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Plant regeneration and *in vitro* flowering from shoot tip of *Basilicum polystachyon* (L.) Moench -An important medicinal plant

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Direct organogenesis and *in vitro* flowering was obtained in *Basilicum polystachyon* (L.) Moench. High frequency and maximum number of multiple shoots were obtained from shoot tip explants on MS medium supplemented with BAP (2.22-13.32μM) and KN (2.32-13.92μM). Regenerates, when transferred to rooting medium IBA (2.46-14.76μM) and IAA (2.85-17.13 μM), they initiated flowering along with rooting. *In vitro* flowers set viable seeds. Rooted plantlets were hardened and transferred to green house with 100% survivability. This finding has significant role in Pharmaceutical industries and *in vitro* flowering facilities *in vitro* pollination and fertilization, further it also facilities in advancing the generation at much faster speed under limited progeny size in the segregating generation of *B. polystachyon* (L.) Moench.

Key words: *Basilicum polystachyon* (L.) Moench., Organogenesis, Shoot tip, Plant regeneration, *in vitro* flowering

Introduction

Basilicum polystachyon (L.) Moench. an aromatic herb, belongs to the family *Lamiaceae*, has a wide range of medicinal values. Volatile basil oil obtained from the plant contains various terpenoids and flavanoids and possesses antibacterial, mostquitocidal and ovicidal activities (Rajkumar and Jabanesan, 2005 and Chakraborti *et al.*, 2007). The leaf decoctions are given for epilepsy, palpitations of the heart, neuralgia and convulsions. Medicinal plants are of great interest to the researchers in the field of biotechnology as most of the drug industries depend in part, on plants for the production of

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pharmaceutical compounds (Chand *et al.,* 1997). Organogenesis is a suitable method for obtaining a large quantity of genetically homogenous and healthy plant material, which can be used for planting. Plant cell and tissue culture has become a major tool in the study of an increasing number of fundamental and applied programs in plant science. Tissue culture techniques are being used globally for the exist conservation of plants. The endeavor is to adopt the method to multiply the medicinal herbs and monitor their secondary metabolites. Conservation of endangered medicinal plants has also been achieved through cell cultures with significance (Rao *et al.,* 1996). Reports of *in vitro* plant regeneration from tissues of medicinal plants are available (Gupta *et al.,* 1997; Verma and Kant, 1996; Hoque *et al.,* 2000; Nichol *et al.,* 1991 and Palai *et al.,* 2000). However there is no report on *in vitro* regeneration of *Basilicum polystachyon* (L.) Moench.

The present study was to establish an efficient protocol for high frequency of plant regeneration and *in vitro* flowering in *Basilicum polystachyon* (L.) Moench.

Materials and methods

Plant material

Field grown young healthy plants were used as source of explants. Shoot tips were selected as explants for direct regeneration. The explants were washed in running tap water to remove surface adhered particles and then with 5% Teepol for 5 min and in 70% ethanol for 30 sec and rinsed in distilled water (3-4times), followed by 0.1% (w/v) $HgCl_2$ for 5 minutes. Finally, the explants were washed in sterile distilled water for 3-5 times to remove the residual $Hgcl_2$ and cultured on MS (Murashige and Skoog, 1962) basal medium containing 3% (W/V) sucrose, 0.8% (W/V) agar. The pH of the medium was adjusted to 5.7±1 before autoclaving for 15 minutes at 121^{o}C. All the cultures were incubated at 25±2^{o}C under 16 hour light (2,500) lux and 8-hour dark.

Multiple shoot regeneration

The Shoot tips were cultured on MS medium supplemented with BAP (6-Benzyl adenine) at concentration of 2.22-13.32 μM and KN (Kinetin) at concentration of 2.32-13.92 μM.

Root induction

The well-developed shoots were excised and were transferred to MS medium supplemented with IBA (Indole butyric acid) at concentration of 2.46-14.76 μM and IAA (Indole acetic acid) at concentration of 2.85-17.13 μM.

Acclimatization

Rooted plantlets were removed from culture tubes and washed their roots in running tap water and transferred to plastic cups containing, sand and farm yard mixture (1:1:1) for a week and subsequently transferred to pots. All the tissue culture raised plantlets need gradual acclimatization for their survival in the field condition from controlled environment. Instead of transferring directly to the pots, plantlets were left for a week in the plastic cups at controlled temperature (25±2^{o}C) with 60% relative humidity. After initiation of new roots, they were kept in the moist chamber and grown till maturity.

