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In vitro micropropagation of *Ocimum citriodorum* and standardization of growth hormone

K.SANTHI

Department of Botany, Selvamm College of Arts and Science (Autonomous), Namakkal, Tamil Nadu- 637 003

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ABSTRACT

In vitro studies on micropropagation protoplast culture on Ocimum citriodorum was investigated at Helixium research laboratory, Tiruchirappalli during December 2017. The healthy plants of Ocimum citriodorum were collected in Kodaikanal were raised and maintained at Poonga Biotech Research Centre, Chennai. Maximum of 75% callus induction was recorded at 4.52 µM 2,4-D, 40 % at 2.69 µM NAA, and 30% under 8.8 µM of BA. Among 16 different concentrations and combinations of BA and NAA, MS medium supplemented with BA (4.44 µM) and NAA (0.14 µM) recorded 75 % shoot regeneration producing highest number of total shoots (8.33 ± 1.15) with an average shoot length of 5.43 ± 0.06 cm from nodal explants after six weeks of culture. Among 5 different concentrations of IBA (0.49, 0.98, 2.46, 4.92 and 12.30 µM), the optimal rooting response was observed on half strength MS medium supplemented with IBA (2.46 µM) in terms of average number of roots (6.0± 1.0) with mean root length of 4.90 ± 0.26 cm per shoot with 75±5.0 % of rooting response recorded after 30 days of culture.

Keywords: Micropropagation, Callus, growth factor, auxins, green house

INTRODUCTION

Plants are one of the most important sources of drugs which are formulated by pharmaceutical company. Today the large numbers of drugs in use are derived from plants and are used in anticancer, antioxidant, analgesic and antidiabetic. The plants rich in secondary metabolites contain essential oils of therapeutic importance (Prakash and Gupta, 2005). *Ocimum basilicum* L. (sweet basil) belongs to the family *Lamiaceaea,* a herbaceous species rich of essential oils with aromatic (Patel *et al.*, 2016) culinary and medicinal importance. The plant is widely used as stomatic, antihelminthic, antipyretic, diaphoretic, expectorant, carminative, pectoral and stimulant. It is a herb grown primarily in northeastern Africa and southern Asia and its strong fragrant lemon scent is used for cooking. The lemon basil has stems that can grow to 20-40 cm tall. It has white flowers in the late summer to early fall. The leaves are similar to basil leaves. Seeds form on the plant after flowering and dry on the plant. Plant tissue culture refers to the in vitro culture of plants from plant parts (tissues, organs, embryos, single cells, protoplasts, etc.) on nutrient media under aseptic conditions (Altman, 2000). *In vitro* cultures are now being used as tools for the study of various problems in plant sciences. It is now possible to propagate all plants of economic importance in large numbers by tissue culture (Hameed *et al.*, 2006). *In vitro* micropropagation is an effective way to obtain a high progeny uniformity of medicinal plants. Many *in vitro* studies have been conducted on Lamiaceae species, including the Ocimum genus, using different explants, like nodal segments (Begun *et al.*, 2000) and leaf explants (Phippen and Simon, 2000).The widespread screening of plants for possible medicinal and antioxidant properties, the isolation and characterization of diverse phytochemicals and the development and utilization of antioxidants of natural origin (Gulcin *et al.*, 2002). The present study was, therefore, carried out to describe the in vitro testing of impact of growth hormone on micropropagation of aromatic medicinal plant *Ocimum citriodorum.*

MATERIALS AND METHODS

Collection of *Ocimum citriodorum:* Healthy plants of *Ocimum citriodorum* collected in Kodaikanal were raised in pots containing soil and farmyard manure (1:1) under green house condition at Poonga Biotech Research Centre, Chennai. The explants were taken from these plants for all experiments.

Corresponding author email: helixiumresearch@gmail.com

Explant preparation (Jananrthanam *et al.*, 2010): The explants namely node, internode, juvenile leaf and shoot tips were washed thoroughly in running tap water and then immersed in 0.1% (w/v) mercuric chloride ($HgCl_2$) for 8 minutes for surface sterilization and then washed repeatedly several times. The cut ends of the explants were again trimmed with the help of sterile blade to eliminate any possible residue of sterilant and the explants were used for culturing.

Culture conditions: Cultures were maintained in the culture room at 28 ± 1°C under 16 h photoperiod provided by cool white fluorescent light followed by 18 h dark period for 45 days. Three repetitions per treatment were used and the data were submitted to variance analysis.

