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Somatic embryogenesis and plant regeneration from leaf callus of *Ocimum basilicum* L.

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Abstract

A effective protocol for complete plant regeneration via somatic embryogenesis has been developed for *Ocimum basilicum* L. Callus was initiated from leaf explant of young plant on Murashige and Skoog's medium (MS) (1962) supplemented with 2,4-dichlorophenoxyacetic acid (2,4-D) 1.0 mg l^{-1}, 3% sucrose and 0.9% agar. The calli showed differentiation of globular structure embryos when transferred to MS medium containing 2,4-D 0.5 mg l^{-1} and BAP 1.0 mg l^{-1}. The maximum globular structure embryos were further enlarged and produced somatic embryos in MS basal medium supplemented with BAP 1.0 mg l^{-1} + NAA 1.0 mg l^{-1} + KN 0.5 mg l^{-1}. Continued formation of globular embryo and germination of embryos occurred in this medium. Complete plantlets were transferred onto specially made plastic cup containing soilrite followed by their transfer to the garden soil. Survival rate of the plantlets under *ex vitro* condition was 80%.

Keywords: *Ocimum basilicum*; Callus induction; Somatic embryogenesis; Plant regeneration; Leaf explant

1. Introduction

Ocimum basilicum L. (sweet basil) of the member Lamiaceae is an important herbaceous plant species, which contains aromatic essential oils with eugenol, methyl eugenol, cervacrol and caryophyllin (Anon., 1988). It is valuable for its pharmaceutical, aromatic and culinary properties. The plant is stomatic, antihelminthic, antipyretic, diaphoretic, expectorant, carminative, stimulant and pectoral (Sahoo et al., 1997; Phippen and Simon, 2000).

O. basilicum is also a globally important economic crop producing annually ca. 100 tonnes of essential oil world wide and with a trade value as a pot herb of around US $15 million per year (Begum et al., 2002). It is conventionally propagated through seeds and cuttings; the progeny shows variability due to cross pollination (Heywood, 1978; Viera et al., 2001). So far, there is no earlier report on *in vitro* plant regeneration via embryogenesis of this species. Somatic embryogenesis

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leading to embryo production and plant regeneration offers advantage for mass propagation and also provides a useful system for genetic manipulation for the example in bananas (Israeli et al., 1995; Sagi et al., 1997). Many *in vitro* studies have been carried out on *O. basilicum* using nodes as explant (Begum et al., 2002), leaves as explant (Dode et al., 2003) and *in vitro* flowering (Sudhakaran and Sivashankari, 2002), The present experiment describes the protocol for the plant regeneration via somatic embryogenesis of *O. basilicum* using leaf callus under *in vitro*.

2. Materials and methods

Young leaves of *O. basilicum* were collected and washed in running tap water for approximately 1 h. They were surface sterilized with 70% ethanol for 20 s and followed by 0.05% mercuric chloride treatment for 5 min. After washing in double distilled water twice or thrice, disc of 0.7 cm diameter were cut from the young leaves by using a sterile cork borer. These discs were cultured on Murashige and Skoog's medium (1962)MS medium supplemented with 3% sucrose, 0.9% agar and different concentrations (0.2–3.0 mg l^{-1}) and alone combination of 2,4-dichlorophenoxyacetic acid (2,4-D), α-naphthalene acetic acid (NAA), 6-benzylamino purine (BAP) and kinetin (KN) for callus initiation, somatic embryogenesis and complete plant regeneration with shoot and roots.

The pH of the culture media was adjusted to 5.7 ± 0.1 before addition of agar and sterilized to by autoclaving for 20 min under 1.1 kg/cm^2 pressure at 121 °C. Cultures were maintained in the culture room at 25 ± 1 °C with light intensity of 2000–3000 lux provided by cool white fluorescent light for 16 h photoperiod regulated by a timer.

Regenerated plants were removed and transferred to soilrite mixture. All the treatments were replicated thrice with 20 culture tubes in each set. The number of shoots developed on embryogenic callus was recorded and analyzed statistically.

3. Results and discussion

3.1. Induction of callus

Leaf explants from field-grown young plant of *O. basilicum* were cultured on MS medium with various levels (viz. 0.2, 0.5, 1.0, and 2.0 mg l^{-1}) of 2,4-D alone and in combination with BAP for induction of callus. After 3 weeks of culture incubation indicated that medium with high concentration of 2,4-D alone upto

Table 1
Effects of different concentrations of 2,4-D and in combination with BAP for callus induction from young leaf explant of *O. basilicum*

Growth regulators (mg l^{-1})	Percentage of explant induced callus	Callus colour and morphology	Degree of callus response
2,4-D alone			
2.0	50	W	[a]
0.5	75	Cr + S	[b]
1.0	65	Cr W + S	[c]
0.2	55	W + S	[b]
2,4-D + BAP			
0.5 + 0.5	65	PG	[a]
0.5 + 1.0	75	G	[c]
0.5 + 2.0	70	G	[c]
1.0 + 0.5	60	PG	[b]
1.0 + 1.0	65	G	[b]
1.0 + 2.0	70	G	[a]

W: white; Cr: creamy; PG: pale green; G: green; S: spongy 20 explants were maintained in each treatment and data were recorded upto 7 weeks of culture.
[a] Slight callusing.
[b] More callusing.
[c] Profuse callusing.