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In vitro regeneration of *Majorana hortensis* Moench from callus and nodal stem segments

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Abstract

Rapid multiplication of *Majorana hortensis* was achieved through plant regeneration from nodal stem explants and callus. Nodal stem explants cultured on Murashige and Skoog's medium supplemented with 2 mg/l benzylaminopurine and maltose as source of carbon, yielded up to 40 shoots. The shoots produced roots in the presence of 0.2 mg/l indole-3-butyric acid. Callus was induced in stem explants in medium containing 0.4 mg/l 2,4-dichlorophenoxy acetic acid. This callus showed organogenesis in Murashige and Skoog's medium containing 3 mg/l benzylaminopurine and 0.2 mg/l indole-3-butyric acid.

Key words: *in vitro* regeneration, marjoram, *Majorana hortensis.*

Abbreviations : BAP : Benzylaminopurine; 2,4-D : 2,4-Dichlorophenoxyacetic acid; IAA : Indole acetic acid; IBA : Indole-3-butyric acid; KN : Kinetin; MS : Murashige and Skoog's medium; NAA : α-Naphthaleneacetic acid.

Introduction

Sweet marjoram, *Majorana hortensis* Moench (syn. *origanum majorana* L.) is a perennial herb known for its aromatic and medicinal values. Although callus formation has been reported in this species (Becker 1970), it has been one of the under-exploited species of aromatic plants. The plant is propagated by seeds and by cuttings and the demand for the plant by the perfumery industry is high. Therefore *in vitro* regeneration of this plant was studied and the results are presented here.

Materials and methods

The plant was obtained from a nursery in Bangalore. The stem segments were treated with 95% ethanol for 1 min and washed with sterile distilled water and surface sterilized with 2% sodium hypochlorite for 3 min. The surface sterilized explants were washed with sterile distilled water thrice and then used.

MS medium (Murashige and Skoog 1962) with 3% maltose and 0.8% agar was used as the basal medium after addition of growth regulators and sugar. The pH of the medium was adjusted to 6 with 0.1M NaOH and autoclaved at 15 psi for 20 min in tubes (150 mm x 15 mm), each containing about 12 ml of the medium. The tubes were incubated at 25 ± 2°C with a photoperiod of 12 h and relative humidity was maintained at 65%.

Various sugars including maltose, sucrose and glucose were tested for their effect on regeneration. Different combinations of BAP and IBA were also tested for their effect on regeneration of the callus. Rooting of shoots was carried out using 0.2 mg/l IBA in MS medium. Callusing of the explants was attempted using different concentrations of 2,4-D (0.01–1.00 mg/l). Hardening of the plantlets was carried out using sterile soil moistened with MS medium (without agar and hormones) for 15 days. The experiments were carried out in triplicates of 8 tubes and repeated thrice. The plantlets were than transferred to soil and maintained at 25°C with a relative humidity of 65% and 12 h photoperiod.

Results and discussion

Shoot induction in nodal explants

The nodal stem segments were earlier shown to regenerate shoots with BA (2 mg/l) and roots with IBA (0.2 mg/l) (Iyer & Pai 1998). Sugars as carbon source in the medium can have an effect on shoot initiation. In the presence of sucrose there were 4 shoots per node but in the presence of maltose the number of shoots increased to 10 per node after 30 days of culture (Table 1). On subculture, the number of shoots in case of maltose increased to 20 per node whereas there was no increase in the case of sucrose. However, 2 shoots were observed in the presence of glucose.

Table 1. Effect of carbon source on shoot regeneration in nodal explants of *Majorana hortensis*

Parameter	Glucose	Sucrose	Maltose	Mannitol	No sugar
No. of shoots	2.0	4.0	10.0	0	0
Shoot length (cm)	1.0	2.3	4.5	-	-
No. of leaves	4.0	12.0	30.0	-	-
Leaf length(cm)	0.2	0.2	0.3	-	-

These results clearly indicate that substitution of sucrose with maltose is useful to increase the number of shoots. Maltose has been reported to be a good substitute for sucrose in culture medium (Straus & La Rue 1954; Sievert & Hildebrandt 1965). When different concentrations of maltose (10–50 g/l) were tested, 30 g/l maltose was found to be optimum for shoot regeneration (Table 2). There was no shoot formation without maltose. Although, further increase in maltose concentration above 30 g/l did not increase the number of shoots or the number of leaves, increase in size of leaves and length of shoots was observed.

