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In vitro Rapid Clonal Propagation of *Ocimum basilicum* L.

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Abstract

In vitro regenerated shoots of *Ocimum basilicum* L. were excised, sectioned into nodal pieces and subcultured individually in the nutrient medium which produced in an average of eight multiple shoots per transfer. For rooting, *in vitro* grown shoots were excised from the culture flask and implanted individually on root induction medium containing half strength of MS salts and 0.1 - 1.0 mg/l NAA, IBA or IAA. The highest frequency and healthy rooting was observed on MS medium containing 1.0 mg/l NAA. *In vitro* regenerated plantlets were transferred on to specially made plastic tray containing coco-peet as potting mix and thereafter successfully established under *ex vitro* condition. The survival percentage of transplanted plantlets was 75.

Introduction

Ocimum basilicum L. is an evergreen herb of the family Labiatae believed to have originated in the warmer parts of the Indo-Malayan regions. It is now found growing throughout tropical and hotter parts of the Indian subcontinent as a sacred homestead plant. The plant is found growing in different habitats including wastelands and on hills and ridges of submontane regions of India. *O. basilicum* is presently grown as a homestead plant in almost all tropical countries. In Bangladesh it is often cultivated in homestead gardens and as pot plants. It also grows semi-wild in different village groves (Hooker 1885, Ghani 1998).

In Bangladesh, the plant has been considered ethnobotanically important due to its use in traditional healthcare system, as a stomachic and anthelmintic as well as in purulent discharge of the ear, bronchitis, hiccough and diseases of the heart and brain. The plant is used as a diuretic and also in chronic pain in the joints, asthma and enlarged spleen. In the Yunani system of treatment, it is believed that the juice gives luster to the eye, good for

toothache, earache, headache and cure of ringworm. It is also used in the treatment of snakebite and other similar purposes. The seeds are mucilaginous and used for treatment in chronic dysentery. They are also given internally with "Sherbot" in cases of habitual constipation and in internal piles (Kirtikar and Basu 1994).

Ocimum basilicum is also a globally important economic crop producing annually ca. 100 tonnes of essential oil worldwide and with a trade value as a pot herb of around US $ 15 million per year. It is also widely used in systems of indigenous medicine (Paton 1996). Although it is conventionally propagated through seeds, the seedling progeny show variability due to cross-pollinated nature of the plant (Heywood 1978). So far there is no earlier report on *in vitro* plant regenertion of this species. It is, therefore, necessary to develop efficient and reliable cultural techniques and formulate appropriate media composition for specific organ as a prerequisite for *in vitro* manipulation of the plant. The present investigation was conducted to establish a suitable regeneration protocol for *O. basilicum* growing in Bangladesh using tissue culture technique.

Materials and Methods

Nodal and shoot tip segments from mature plants of *Ocimum basilicum* L. were used in the present study. The explants were collected, washed thoroughly under running tap water for 15 min, treated in 1% Savlon for five min and were surface sterilized with 01.% $HgCl_2$ for different lengths of time to ensure contamination-free culture. Thereafter the explants were washed three - four times with autoclaved distilled water to remove traces of $HgCl_2$ inside a laminar air flow cabinet. The surface sterilized shoots were cut into 1.0 - 1.5 cm long segments, each containing a single node. The cut segments were then cultured individually on half strength of MS medium containing different levels (0.1 - 2.0 mg/l) of BAP or Kn. For regeneration of complete plantlets, isolated shoots were rooted on half strength of MS medium with NAA, IBA or IAA either singly or in combination. Both proliferation and rooting media contained 3% sucrose and gelled with 0.8% agar (Hi-Media, India). The pH was adjusted to 5.7 ± 0.1. All media were steam sterilized under 1.1 kg/cm^2 pressure at 121_C. Cultures were grown at 26 ± 1_C under 16 h photoperiod with a light intensity of 2000 - 3000 lux.

Results and Discussion

Nodal and shoot tip explants from field-grown mature plants of *Ocimum basilicum* were cultured on modified MS (half strength of major and full

strength of minor salts) medium supplemented with BAP and Kn at different concentrations (viz. 0.1, 0.2, 0.5, 1.0 and 2.0 mg/l) for proliferation of axillary shoots. Initial shoot segments containing nodal zone produced multiple shoot buds on all the cytokinin-supplemented media. Explants from the *in vitro* grown shoots were also incorporated in this experiment along with explants of the mature field grown plants for proliferation of axillary shoots.