Callus induction: The surface sterilized leaf segments were cultured on MS medium (Kintzios *et al.*, 2004) containing various concentration of 2, 4-D (0.45, 2.26, 4.52, 11.31and 22.62 µM); NAA (1.34, 2.69, 5.37, 13.43 and 26.85 µM) and BA (1.11, 2.22, 4.44, 8.88 13.32 and 22.20 µM) for callus induction.

Shoot proliferation: Explants from node and leaf derived callus explants of *Ocimum citriodorum* plant were inoculated on MS basal medium supplemented with individual concentrations and combination of BA (1.11, 2.22, 4.44, 8.88 and 11.10 µM) NAA (0.054, 0.14, 0.27 and 0.54 µM) and IAA (0.06 - 0.57 µM) for shoot multiplication. At the end of the experiment, percentage of shooting, shoot length and the number of shoots per explants were recorded. After six weeks, the micro shoots were transferred to rooting medium.

Root proliferation: The healthy shootlets were transferred to half strength MS basal medium supplemented with individual concentrations of IBA (0.49, 0.98, 2.46, 4.92 and 12.30 µM) for root initiation. The percentage of rooting, root length and the number of roots per individual shoots were recorded.

RESULTS AND DISCUSSION

Callus Initiation

The response for the growth and development of calli varied with different explants. Callus was developed when the leaf explants *Ocimum citriodorum* were cultured on 2,4-D. Lower concentrations callus induction medium with 2,4–D showed better callus induction and proliferation. The higher concentration of 2, 4-D (22.62 µM) did not show much effect on callus induction. Callus in the 2, 4- D (4.52 µM) supplemented medium was found well developed, spongy, and loosely arranged. The moisture content of callus was also high as compared to other auxins supplemented media. In NAA supplemented medium the callus was pale, yellowish green in colour, more friable, hard and granular. Callus grown on medium supplemented with BA was green in colour more compact, hard and granular. Among three plant growth regulators studied, the response of callus was good in 2, 4-D (4.52 µM) compared to others (Table.1). The callus response on MS media supplemented with 2, 4 –D (4.52 µM) was 75% and the rate of proliferation was recorded from 12th day after inoculation up to 40 days with four day intervals. While the colour of callus changed with the concentration of 2, 4-D used, the callus was found to be creamish in 2, 4-D (4.52µM), and in the higher concentration it was appeared yellowish to brown in colour. These findings harmonized with those of Uddin *et al.* (2006) in *Stevia rebaudiana*. The performance of nodal segments was much better than the shoot tips. The most commonly used explants in shoot proliferation of *Ocimum* are nodal stem segment, where in the axillary bud is made to proliferate multiple shoot (Shahrzad and Siddiqui, 2000).

Table.1: Impact of auxin and cytokinin on callus induction

PGR Concentration (µM)			Juvenile leaf % Response
2,4-D	NAA	BA	
0.45	-	-	40 ± 10
2.26	-	-	50 ± 0.0
4.52	-	-	75 ± 5.0
11.31	-	-	55 ± 0.0
22.62	-	-	45 ± 0.0
-	1.34	-	25 ± 5.0
-	2.69	-	40 ± 0.0
-	5.37	-	30 ± 0.0
-	13.43	-	25 ± 5.0
-	26.85	-	15 ± 5.0
-	-	1.11	20 ±5.0
-	-	2.22	25 ±0.0
-	-	4.44	35 ± 0.0
-	-	8.88	30 ± 0.0
-	-	13.32	20 ±0.0

Combined effect of BA and NAA for shoot proliferation

The synergistic effect of BA and NAA in varying concentrations was studied for shoot multiplication of *Ocimum citriodorum*. Among the various concentrations and combinations of BA and NAA, MS medium supplemented with BA (4.44 µM) and NAA (0.14 µM) recorded 75 % shoot regeneration producing highest number of total shoots (8.33 ± 1.15) with an average shoot length of 5.43 ± 0.06 cm from nodal explants after 35 days of culture (Table 2).

