

Caulogenesis in *Heracleum candicans* Wall.

R.K. SHARMA

Accepted : February, 2009

SUMMARY

Callus was initiated from petiole explants on MS medium supplemented with 0.5 mg l⁻¹ 2,4-D and 0.5 mg l⁻¹ BAP. The best combination of growth regulator for maximum callus growth was obtained upon subculture of callus to a medium supplemented with 1 mg l⁻¹ 2,4-D, 0.25 mg l⁻¹ Kn, 1% sucrose and 825 mg l⁻¹ NH₄NO₃.

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% HgCl₂ 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

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

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, NH₄NO₃ and KNO₃ 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°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 0.5 mg l⁻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 (mg l ⁻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

Correspondence to:

R.K. SHARMA, Department of Botany, G.G.M. Science College, JAMMU (J&K) INDIA

significantly enhanced the callus initiation response.

Callus initiation is known to be dependent on type and size of explant (Hirai *et al.*, 1997). Callus initiation is known to be dependent on type and size of explant (Davies and Dale, 1979). Among the explant types and sizes examined, 6 mm long petiole explants exhibited high callus initiation response. Variation exhibited by explants type

Table 2 : Effect of 2,4-D and Kn on callus growth of *Heracleum candicans* after 4 weeks of culture

2,4-D (mg ^l ⁻¹)	KN (mg ^l ⁻¹)	Increase in FW/culture (mg) (mean \pm s.d)	Growth rate
0.5	0.0	295.6 \pm 42.2 ^b	0.7
1.0		1312.2 \pm 92.6 ^c	3.0
2.0		1924.8 \pm 97.9 ^h	5.0
4.0		167.6 \pm 11.0 ^a	0.4
0.5	0.1	1503.4 \pm 70.0 ^f	4.0
1.0		1693.8 \pm 51.9 ^g	4.0
2.0		531.0 \pm 53.5 ^c	1.0
4.0		187.0 \pm 22.9 ^a	0.5
0.5	0.25	1886.8 \pm 72.5 ^h	5.0
1.0		2419. \pm 114.9 ⁱ	6.0
2.0		410.4 \pm 23.8 ^c	1.0
4.0		181.0 \pm 11.9 ^a	0.5
0.5	0.5	536.0 \pm 76.21 ^c	1.0
1.0		1269.4 \pm 76.2 ^e	3.0
2.0		1561.2 \pm 96.8 ^f	4.0
4.0		783.6 \pm 65.2 ^d	2.0

Means with same superscript in a column are not significantly different from each other at 5% level.

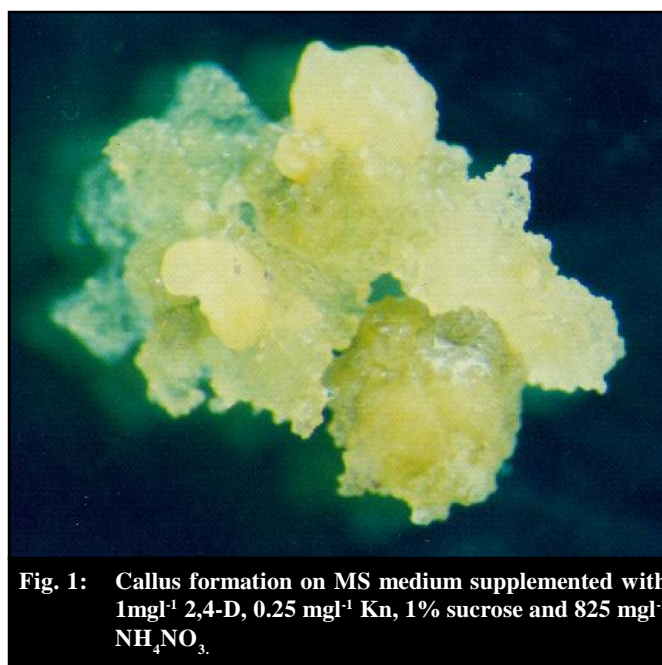


Fig. 1: Callus formation on MS medium supplemented with 1mg^l⁻¹ 2,4-D, 0.25 mg^l⁻¹ Kn, 1% sucrose and 825 mg^l⁻¹ NH₄NO₃.

with respect to callus initiation may be due to differences in endogenous hormone level (Krens and Jamer, 1989; Goyal and Maheshwari, 1990). Maximum callus growth was obtained upon transfer of callus from 0.5 mg^l⁻¹ 2,4-D and 0.1mg^l⁻¹ BAP supplemented medium to a medium containing 1mg^l⁻¹ 2,4-D and 0.5mg^l⁻¹ Kn (Table 2; Fig. 1). Among different carbon sources used for callus growth, sucrose (1%) gave best results (Table 3) whereas high concentrations (2-6%) retarded the callus growth.

