5) + BAP (0.5)	360	205	50

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