Table 2: Effect of cytokinin (BA) and auxin (NAA) on shoot multiplication

Plant growth regulator (µM)		Shoot induction (%)	Number of shoot per explants	Shoot length (cm)
BA	NAA			
1.11	0.054	25.00 ±5.00	2.00 ±1.00	2.07±0.38
	0.14	36.67 ±2.89	2.67 ±0.58	1.90±0.17
	0.27	40.00 ±0.00	4.33 ±1.15	3.33±0.15
	0.54	25.00 ±5.00	2.67 ±0.58	1.93±0.12
2.22	0.054	40.00 ±0.00	2.00 ±1.00	1.90±0.10
	0.14	46.67 ±2.89	3.67 ±0.58	2.93±0.06
	0.27	41.67 ±5.77	2.00 ±0.00	2.00±0.50
	0.54	30.00 ±10.0	1.67 ±1.15	2.03±0.23
4.44	0.054	31.67 ±2.89	2.67 ±0.58	2.67±0.29
	0.14	75.00 ±0.00	8.33 ±1.15	5.43±0.06
	0.27	50.00 ±5.00	4.00 ±1.00	3.87±0.21
	0.54	40.00 ±5.00	3.33 ±1.53	2.43±0.06
11.10	0.054	25.00 ±5.00	2.67 ±1.15	1.93±0.06
	0.14	26.67 ±2.89	2.67 ±0.58	2.23±0.31[c]
	0.27	35.00 ±5.00	3.00 ±1.73	1.93±0.1
	0.54	30.00 ±.00	1.00 ±0.00	1.47±0.06

The combination of BA (4.44 µM) with NAA (0.27 µM) was found to show 50% response producing 4.00 ± 1.00 shoot lets per explants with an average shoot length of 3.87±0.21cm. At lower concentration of BA (1.11 µM) and NAA (0.27 µM), the results showed only 40% response, producing (4.33 ± 1.15) shootlets per explants with an average length of 3.33 ± 0.15cm. When the concentration of BA (11.10 µM) and NAA (0.54 µM) was increased, a gradual fall in the number of shoots per explants was recorded. Similarly on increasing the concentration of NAA (0.27 µM and 0.54 µM), the response was low due to the basal calli formation in the cut ends of the explant. Nodal explants on MS medium without plant growth regulators did not show any response. Guirgis *et al.* (2007) observed a quickest callus growth of *O. basilicum* on medium fortified with 1 mg/l 2,4-D.The synergistic combination of auxin and cytokinin combinations on organogenic differentiation has been well established in several systems (Thomas and Puthur, 2004). In *Ocimum citriodorum,* the synergistic effect of BA (4.44 µM) and NAA (0.14 µM) recorded 75 % shoot regeneration producing highest number of total shoots (8.33 ± 1.15) with an average shoot length of 5.43 ± 0.06 cm per shoot. The result corroborates with the findings of Khosravi *et al.* (2007).BA has been considered to be one of the most active cytokinins in organogenic differentiation in plant tissue culture (Gururaj *et al.*, 2007; Baskaran and Jayabalan, 2005).

Effect of IBA on root proliferation from *in vitro* shoot

For root induction individual microshoots (6.0 cm) taken from the *in vitro* proliferated shoots were placed in half strength MS medium supplemented with various concentrations of IBA (0.49, 0.98, 2.46, 4.92 and 12.30 µM). The excised shoots did not show rooting on culture medium without plant growth regulators. The first roots appeared after two weeks of culture, and after four weeks, the root system was well developed. Rooting occurred in all concentrations but with different rooting percentages, and the optimal response was observed on half strength MS medium supplemented with IBA (2.46 µM) in terms of average number of roots (6.0± 1.0) with mean root length of 4.90 ± 0.26 cm per shoot with 75±5.0 % of rooting in a decrease in the percentage of root induction response with decreased number of roots and root response recorded after 30 days of culture (Table 3). The maximum rooting response was achieved on medium supplemented with 2.46µM IBA, 75% response; 6.0 ± 0.25 number rootlets per shootlets and an average root length of 4.9 ± 0.2 cm in 30 days old cultures. The observation on the reduction of MS salts strength to one half to enhance the rooting frequency is in agreement with earlier finding in *P. niruri* and *S. rebaudiana* (Catapan *et al.*, 2000). In the present study root induction was obtained with lower concentration of IBA.

Table 3: Effect of concentration of auxin (IBA) on rooting response of *Ocimum citriodorum*

IBA (µM)	Rooting response (%)	Number of roots per shoot	Root length (cm)
0.49	30.00 ±5.00	2.67 ±0.58	3.07±0.51
0.98	31.67 ±2.89	2.67 ±1.15	3.07±0.25
2.46	75.00 ± 5.00	6.00 ±1.00	4.90±0.26
4.92	45.00 ±5.00	2.67 ±0.58	2.30±0.30
12.30	36.67 ±2.89	2.00 ±1.00	2.00±0.20

The results concluded that the significant induction of shoot multiplication for *Ocimum citriodorum* was recorded on MS basal medium supplemented with combination of BA + NAA. The overall number of shoots in this study produced was significantly higher in the presence of BA. However, higher concentrations of BA favoured callus proliferation and subsequent culture of the callus favoured better shoot morphogenesis.

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