The proliferation efficiency of nodal explants from mature plants was significantly higher than that of shoot tip explants when evaluated six - seven weeks after proliferation. As a supplement, 0.2 mg/l BAP showed the best performance of proliferation that induced shoot in 100% cultured explants (Fig. A). The explants produced the highest number of 7.5 ± 0.48 shoots per culture on the medium with 5.5 ± 0.26 cm average length of shoots per culture (Fig. C). On the same medium, shoot tip explants, produced shoots in 75% of the cultures (Fig. B). The shoot tip explants produced the highest number of 4.1 ± 0.23 shoots per culture on the same medium, their average lengths being 3.0 ± 0.43 cm (Fig. D).

When the explants were cultured on Kn based medium only 16 - 75% of them showed response to proliferation. In this treatment the height, number of shoots per explant and average shoot lengths were 4.0 ± 0.35 and 3.1 ± 0.15 cm for nodal explants and 2.2 ± 0.46 and 2.5 ± 0.56 cm for shoot tip explants, respectively.

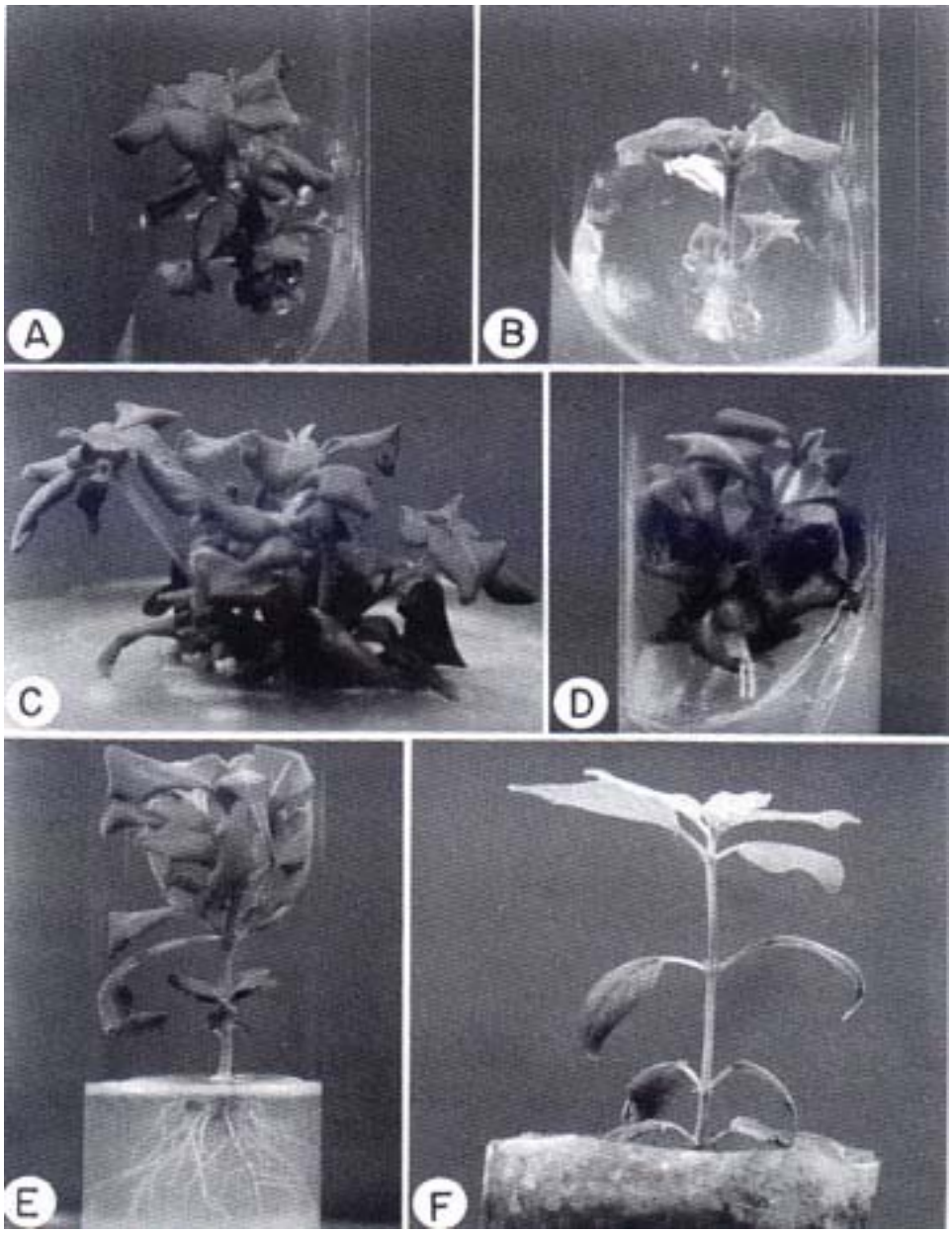
The nature and concentration of cytokinin used in this study influenced proliferation of axillary shoots derived from nodal and shoot tip segments of mature plants and *in vitro* raised shoots. BAP, at most of the concentrations, was comparatively more effective in inducing proiferation of axillary shoots whilst Kn was considerably less effective. The percentage of explants showing proliferation and the number of shoots per culture increased gradually with an increase of cytokinin concentrations from 0.1 to 0.5 mg/l. Further increase in cytokinin concentration from 0.5 to 2.0 mg/l did not improve any of the parameters but reduced shoot proliferation. At the highest level of cytokinin (2.0 gm/l), the explants failed to proliferate any shoots. The results of this experiment also indicate that 0.2 mg/l BAP was more suitable than 0.2 mg/l Kn for shoot proliferation (Table 1).

