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In Vitro Flowering Response of *Ocimum basilicum* L.

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Abstract

Nodal explants of *Ocimum basilicum* L. (Sweet basil, Lamiaceae), showed shoot proliferation after 7-10 days on MS media containing 1.5 mg/L kinetin. *In vitro* flowering was achieved from 90% of the shootlets which were sub cultured on a half strength MS media fortified with 5 mg/L BAP and 1 mg/L IAA. Cytokinin alone or in combination with GA_3 and NAA resulted in shoot proliferation only. For rooting the plantlets were subcultured on MS basal medium supplemented with 3 mg/L NAA and rootlets emerged after 10 days of incubation. The survival percentage of transplanted plantlets was 70%.

Introduction

Ocimum basilicum L. (Sweet basil, *Lamiaceae*), is a multipurpose medicinal herb. It contains volatile oil with eugenol, methyl eugenol, cervacrol and caryophyllin (Anonymous, 1988). It is mainly propagated only through seeds and vegetative propagation is also not advisable. There are earlier reports on *in vitro* systems for *O. basilicum* toward regeneration of shoots and roots but there is no report about *in vitro* flowering (Ahuja et al., 1982; Sahoo and Chand, 1998; Shahzad and Siddiqui, 2000; Begum et al., 2002). The ability of explants to form flowers *in vitro* depends on numerous internal, external, chemical and physical factors and virtually all these factors interact in various complex and unpredicted ways (Tran than van, 1973; Scorza and Janick, 1980; Croes et al., 1985; Compton and Vielleux, 1992). Combination of genetic and environmental factors also play a role in flowering response *in vitro* (Tisserat and Galletta, 1993). Flowering *in vitro* can occur on explants from flowering plants of some woody species (Scorza, 1982). It has been established that in budding and early flowering stages the basil oil was rich in monoterpenes; the quantity of sesquiterpenes and phenylpropane derivatives increased only in later stadiums (Lemberkovics et al., 1998). Since there is no report on *in vitro* flowering from the *O. basilicum* explants, the present investigation was aimed to induce *in vitro* flowering from nodal explants.

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Materials and Methods

Young nodal explants after removing the leaves were washed thoroughly under running tap water for half an hour to remove surface adhering contaminants. The explants were then treated with detergent solution for 5 min followed by surfactant (Tween 20) treatment for 10 min. Then with alcohol wash for just washing off the contaminants followed by 0.1% mercuric chloride treatment for 2-3 min. The explants were then rinsed in 0.5% antifungal (zole) solution for 10 min. The materials were then thoroughly washed 2-3 times with sterile distilled water. The explants were then cultured on half strength MS basal medium supplemented with 1.5 mg/L kinetin and incubated under 3000 lux and 16 hrs photoperiod.

Results and Discussions

Shoot proliferation was observed after 7-10 days on MS media containing 1.5 mg/L kinetin. The shoots were then subcultured on to half strength MS media fortified with various combinations and concentrations of growth regulators as illustrated in Tables 1 and 2. Flowering was observed from 90% of the shootlets on half strength MS

Table 1. *In vitro* responses of nodal explants of *O. basilicum* on half strength MS medium supplemented with various hormonal combinations.

Serial No.	BAP (mg/L)	Other Growth regulators	Response
1	0	0	No Response
2	2	-	Shooting Regeneration with callusing at the base
3	-	KN (1.5 mg/L)	Shoot Regeneration
4	2	GA3 (1 mg/L)	Shoot elongation
5	5	1AA (1 mg/L)	Shoot Proliferation with Flowering
6	7	1AA (1 mg/L)	Shoot Proliferation
7	5	NAA (1 mg/L)	Shoot Proliferation
8	-	NAA (3 mg/L)	Rooting

Table 2. *In vitro* flowering responses of nodal explants of *O. basilicum* on half strength MS medium.

Serial No.	Growth regulators	Flowering Response
1	BAP (3mg/L) + 1AA(1mg/L)	No flowering response
2	BAP (5mg/L) + 1AA(3mg/L)	1.414±0.58
3	BAP (7mg/L) + 1AA(5mg/L)	No flowering response

Data points are Standard Deviation ±SE. of 3×12 replicates

media fortified only with 5 mg/L BAP and 1 mg/L IAA (Figure 1A and 1B). Flowering buds emerged and developed into inflorescence after 20 days of incubation. Similar results were reported in Vitex where they used 1.5 mg/L BAP in combination with 0.1 mg/L NAA (Thiruvengadam and Jayabalan, 2001). Various factors such as carbohydrates, growth regulators, light and pH of the culture medium are playing a major role in flowering (Heylon and Vendrig, 1988). Jumin and Ahmed (1999) recommended 0.01 mg/L BAP to induce flowers in *Murraya*. Stephen and Jayabalan (1998) induced maximum number of flowers from *Coriandrum* on culture with 0.15 mg/L NAA and 0.5 mg/L GA_3. Flower bud maturation was observed on the BAP (5 mg/L) + IAA (1 mg/L) medium which is supported by the study of Patil et al. (1993), which indicated that the exogenous cytokinin stimulates flowering by activation of endogenous cytokinin in ascending xylem sap. Earlier studies indicated that during budding and early flowering stages the basil oil was rich in monoterpenes (Lemberkovics et al., 1998). In our study *in vitro* grown shootlets of *O. basilicum* and *O. gratissimum* were found to be more suitable than *in vivo* leaf, callus and cell free supernatant for secondary metabolite productivity in general and eugenol productivity in particular (Unpublished data). The present work on *in vitro* flowering will go a long way in the determination of stages at which we can exploit the secondary metabolites of our choice. Shootlets were sub-