Statistical analysis

Experiments were setup in a Randomized Complete Block Design (RCBD) and each experiment was repeated twice times. Data were recorded on the percentage of response, number of shoots per explants, shoot length and number of roots per shoot. Means and Standard errors were carried out for each treatment.

Result and discussion

Basilicum polystachyan (L.) Moench. plants were efficiently regenerated from shoot tips. Explants were capable of directly developing multiple shoots on MS basal medium containing different concentrations of cytokinins. Multiple shoot initiation from shoot tip explants was observed within 10-15 days after inoculation (Fig.1A). The highest number of shoots (66/ explant) was observed in the medium containing BAP 8.88 μM/l followed by KN 9.28 μM/l (58/ explant). Of the two cytokinins (BAP and KN), BAP was found to be more suitable than KN for initiation and proliferation of multiple shoot buds (Table 1) (Fig.1B).

The elongation of shoots and proliferation of nodes were achieved on the same parental medium. In the present study, the relative effectiveness of BAP and KN varied for *in vitro* multiple shoot regeneration from shoot tip explants. BAP (8.88 μM/l) was found to be the best concentration for regeneration of

multiple shoots buds (33 – 66). The maximum number of multiple shoot proliferation was observed on MS medium containing 8.8 μM/l BAP (66.68±1.88) and 9.24 μM/l KN (58.66±4.77) after 30 days of culture. Shoots were harvested every 30 – 40 days and new shoot lets were harvested periodically. All the plantlets produced roots on the rooting medium containing IBA 9.84 μM/l and IAA 11.42 μM/l after a week (Fig 1C; Table 2).

In vitro flowering

Flowering was considered to be a complex process regulated by both internal and external factors and its induction under *in vitro* culture is extensively rare. *In vitro* flowering was observed with in 20 days of culture (Fig 1C), when *in vitro* shoots were transferred to rooting medium. For *in vitro* flowering, the response of IBA is better than IAA. The *in vitro* regenerated plantlets were successfully transferred to plastic cups and than to the field (Fig ID).

The capacity of shoot bud differentiation and shoot proliferation from shoot tip explants of *B. polystachyon* (L.) Monech. depended on hormonal variation. There was good shoot bud induction and proliferation response only in the presence of cytokinin and no response in the basal medium. Similar results are well documented in several medicinal plants (Pattnaik and Chand, 1996), *Bixa ovellana* L. (Sharon and Marie, 2000), *Emblila officinale* (Verma and Kant, 1996) and *Withania somnifera* (Deka *et al*., 1999). From our study it was clear that 8.88 μM/l BAP and 9.28 μM/l KN were significantly more effective for inducing shoot organogenesis. Well-developed shoot lets when transferred to rooting medium containing 9.84 μM/l IBA induced higher frequency of roots than 11.42 μM/l IAA. Similar effect of IBA was reported in *Ocimum americanum*, *O.canum* and *O.sanctum* (Pattnaik and Chand, 1996) and also in *Heracleum candicans* (Wakhlu and Sharma, 1999). However, IBA 9.84 μm /l was found to be the best rooting hormone than other auxins. Auxin support *in vitro* flowering, similar results were observed in Sunflower (Patil *et al.*, 1993), in Mulberry (Naik and Lata, 1996) and in Coriander (Stephan and Jayabalan, 1998).

From our experimental data, it is evident that BAP and KN are the best suited for inducing multiple shoots and IBA for rooting and *in vitro* flowering. In conclusion, this communication describes an efficient rapid propagation system of *B. polystachyon* (L.) Moench.

Table 1. Effect of BAP and KN on shoot Multiplication from shoot tips explants of *Basilicum polystachyon* (L.) Moench.