Root formation was induced in the *in vitro* regenerated shoots by culturing them on half strength of MS medium with 0.1 - 1.0 mg/l either of IBA, NAA and IAA. Among the three types of auxin, NAA was found to be most effecive at different concentrations tested for producing roots on the shoot cuttings (Fig. E).

Table 1. Effects of different concentrations of cytokinin in half strength of MS medium in direct regeneration of shoots from nodal segments of *Ocimum basilicum*.*

Growth regulators (mg/l)	% of explant showing proliferation	No. of shoots/ culture	No. of usable shoots/culture	Average length of shoots (cm)	% of explants showing proliferation	No. of shoots/ culture	No. of usable shoots/culture	Average length of shoots (cm)
	Nodal explant				Shoot tip explant			
BA								
0.1	70.0	4.0 ±0.21	3.5 ±0.45	3.1 ±0.20	50.0	3.0 ±0.41	2.1 ±0.37	2.0 ±0.32
0.2	100.0	7.5 ±0.48	6.5 ±0.37	5.5 ±0.26	75.0	4.1 ±0.23	3.5 ±0.31	3.0 ±0.43
0.5	83.0	5.8 ±0.63	4.3 ±0.16	4.0 ±0.16	60.0	3.2 ±0.24	2.5 ±0.15	2.2 ±0.16
1.0	58.3	2.3 ±0.39	2.0 ±0.21	1.1 ±0.12	45.0	1.7 ±0.53	1.5 ±0.23	1.5 ±0.23
2.0	41.6	1.5 ±0.23	1.0 ±0.11	1.0 ±0.17	30.0	1.3 ±0.21	1.0 ±0.12	1.0 ±0.06
Kn								
0.1	53.0	2.1 ±0.05	2.0 ±0.09	2.0 ±0.03	42.0	1.8 ±0.32	1.5 ±0.31	1.4 ±0.23
0.2	75.0	4.0 ±0.35	3.0 ±0.28	3.1 ±0.15	60.0	2.2 ±0.46	2.0 ±0.16	2.5 ±0.56
0.5	60.0	3.1 ±0.41	2.6 ±0.06	3.0 ±0.31	45.0	2.0 ±0.23	1.7 ±0.21	2.1 ±0.51
1.0	25.0	2.0 ±0.16	1.5 ±0.13	1.8 ±0.08	20.0	1.5 ±0.41	1.2 ±0.27	1.6 ±0.21
2.0	16.6	1.0 ±0.30	–	1.0 ±0.02	13.0	1.0 ±0.16	–	1.1 ±0.51

*Ten - 15 explants were used for each treatment and data ($\bar{X}$ ± S.E.) recorded five - six weeks after culture.