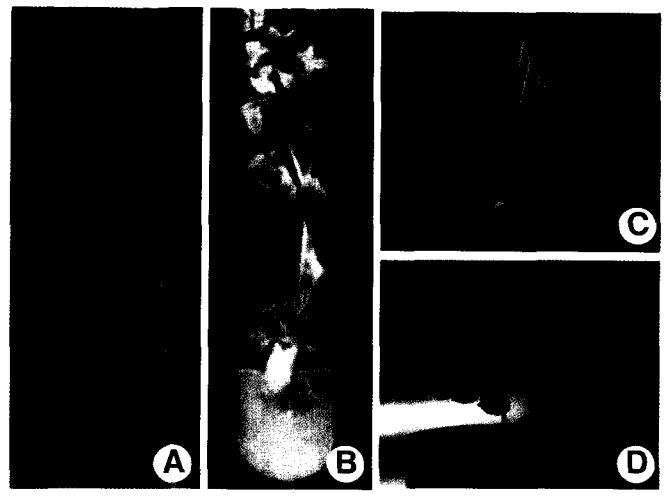

Figure 1. A and B: *In vitro* flowering from the nodal explants, C: *In vitro* plantlet with flowers and rootlets, D: Plantlet after hardening.

cultured on to the MS basal media supplemented with various concentrations of NAA, of which NAA (3 mg/L) was found to be more suitable for rootlet formation (Figure 1C), Whereas in *O. sanctum* rooting was reported on 1 mg/L NAA and in *O. americanum* on 1 mg/L IBA (Patnaik and Chand, 1996). Hardened plantlets (Figure 1D) were successfully transferred to the field conditions.

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References

Ahuja M Verma, Gerwal S (1982) Clonal propagation of *Ocimum* species by tissue culture. Indian Journal of Experimental Biology 20: 455-458.

Anonymous (1988) Pharmacognosy (Eds. S.S Handa and V.K. Kapoor): Vallabh Prakasan Delhi.

Begam F, Amin MN, Azad MAK (2002) *In vitro* rapid clonal propagation of *O. basilicum* L. Plant Tissue Cult 12: 27-35.

Compton ME, Vielleux RE (1992) Thin cell layer morphogenesis. Hort Rev 14: 239-264.

Croes AF, Creemer Molenaar T, Vanden Ende G, Kemp A, Barendse GMW (1985) Tissue age as an endogenous factor controlling *in vitro* bud formation in explants from the inflorescence of *Nicotiana tobacum* L. J Exp Bot 36: 1771-1779.

Heylon C, Vendrig JC (1988) The influence of different cytokinins and auxins on flower formation in thin cell layers of *Nicotiana tobacum* L. Plant Cell Physiol 29: 665-

671.

Jumin HB, Ahmed M (1999) High frequency *in vitro* flowering of Murraya paniculata (L.) Jack. Plant Cell Rep 18: 764- 768.

Lemberkovics E, Kery A, Marczal G, Simandi B, Szoke E (1998) Phytochemical evaluation of essential oils, medicinal plants and their preparations. Acta Pharm Hung 68: 141-149.

Patil MS, Ramaswamy NM, Sree Rangaswamy SR (1993) *In Vitro* flowering in sun flower (*H. annus* L.) Curr Sci 65: 565-566.

Pattnaik SK, Chand PK (1996) *In Vitro* propagation of the medicinal herbs *cimum americanum* L. Syn. *O. canum* Sims. (Hoarybasil) and *O. santum* L. (Holybasil) Plant Cell Reports 15: 846-860.

Scorza R, Janick J (1980) *In Vitro* flowering of *Passiflora suberosa* L. J Am Soc Hort Sci 105: 982-997.

Scorza R (1982) *In Vitro* flowering. Hort Rev 4: 106-127.

Shahzad A, Siddiqui SA (2000) *In Vitro* organogenesis in *O. santum* L.-a multipurpose herb. Phytomorphology 50: 27-35.

Stephen R, Jayabalan N (1998) *In Vitro* flowering and seed setting formation of *Coriandrum sativum* L. Curr Sci 74: 195-197.

Thiruvengadam M, Jayabalan N (2001) *In Vitro* flowering of *Vitex negundo* L. A medicinal Plant. Plant Cell Biotechnology and Molecular Biology 2 (1 and 2): 67-70.

Tisserat B, Galletta PD (1993) Production of Cucumber fruits from the culture of "Market more-76" Plantlets. Plant Cell Rep 13: 37-40.

Tran than van M (1973) Direct flower neoformation from superficial tissue or small explants of *Nicotiana tobacum* L. Planta 115: 87-92.