Growth Regulators μM/l	Percentage of cultures with induced shoots	Number of shoots per explants	Shoot length (cm)
BAP			
2.22	85	33.34±4.21^{h}	2.42±0.19^{j}
4.44	90	40.00±6.34^{f}	4.48±0.40^{g}
6.66	95	55.98±4.45^{c}	5.40±0.20^{ef}
8.88	100	66.68±1.88^{a}	8.78±0.40^{a}
13.32	80	54.66±2.22^{cd}	6.93±0.69^{c}
KN			
2.32	70	20.00±2.68^{j}	2.96±0.04^{i}
4.64	80	32.00±2.22^{i}	4.40±0.11^{gh}
6.96	90	40.02±3.26^{fg}	5.54±0.43^{e}
9.28	95	58.66±4.77^{b}	8.38±0.64^{b}
13.92	75	49.34±1.44^{e}	6.04±0.34^{d}

Each value represents the mean ± SD of 30 replicates and each experiment was repeated at least thrice. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

Table 2. Effect of different concentrations of IAA and IBA on rooting of *in vitro* regenerated shoots of *Basilicum polystachyon* (L.) Moench.

Plant Growth Regulators μM/l	Percentage of Root Induction	Number of Roots per Shoot
IAA		
2.85	48	5.02±0.37^{h}
5.71	82	7.70±0.22^{ef}
11.42	90	12.68±0.96^{b}
17.13	54	7.94±0.57^{e}
IBA		
2.46	64	6.56±0.37^{fg}
4.92	84	1.76±0.97^{c}
9.84	96	14.38±1.37^{a}
14.76	56	9.46±0.56^{cd}

Each value represents the mean ± SD of 30 replicates and each experiment was repeated at least thrice. Values with the same superscript are not significantly different at the 0.05% probability level according to DMRT.

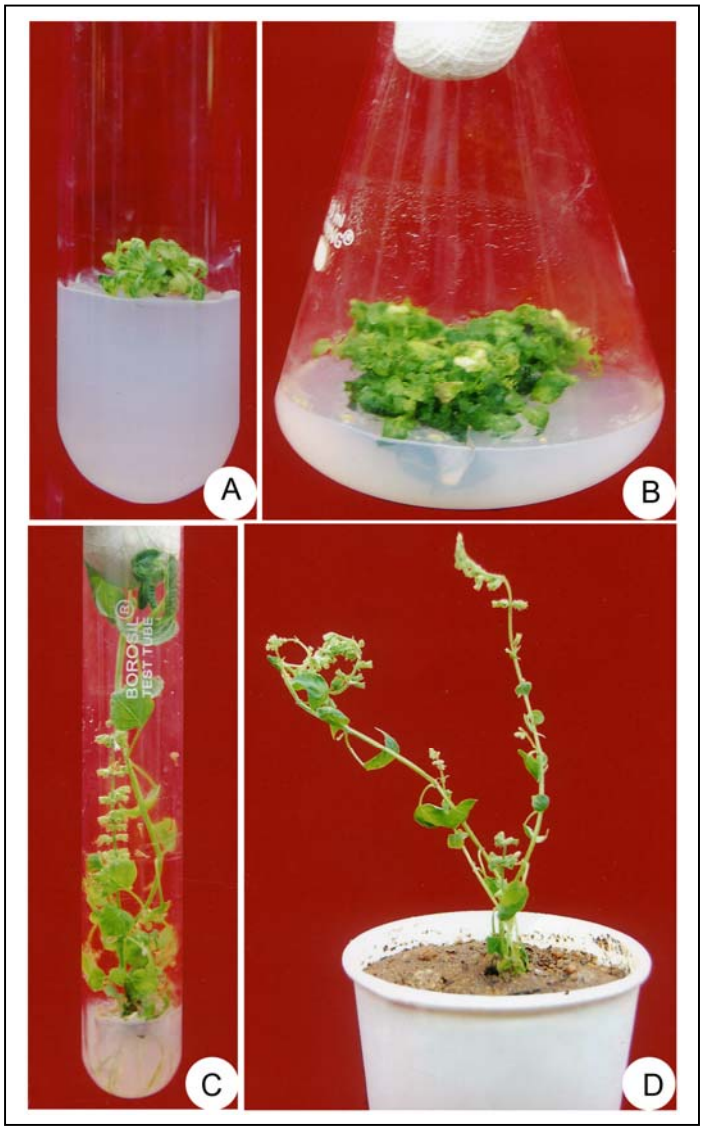

Fig. 1. A. Multiple shootbud initiation, B. Multiple shootbuf proliferation, C. Root induction and in vitro flowering and D. Harderning.

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