Figs. A-F : *In vitro* regeneration of plantlets from the explants of mature plants of *Ocimum basilicum* L. A-B: Development of axillary shoots from the nodal (A) and shoot tip (B) explants, respectively after six and four weeks of culture. C - D: Proliferation and elongation of multiple shoots from the nodal (C) and shoot tip (D) explants, respectively after ten and eight weeks of culture. E. Development of roots from the base of a microcutting on half strength of MS + 1.0 mg/l NAA. F : Growth of transplanted plants on the soil after seven weeks of transfer under *ex vitro* condition.

Table 2. Effects of different auxins for the formation of root from the *in vitro* grown microshoots cultured on half strength of MS medium. Ten - 15 microshoots were used for each treatment and data ($\bar{X} \pm$ S.E.) collected after five - six weeks of culture.

Auxins	Concentration of auxins (mg/l)	Percentage of cutting rooted	Number of roots/rooted cuttings	Average length of roots (cm)	Days to emergence of roots
IBA	0.1	100.0	5.0 ±0.13	5.1 ±0.27	6 - 12
	0.2	90.0	3.5 ±0.21	3.9 ±0.17	7 - 10
	0.5	70.0*	2.5 ±0.21	2.8 ±0.11	8 - 12
	1.0	-***	-	-	-
NAA	0.1	100.0	5.5 ±0.05	5.4 ±0.03	8 - 12
	0.2	100.0	5.0 ±0.15	5.1 ±0.25	10 - 14
	0.5	75.0*	2.6 ±0.12	3.5 ±0.31	10 - 15
	1.0	-***	-	-	-
IAA	0.1	60.0	3.5 ±0.18	3.1 ±0.06	5 - 16
	0.2	51.0*	2.5 ±0.11	2.9 ±0.19	7 - 12
	0.5	40.0**	2.1 ±0.06	2.2 ±0.23	6 - 10
	1.0	-***	-	-	-

'-' indicates no response; *indicates intensity of basal callusing. viz. *slight callusing; **considerable callusing; and ***profuse callusing.

The *in vitro* derived plants acclimated better under *ex vitro* conditions when they were transferred on specially made plastic trays containing coco-peet as potting mix and moistened uniformly at periodic intervals taking special care not to damage the roots. In the first week of transplantation, plantlets were kept covered in a polythene tent for providing the condition of high humidity and sufficient light. The polythene cover was removed periodically and progressively whenever leaves appeared water soaked. After new growth started on soil two - three weeks after transfer regenerated plantlets were transferred to larger earthen pots filled with organic manured soil. Through this process of acclimatization, regenerated plantlets were establised under *ex vitro* condition. The survival percentage of the transplanted plantlets was 75.

For axillary shoot proliferation, nodal explants from the mature field grown plants were found to be better than those of *in vitro* grown seedlings. Nodal explants of mature plants produced significantly a higher number of shoots in culture. Many authors reported that explants from mature field grown plants were better than seedling explants. Such instances were recorded in *Asclepias curassavica* (Paramanik and Datta 1986), *Prosopis juliflora* (Nandwani and Ramawat 1991), *Terminalia belerica* (Roy et al. 1987), *Solanum nigrum* (Jahan and Hadiuzzaman 1996), *Carica papaya* (Hossain et al. 1991) and *Gmelina arborea* (Sen et al. 1992).

The shoot tip and nodal explants were cultured on half strength of MS medium supplemented with BA and Kn at a concentration range 0.1 - 2.0 mg/l for assessing the optimum concentration of cytokinins for early sprouting and maximum proliferation of axillary shoots. The proliferation efficiency of nodal explants was significantly higher than that of shoot tip explants, when evaluated five - six weeks after proliferation. Xin and Zhang (1987) obtained better *in vitro* plantlet regeneration from nodal explants. Lundergan and Janick (1980) also reported that BA was more effective than Kn for proliferation and the development of apple shoots.

