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IN VITRO MULTIPLICATION OF *CORIANDRUM SATIVUM*(L.) FROM SHOOT TIP AND NODAL EXPLANTS

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ABSTRACT

Coriandrum sativum (L) (coriander) is a valuable medicinal plant. The plantlet regeneration protocol of this plant through nodal segments, were employed in microcloning and conservation. Standardization of *in vitro* culture protocol for induction of shoot from shoot tip and nodal explants were used for shoot multiplication. Among these two explants, nodal produced maximum number of shoot buds. BAP (Benzyl Amino Purine) (2.0mg/l) was more effective than KN(Kinetin) (2.0mg/l) to produce multiple shoots. Rooting was best in half-strength MS medium with 1.0mg/l IBA. Almost 96% of the rooted shoots survived hardening under glass house and transferred to the field. The regenerated plants did not show any morphological change and variation in levels of secondary metabolites when compared with the mother stock.

KEYWORDS: *Coriandrum sativum*, Explant, MS medium, plant growth hormones.

INTRODUCTION

Coriander (*Coriandrum sativum* L.) which belongs to the family Apiaceae (Umbelliferae) is mainly cultivated from its seeds throughout the year (Mhemdi *et al*., 2011). India is the biggest producer, consumer and exporter of cori-ander in the world with an annual production of around three lakh tonnes. It is an annual, herbaceous plant which originated from the Mediterranean and Middle Eastern regions and known as medicinal plants. It contains an essential oil (0.03 to 2.6%) (Nadeem *et al*., 2013). All parts of this herb are in use as

flavoring agent and/or as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations (Sahib *et al*., 2012). Coriander closely resembles flat leaf parsley. This resemblance makes many people confused between the two however, coriander has strong fragrance and parsley has mild fragrance. It grows best in dry climates however it can grow in any type of soil like light, well drained, moist, loamy soil, and light to heavy black soil (Verma *et al*., 2011). Its seeds are almost ovate, globular and have a mild, sweet, slight pungent like citrus flavor with a hint of sage. The most important constituents of its seeds are the essential oil and fatty oil. It is highly reputed ayurvedic medicinal plant commonly known as "Dhanya" in India. This plant is highly aromatic and has multiple uses in food and in other industries. Plants have played a critical role in maintaining human health and civilizing the quality of human life for thou-sands of years (Dhankar *et al*., 2011). Coriander has been reported to posses many pharmacological activities like antioxidant (Darughe *et al*., 2012), anti-diabetic (Eidi *et al*., 2012), anti-mutagenic (Cortes *et al*., 2004), anti-lipidemic (Sunil *et al*., 2012), anti-spasmodic (Alison *et al*., 1999). Moreover, coriander oil is used as an antimicrobial agent as it possesses broad spectrum antimicrobial activity (Silva *et al*., 2011). This oil can be encapsulated in alginates, chitosan etc. so as to enable isolation, protection, transport and release of its active components like vitamins, flavours, peptides, minerals, fatty acids, polyunsaturated fatty acids, antioxidants, enzymes and living cells (Cristian, 2013). The present study reports an efficient micro propagation system for regenerating a large number of plants directly from shoot tip and nodal explants of *Coriandrum sativum* which would form a strategy in the conservation of this important medicinal plant.

MATERIALS AND METHODS

The methods of plant tissue culture were the standard method as described in Plant Cell, Tissue and Organ Culture Fundamental Methods (Gamborg and Phillips, 2004). The plant materials selected for the present investigation was *Coriandrum sativum* (coriander) belong to the family umbellifereae. (Fig 1)

SOURCE OF EXPLANT

The explants were selected from 3 month old matured plants of *Coriandrum sativum* growing in the Botanical Garden of AVVM Sri Pushpam College, Poondi, Thanjavur district, Tamil Nadu, India.

CULTURE MEDIUM

Nodal and shoot tip explants were used for direct regeneration on MS medium (Murashige and Skoog, 1962). The explants were first washed with tap water for about half an hour, followed by 2-4 drops of liquid soap for 10-20 min. After rinsing with tap water thoroughly, the explants were surface sterilized with 0.1% mercuric chloride solution for 2-3 min. This was followed by washing with sterile distilled water 3-4 times to remove the traces of $HgCl_2$ solution. The shoot tip and nodal explants were inoculated by inserting their cut ends in the MS medium supplemented with 0.5, 1.0, 1.5, 2.0, 2.5 and 3 mg/l of BAP or KN individually include multiple shoots. The medium contained 3% (w/v) sucrose and solidified with 0.8% (w/v) agar. The pH of the medium was adjusted to 5.6 before gelling with agar and autoclaved at 121^{o}C at 15 lb pressure for 20 min. The cultures were maintained at 25 ±2^{o}C under the light intensity of 2000 lux provided by cool white fluorescent lamps. The shoots (5-6 cm long) bearing at least 4.5 internodes were excised from the mass of proliferated shoots and transferred to the rooting medium containing 0.5, 1.0, 1.5 and 2.0 mg/l of IBA. Rooted plantlets were transferred to polycups and PVC pots containing sterile soil and perlite (1:1) and covered with plastic bags to maintain 85 – 92% humidity. Subsequently, the plantlets were transferred to glass house after one month. The plantlets were planted in the soil after one month period of hardening. Experiments were set up in completely randomized block design. Ten cultures were raised for each treatment and all experiments were conducted thrice. Data on number of shoots, shoot length and number of roots and root length were determined.

