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A CASE STUDY

Callus formation in *ferula jaeschkeana* vatke

■ **R.K. SHARMA**

SUMMARY

Callus was initiated from the petiole explants of *Ferula jaeschakeana* on a medium containing 2,4-D. The highest percentage of explants forming callus (92.5) and callus index (258) were obtained on medium supplemented with 1 mgl^{-1} 2,4-D. Among explants sizes and type tested for callus initiation, 10 mm long petiole gave maximum callus index. Small and large explants initiated a slight amount of callus. Addition of Kn in combination with 2,4-D enhanced the callus formation from the explants.

Key Words : Callus, *Ferula jaeschkeana*, Tissue culture

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Ferula jaeschkeana Vatke is an important medicinal perennial herb known as Jangli Heeng grows wild on the dry sunny mountain slopes of Kashmir Himalaya at an altitude ranging from 2000-4000m. The roots of the plant yields oleo-resin gum which is traditionally used as antihistaminic, antiseptic and abortifacient (Hussain *et al.*, 1988). Its gum has been found effective in treating wounds and bruises (Lewis and Elvin-Lewis, 2003). The species is an out-breeding and propagates by seed. Preliminary studies reveals that the seed germination is poor in this species. This coupled with unscrupulous collection of roots and fruits from the wild populations for trade purpose has resulted in the depletion of its natural populations. The species is now listed as vulnerable by International Union for Conservation of Nature and Natural Resources and the need of immediate attention to conserve this plant has become imperative (Dubey *et al*., 2004). Tissue culture methods which have been used to propagate and conserve several medicinal plants (Arumugam and Bhojwani, 1990; Constable,1984; Krishnan *et al*., 1995; Wakhlu and Sharma, 1998 and Wakhlu *et al*., 1990) can be a useful alternative method for mass propagation and ex-situ conservation of this species as well. For the utilization of techniques, it is important to optimize *in vitro* conditions for regenerating plants from the cultured tissue. There is, however, no information available about tissue culture response and plant regeneration on this plant. Hence, the present study.

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MATERIAL AND METHODS

Plants of *Ferula jaeschkeana* Vatke were collected from wild populations located at Banihal (2,800m altitude) Jammu (J&K) between April to June 2011. They were kept in bags in a refrigerated at 5-7 ^{0}C. Leaf petiole (1–2 cm) explants were excised and washed with tap water. They were rinsed 4 to 5 times with tap water. Explants were then surface sterilized with 70 per cent (v/v) ethanol for 30 sec., 1 min in 0.1 per cent mercuric chloride (w/v) solution and were rinsed 5 to 6 times with sterilized distilled water. The effects of auxins (0.5-4.0 mgl^{-1} 2,4-D, NAA, IBA and 1AA), cytokinins (0.5-4.0 mgl^{-1} BAP, Kn) and explant type and size (5-20 mm) were tested. The response was recorded as percentage of explants forming callus and size of callus formed on each of the cultured explant. Callus initiation was visually assessed on a scale of 1-4 (smallest to largest). Scale '0' was given when no callus was produced.

Callus index was calculated as :

$$\frac{n \times G}{N} \times 100$$

where,

n = total number of explants callused,

G = average callus rating on explants and

N = total number of explants cultured.

Five explants per treatment used which was replicated once.

The effects of 2,4-D (0.5-4.0 mgl^{-1}) and Kn (0.5-1.0 $mg1^{-1}$) were tested. The growth of calli was estimated by measuring their fresh and dry weight. Fresh weight. (FW) was obtained by weighing calli immediately after the removal of residual agar. Dry weight (DW) was measured after drying the callus at 80°C for 24h. The growth of callus was measured in terms of increase in fresh weight and dry weight. The growth was expressed as the ratio of the increase in fresh weight to the initial fresh weight of callus. The growth rate was calculated as:

$$\text{Growth rate} = \frac{\text{Final fresh weight} - \text{Initial fresh weight}}{\text{Initial fresh weight}}$$

Five callus pieces (400 ± 50 mg FW) per treatment were used and replicated once. Cultures were maintained in 16 h photoperiod (30 $\mu EM^{-2}S^{-1}$) provided by cool white fluorescent tubes (40 W Bajaj India Ltd) at a temperature of 23±2°C and 50± 5 per cent relative humidity. Experiments were repeated at least once. Data were taken after 4 weeks of culture and analyzed by analysis of variance (ANOVA) (Cochran and Cox, 1957).