Table 3 : Effect of carbohydrates on callus proliferation in *Heracleum candicans* after 4 weeks of culture

Carbohydrates (%)	Increase in FW (mean \pm S.D.)	Growth rate
Sucrose		
1	2728 \pm 38.1 ⁱ	7.0
2	2569.6 \pm 66.2 ^h	6.0
3	2368 \pm 70.0 ^g	6.0
4	2186.4 \pm 55.7 ^f	5.0
Sorbitol		
1	1821.6 \pm 146.6 ^d	5.0
2	2350.6 \pm 60.3 ^g	6.0
3	2150.4 \pm 97.7 ^g	5.0
4	1823 \pm 52.9 ^d	4.0
Mannitol		
1	156.8 \pm 16.0 ^a	0.4
2	231.2 \pm 8.1 ^a	0.6
3	352.4 \pm 39.8 ^b	0.9
4	223.4 \pm 19.4 ^a	0.6

MS medium supplemented with 1.0mg^l⁻¹ 2,4-D and 0.25 mg^l⁻¹ Kn was used.

Means within column followed by the same superscript are not significantly different from each other at 5% level.

Table 4 : Effect of ammonium nitrate and potassium nitrate on callus proliferation in *Heracleum candicans* after 4 weeks of culture

Macronutrients	Increase in FW (mean \pm s.d)	Growth rate
KNO₃		
950	2137.8 \pm 84.3 ^b	5.0
1900	2710.6 \pm 36.2 ^c	7.0
2850	3012.8 \pm 115.8 ^e	8.0
3800	2200.6 \pm 46.3 ^f	6.0
NHNO₃		
825	3088.8 \pm 56.9 ^e	8.0
1650	2741.6 \pm 65.8 ^d	6.0
2475	2528.6 \pm 70.0 ^c	6.0
3300	1956.2 \pm 67.7 ^a	5.0

MS medium supplemented with 1.0mg^l⁻¹ 2,4-D, 0.25 mg^l⁻¹ Kn and 1% sucrose was used.

Means within column followed by the same superscript are not significantly different from each other at 5% level.

Maximum callus growth was obtained on a medium supplemented with reduced concentration of ammonium nitrate (50% of that MS medium) or increased concentration of potassium nitrate (50% of that MS medium) (Table 4).

This observation is in accordance with the results obtained in *Haplopappus gracilis* (Eriksson, 1965). The growth rate of callus increased from 1-4 weeks of culture

after which showed decline. The present protocol can be used by those who would like to exploit the callus of *Heracleum candicans* for the production of xanthotoxin.

Acknowledgment:

Authors thanks Head of the Department of Botany for providing laboratory facilities.

REFERENCES

- Davies, M.S and Dale, M.M. (1979). Factors affecting *in vitro* shoot regeneration on leaf discs of *Solanum laciniatum*. *Z. Pflanzenphysiol*, **92** : 51-60.
- Duncan, D.B. (1955). Multiple range and multiple F-tests. *Biometrics*, **11**: 1-42.
- Eriksson, T. (1965). Studies on the growth requirement and growth measurements of cell cultures of *Haplopappus gracilis*. *Physiol. Plant*, **18** : 976-993.
- George, E.F. (1993). Plant propagation by tissue culture. Exegetics Ltd. Edington, England.
- Gharyal, P.K. and Maheshwari, S.C. (1990). Differentiation in explants from mautre leguminous trees. *Plant Cell Rep.*, **8** : 550-553.
- Hirai, G., Kasai, N. and Harada, T. (1997). Somatic embryogenesis in mature zygotic embryo culture of *Glehnia littoralis*. *Plant Cell Tiss. Org. Cult.*, **48** : 175-180.
- Khoder, M., Villemur, and Jonard, R. (1970). Micropropagation et bouturage *in vitro* chez le jasmine (*Jasminum officinale* L.) *Compt. Rend. Acad. Sci. Paris*, **288** : 323-326.
- Krens, F.A. and Jamer (1989). The role of explant source and culture conditions on callus induction and shoot regeneration in sugar beet (*Beta vulgaris* L.). *J. Plant Physiol.*, **134** : 651-655.

