Journal of Spices and Aromatic Crops 7 (1) : 47-49 (1998)

Micropropagation of sweet marjoram (*Majorana hortensis* Moench)

P V IYER & J S PAI[1]

Food and Fermentation Technology Division
University of Bombay
Matunga, Mumbai - 400 019, India.

Abstract

Multiple shoots were obtained in sweet marjoram (*Majorana hortensis)* when nodal stem explants were cultured on Murashige and Skoog medium containing 2 mgl^{-1} 6-benzylaminopurine. Rooting of shoots was induced with 0.2 mgl^{-1} indolebutyric acid in the same medium.

Key words : *Majorana hortensis,* micropropagation, marjoram.

Abbreviations

BAP : 6-Benzylaminopurine
IBA : Indolebutyric acid
MS : Murashige & Skoog medium

Sweet marjoram (*Majorana hortensis* Moench, syn. *Origanum majorana* L.), a perennial plant cultivated as an annual herb, is known for its aesthetic, medicinal and condimental values and also finds wide application in perfume and liquor industries. The present study was undertaken for rapid multiplication of the plant and the results are reported here.

Stem segments of the plant used for the experiments 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 stem was washed with sterile distilled water thrice and then used as explants. MS medium (Murashige & Skoog 1962) with 2 % sucrose and 0.8 % agar was used as basal medium. After addition of ingredients including growth regulators and sugar, pH was adjusted to 6 with 0.1M NaOH. Agar was added thereafter and the medium was autoclaved at 15 psi for 20 min in tubes (150 mm x 15 mm) each holding 12 ml of medium. The tubes were incubated in an environmental chamber at 25±2°C with light/dark cycles of 12 h each and the Relative Humidity was maintained at 65 %. The explants were cultured in basal medium containing 3 % surrose and various concentrations of BAP ranging from 0.01 to 10 mgl^{-1} for multiple shoot induction. For rooting of shoots, the basal

[1]Corresponding author

medium containing 2 % and 3 % surose were used with various concentrations of IBA ranging from 0.01 to 1.00 mgl^{-1}. Hardening of plantlets was carried out under controlled conditions in sterile soil with MS medium (with sucrose but without hormones) for 15 days.

Shoot induction

Axillary shoots were formed from nodal segments but adventitious shoots were not seen. Though different auxins and cytokinins were tested alone or in combinations in this preliminary study, only BAP could induce shoot formation. Similar results have been reported in *Leucoseptrum cannum* (Pal *et al.* 1985), peppermint and orangemint (Van Eck & Kitto 1992). With increasing concentration of BAP, the number of shoots per node increased but the length of the shoots decreased. While the number of leaves increased with increasing concentration of BAP, the size of the leaves decreased (Table 1). BAP 2 mgl^{-1} was the best with respect to number of shoots, shoot size and number of leaves (in comparison with other concentrations of BAP used) (Fig 1). Initiation of shoots took 31 days after which there was no further increase in the number of shoots nor the size of leaves. This response was observed in 85 per cent of explants.

Table 1. Response of nodal stem explants of sweet marjoram to BAP in MS basal medium

BAP (mgl^{-1})	No. of shoots per node	Length of shoots (cm)	No. of leaves per culture	Size of leaves (cm)
0	-	-	-	-
0.1	1	5.0	6	0.40
0.2	1	4.7	6	0.40
0.3	1	4.0	8	0.30
0.4	1	3.5	8	0.25
0.5	2	2.7	8	0.25
0.6	2	2.5	10	0.20
0.7	2	2.8	10	0.30
0.8	2	2.5	10	0.30
0.9	3	2.5	10	0.25
1.0	3	2.5	10	0.20
2.0	4	2.3	12	0.20
3.0	4	2.1	12	0.15
4.0	4	1.7	12	0.20
5.0	4	1.4	12	0.15
6.0	5	1.2	>12	0.12
7.0	5	1.1	>12	<0.12
8.0	5	1.1	>12	<0.12
9.0	5	1.0	>14	<0.12
10.0	5	0.7	>14	<0.12

Results are averages of 3 experiments each with 12 tubes

Table 2. Response of shoots of sweet marjoram to IBA in MS basal medium

IBA	No of days needed for rooting	Callusing of shoots
0	No rooting	No
0.1	15	No
0.2	12	No
0.3	12	No
0.4	12	Yes
0.5	10	Yes
0.6	10	Yes
0.7	10	Yes
0.8	10	Yes
0.9	9	Yes
1.0	9	Yes

Results are averages of 3 experiments each with 12 tubes

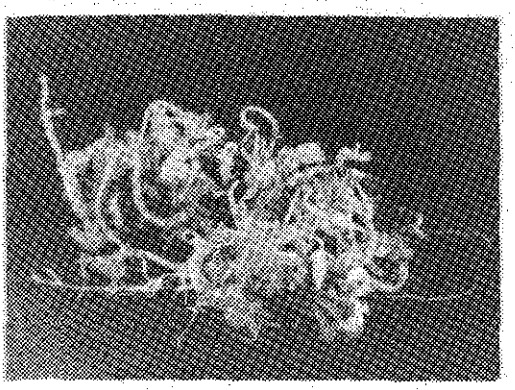

Fig. 1. Multiple shoot induction in sweet marjoram

Rooting of shoots

Though IBA induced rooting at all concentrations in 9-15 days, a concentration of 0.2 mgl^{-1} was the lowest concentration which induced rooting without callusing of the shoot (Table 2). Sucrose concentration at a lower level of 2 % helped rooting of shoots faster than at 3 %. Root formation was observed in around 80 % of the shoots. The shoots when transferred to the rooting medium showed elongation and increase in size of leaves. The plantlets were hardened and transferred to soil; the survival rate of these plantlet was about 50 %.

References

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