RESULTS AND DISCUSSION

The present study revealed that callus can be initiated from explants in the presence of an auxin. 2,4-D was the most potent source of auxin for inducing callogenesis from various explants. Similar effect of 2,4-D on callus initiation response has been reported in other Apiaceace species such as *Bunium persicum* (Valizadeh *et al*., 2008), *Daucus carrota* (Ibaraki *et al*., 2000) and *Ferula assafoetida* (Zare *et al*., 2010). The basal medium lacking growth regulators was not able to induce callus initiation (Table 1). The superior effect of 2,4-D over other auxins for callus initiation has been ascribed to its stable nature in the medium (George,1993). The initiation of callus

Table 1: Effect of growth regulators on callus initiation from petiole explants of *Ferula jaeschkeana* after 4 weeks of culture

Growth regulators (mgl^{-1})	% Explants forming callus	Callus index
2,4-D(0.0)	0.0	0.0
2,4-D(0.5)	80.4±2.0	120±3.4
2,4-D(1.0)	92.5±4.1	258±2.6
2,4-D(2.0)	65.2±3.4	139±2.1
2,4-D(4.0)	52.1±2.6	42±3.1
2,4-D(0.5) +Kn(0.5)	94.3±3.0	278±3.1
2,4-D(1.0) +Kn(1.0)	98.1±3.1	384±4.2
2,4-D(2.0)+ Kn(2.0)	91.2±2.1	305±2.5
2,4-D(4.0)+ Kn(4.0)	86.1±1.4	274±3.1
2,4-D(0.5)+BAP(0.5)	74.3±3.0	218±3.1
2,4-D(1.0)+BAP(1.0)	78.1±2.1	154±3.2
2,4-D(2.0)+BAP(2.0)	61.2±2.1	125±2.5
2,4-D(4.0)+BAP(4.0)	56.1±1.4	74±2.1

Table 2: Effect of 2,4-D and Kn on callus growth in *Ferula jaischkeana* after 4 weeks of culture

2,4-D (mgl^{-1})	Kn (mgl^{-1})	Increase in FW/ Culture (mg) (mean±s.d)	Increase in DW/ Culture (mg) (mean±s.d)	Growth rate
0.5	0.0	265±1.3	17.1±0.5	0.6
1.0	0.0	1216±1.4	78.6±0.5	3.0
2.0	0.0	1820±8.4	121.5±3.5	4.3
4.0	0.0	168±6.4	24.2±4.1	0.4
0.5	0.5	1501±11.1	97.4±2.1	3.4
1.0	0.5	1694±4.3	115.2±4.1	4.3
2.0	0.5	531±3.6	39.1±4.3	1.3
4.0	0.5	188±6.1	12.5±2.3	0.4
0.5	1.0	1681±7.3	109.1±1.5	4.1
1.0	1.0	1890±8.3	128.2±2.4	4.7
2.0	1.0	2069±2.8	138.2±3.5	5.1
4.0	1.0	670±6.3	48.1±4.1	1.7

was significantly improved with the inclusion of Kn (1 mgl^{-1}).

The combined presence of 2,4-D and Kn has also been found to improve callus initiation in *Foeniculum vulgare* (Nagari and Wakhlu, 1989) and *Ferula sinkiangensis* (Zhu *et al*., 2009). Callus initiation has been reported to depend greatly on the type and size of explants (Irvani *et al*., 2010). The results of the present study showed that petiole explants gave maximum callus index, whereas leaf explants gave lowest callus index. The influence of explants type on callus initiation has been earlier demonstrated in *Heracleum candicans* (Wakhlu and Sharma,2001). Variation of callogenic response of various explants types has been reported to be because of the difference in their endogenous hormone level (Krens and Jamer, 1989). The callus forming ability of explants has been shown to depend on the size of explants of *Centella asiatica* (Naidu *et al*., 2010). Among explants sizes tested for callus initiation, 10 mm long petiole gave maximum callus index (Data not presented). Small and large explants initiated a slight amount of callus. Low callus forming ability of small and larger explants has been attributed to an increase or decrease in the ratio of wound to intact cells (Constabel, 1984). Callus growth was optimal when 2 mgl^{-1} and 1mgl^{-1} Kn was used (Fig. 1).

Fig. 1: Callus formation on MS medium supplemented with 2 mgl^{-1} and 1mgl^{-1} Kn after 4 weeks of culture

The enhancement of callus growth due to 2,4-D and Kn . . . . . *Ferula sinkiangensis* (Zhu *et al*., 2009) and *Hydrocotyle bonariensis* (Masoumian *et al.*, 2011). Incubation of callus cultures under 16h photoperiod permitted efficient callus growth. This findings does not agree with Pola *et al.* (2007), who reported that incubation of calli in total darkness encourages their growth in *Sorghum bicolour*.

Among the various concentrations of sucrose, 4 per cent concentration decreased the growth rate of callus. The effect of high level of sucrose level on the callus proliferation may be ascribed to increase in osmotic potential of the medium (George, 1993). The growth rate of callus increased from the first week of culture until the fifth week where after it decreased (Data not presented). The decline in the growth of callus may be due to the exhaustion of some factors or the accumulation of certain toxic metabolites in the culture medium (Bhojwani and Razdan, 2005). Thus, the protocol can be exploited for the regeneration of plants from callus culture for the conservation of this species.

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