Induction of callus and shoot regeneration in callus

The effect of IBA, IAA, NAA, KN and 2,4-D at 0.01–1.00 mg/l concentrations were tested for callus initiation in different explants of the plant. The leaf and the stem showed callus formation but not shoot meristem and roots. Among the hormones used, only 2,4-D showed callus initiation in the explants. With combinations of other hormones, neither callus initiation nor increase in weight of calli was observed. Becker (1970) had earlier reported that 2,4-D promoted callus formation in *Origanus vulgare* in MS medium. The different concentrations of 2,4-D tested ranged from 0.01 to 0.10 mg/l for stem and from 0.01 to 1.00 mg/l for leaf. The optimum concentration of 2,4-D for callus initiation from stem and leaf explant was 0.04 mg/l and 0.20 mg/l, respectively, with maximum callus weight (Table 3). The period required for callus formation was the least at these concentrations for stem and leaf callusing. The period required for leaf and stem callusing was 4 and 2 days, respectively. The calli obtained were nodular and gelatinous at lower concentrations of 2,4-D and were friable at higher concentrations. Initiation of callus formation was observed at the cut ends and margins but later spread throughout in 20 days. The colour of the callus was light brown but turned black when kept in the same medium for 35 days. The calli were subcultured every 20 days to a fresh medium. Various sugars were tested for callus initiation in presence of 2, 4-D and all sugars except mannitol supported callus formation. Maltose supported early callus initiation with higher callus weight compared to other sugars, and hence was used for further studies.

Table 2. Effect of maltose on shoot regeneration in nodal stem explants of *Majorana hortensis*

Conc. of maltose (g/l)	No. of shoots	No. of leaves	Length of shoot (cm)	Leaf length (cm)
0	0	0	0.0	0.0
10	2	3	0.3	0.2
20	4	14	4.5	0.2
30	10	30	4.8	0.3
40	8	25	12.0	0.4

Table 3. Effect of 2,4-D on weight of stem and leaf calli in *Majorana hortensis*

Conc. of 2,4-D (mg/l)	Weight of stem calli (g)	Weight of leaf calli (g)
0.00	0.000	-
0.01	0.056 ± 0.002	-
0.02	0.078 ± 0.001	-
0.03	0.082 ± 0.004	-
0.04	0.094 ± 0.002	-
0.05	0.094 ± 0.004	-
0.06	0.092 ± 0.003	0.102 ± 0.005
0.07	0.089 ± 0.002	0.145 ± 0.004
0.08	0.092 ± 0.002	0.168 ± 0.004
0.09	0.090 ± 0.001	0.288 ± 0.004
0.10	0.095 ± 0.002	0.340 ± 0.005
0.20	-	0.467 ± 0.003
0.30	-	0.445 ± 0.004
0.40	-	0.400 ± 0.006
0.50	-	0.399 ± 0.002
0.60	-	0.389 ± 0.002
0.70	-	0.390 ± 0.001
0.80	-	0.379 ± 0.004
0.90	-	0.379 ± 0.004
1.00	-	0.380 ± 0.006

Values are means ± SD

The calli were transferred to MS medium with BAP, either alone or in combination with IBA, IAA and KN (0.1–10.0 mg/l). The combination of BAP and IBA alone promoted organogenesis. Shoot formation was initiated when the medium was supplemented with 3 mg/l BAP along with 0.2 mg/l IBA. BAP alone could not promote shoot formation but in combination with IBA gave only one shoot per callus and there was no root formation. A combination of auxin and cytokinin was essential for regeneration from callus. Similar observations were made in *Lavendula* spp. (Calvo & Sengura 1989) and *Ocimum* sp. (Ahuja *et al.* 1982). Increase in BAP and/or IBA concentrations did not increase the number of shoots. Only the stem calli showed shoot formation while the leaf callus did not. The shoot was formed only when maltose was used as source of carbon. Regeneration of shoots was not observed when sucrose or glucose was used. A similar preference to maltose rather than sucrose was also observed in mulberry (Smith & Stone 1973; Minocha & Halperin 1974). However, increase in the concentration of maltose did not result in increase in the number of shoots formed and there was no effect on the size and number of leaves. There was no shoot formation with 0, 10 and 20 g/l of maltose. The callus first turned green and a single shoot emerged from the callus. The transfer of the callus to fresh medium did not increase the number of shoots.

Rooting of in vitro *shoots*

Rooting of shoots of marjoram was obtained in the presence of 0.2 mg/l of IBA in MS medium (Iyer & Pai 1998). There was elongation of the shoot in the presence of IBA alone and the length of internodes increased. The effect of sugar on rooting of shoots was also studied. All the three sugars promoted rooting of shoots but maltose showed rooting within 4 days compared to 6 days for sucrose and 7 days for glucose. The concentration of maltose (10–50 g/l) tested varied and roots were obtained with 20 g/l after 4 days of culture. Further increase in maltose concentration did not improve the initiation of roots. At lower concentration namely, 10 g/l maltose, roots could be observed only after 9 days.

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