RESULT AND DISCUSSION

MS medium supplemented with different concentrations of BAP/KN resulted in initiation of callus and shoots from shoot tip and nodal explants (Table 1). Maximum number of multiple shoots were induced in MS medium supplemented with 1.5 mg/l BAP (Fig. 2.a,b) when compared to other and higher concentrations used. Hence it is suggested that this optimum concentration of BAP promotes multiple shoot induction. Similar reports were also obtained with the cultures of *Phyllanthus amarus* (Ghanti *et al*. 2004), *Celastrus paniculatus* (Nair and Seeni, 2001) and *Withania somnifera* (Chandran *et al*. 2007). The higher concentrations of BAP inhibited the formation of shoots, and even when the shoots so formed were short and thick (Fig.3.a,b). Multiple shoots were also induced from shoot tip and nodal explants on MS medium supplemented with different concentrations of KN (0.5, 1.0, 1.5 and 2.0 mg/l). The

number of shoot length was higher on the medium containing 2 mg/1. The higher concentration of KN inhibited the shoot formation from the shoot explants.

For root induction, plantlets were transferred to MS medium supplemented with different concentrations of IBA (0.5, 1.0, 1.5 and 2.0 mg/l) (Table 2). Number of roots per explant and root length were more on the medium containing IBA (1mg/1). The number of roots and root length decreased when the concentrations of IBA. The influence of IBA on enhanced root formation had also been reported in the case of *Phyllanthus amarus* (Nair and Seeni, 2001), *Centella asiatica* (Banerjee, 1999), *Phyllanthus caroliniensis* (Catapan *et al*. 2000) and *Withania somnifera* (Chandran *et al*. 2007).

Table 1: shows Mean number of shoots produced per shoot tip explants on multiplication media with different concentration of growth regulator BAP and KN after 4 weeks of culture

Growth regulator BAP(mg/l)	Culture showing response (%)	Mean number of roots/shoots
0.5	44	1
1.0	38	2
1.5	74	4
2	76	5
2.5	68	3
3.0	42	1
Growth regulator KN(mg/l)		
0.5	44	1
1.0	50	3
1.5	65	2
2	68	5
2.5	47	1
3.0	N.D	N.D

Table 2: Shows Mean number of roots produced per shoot tip explants on multiplication media with various concentration of IBA after 4 weeks of culture

Growth regulator IBA(mg/l)	Culture showing response (%)	Mean number of roots
0.5	54	2
1.0	72	8
1.5	68	4
2	58	2
2.5	52	4
3.0	N.D	N.D

N.D. = Not determined due to nil response

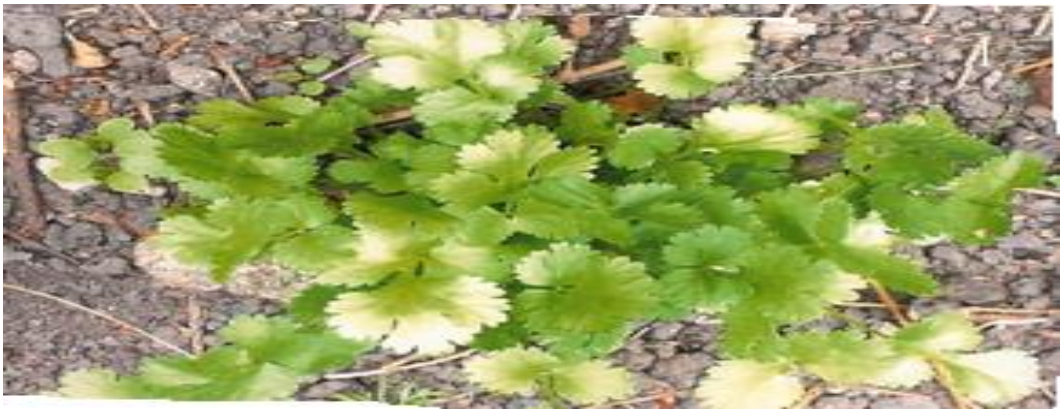

Fig 1: Shows Habit of *Coriandrum sativum* (L.)

Fig 2: (a, b) Multiple shoots were induced in MS medium supplemented with 1.5mg/l BAP after two weeks.

Fig 3: (a, b) Three weeks old culture showing inhibited the formation of shoots, when the shoots were short and thick from shoot tip and nodal explant on MS medium supplemented with 2.5 mg/1 BAP.

CONCLUSION

In the present work has deciphered methods of improving *in vitro* propagation by developing a novel improved protocol highlighting efficient reproducible and reliable techniques for mass multiplication of a medicinally and economically important herb *Coriandrum sativum*. *Coriandrum sativum* has a high morphogenic potential, and the explants readily responded to cytokinins in the culture medium and formed multiple shoot buds. Of the three growth regulators tried, we found BAP to be more suitable than KN as the former resulted in a quicker and better response than the latter. Nodal explants responded better than the shoot tip explants and gave maximum shoots on BAP (1.5 mg/1) with supplemented medium and Mean number of roots per explant (8) on the medium containing IBA (1 mg/1). Thus this proves that this present protocol could successfully be used for large scale clonal propagation without any seasonal constraint and also for conservation and commercial propagation of this medicinal plant in the Indian sub continent. The present study is a stepping stone for *in vitro* production of required active principles of *Coriandrum sativum*. This protocol is novel because of its minimal requirements and cost effectiveness for propagation.

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