Among the three types of auxin used, NAA was found to be the best for root induction. The findings are in agreement with those observed in other plant species such as *Adhatoda vasica* (Amin et al. 1997, Azad and Amin 1998), *Caphaelis ipecacuanha* (Jha and Jha 1989), *Ocimum sanctum* (Begum et al. 2000), *Plantago ovata* (Wakhlu and Barna 1989), *Rehum emodi* (Lal and Ahuja 1989), *Ruscus hypophyllum* (Jha and Sen 1985).

About 70 - 80% of the regenerated plantlets could tolerate and survive under *ex vitro* enviornment or field conditions. A number of plantlets were lost due to damping off and necrosis during acclimatization in *ex vitro* condition. Loss of regenerants due to such symptoms was also observed in *Eucalyptus tereticornis* (Gill et al. 1993), *Solanum nigrum* (Ara et al. 1993), *Rauvolfia serpentina* (Ilahi 1993) and *Rosa damascena* (Kumar et al. 1995). Due to the delicate nature of *in vitro* regnerated plantlets, special arrangement such as controlled green house conditions, use of soil free potting mix like perlite, vermiculite, peat plugs and application of fungicides are needed for easy and successful acclimatization of plantlets (Debergh and Read 1990).

References

Paton A (1996) Basil Taxonomy, Kew Scientist **9** : 7.

Amin MN, Azad MAK and **Begum F** (1997) *In vitro* plant regeneration from leaf-derived callus cultures of *Adhatoda vasica* Nees. Plant Tissue Cult. **7** : 109 - 115.

Amin MN, Islam MN and **Azad MAK** (1999) Regeneration of plantlets *in vitro* from the seedling explants of Pomegranate (*Punica granatum*). Plant Tissue Cult. **9** : 53 - 61.

Ara M, Jahan A and **Hadiuzzaman S** (1993) *In vitro* plant regeneration from leaf explant of *Solanum sisymbrifolium* Lamk in Bangladesh. Intl. Plant Tissue Cult Conf., Dhaka, 19 - 21 Dec. pp. 47.

Azad MAK and **Amin MN** (1998) *In vitro* regeneration of plantlets from internode explants of *Adhatoda vasica* Nees. Plant Tissue Cult. **8** : 27 - 34.

Begum F, Amin MN and **Azad MAK** (2000) *In vitro* clonal propagation of Holy Basil - *Ocimum sanctum* L. Plant Tissue Cult. **10** : 31 - 37.

Datta SK, Datta K and **Datta PL** (1981) Propagation of Yam - *Dioscorea composita* through tissue culture. *In:* A.N. Rao (ed.) Tissue Culture of Economically Important Plants. Singapore : COSTED & ANBS. pp. 90 - 93.

Debergh PC and **Read RE** (1990) Micropropagation. *In:* P.C. Debergh and R.H. Zimmerman (eds.) Technology and Application, Kluwer Academic Publ. Dordrecht, The Netherlands, pp. 1 - 12.

Ghani A (1988) Medicinal plants of Bangladesh: Chemical constituents and uses. Asiatic Society of Bangladesh. pp. 248 - 250.

Gill RIS, Gill SS and **Gosal SS** (1993) Vegetative propagation of *Eucalyptus tereticornis* Sm. through tissue culture. Intl. Plant Tissue Cult. Conf., Dhaka 19 - 21 Dec., pp. 44.

Heywood VH (1978) Flowering Platns of the World. Oxford Univ. Press, UK. pp. 239 - 240.

Hooker JD (1985) Flora of British India. Vol. 6. L. Reeve and Co. Ltd. pp. 608.

Hossain M, Rahman SM and **Joarder OI** (1991) *In vitro* culture of *Carica papaya* L. III. Maintenance proliferating culture. Plant Tissue Cult. **1** : 19 - 25.

Ilahi I (1993) Micropropagation and biosynthesis of alkaloid by *Rauvolfia* cell culture. Intl. Plant Tissue Cult. Conf., Dhaka 19 - 21 Dec. pp. 21.

Jahan MAA and **Hadiuzzaman S** (1996) Callus induction and plant regeneration from different explants of *Solanum nigrum* L. seedlings. Plant Tissue Cult. **6** : 57 - 62.

Jha S and **Jha TB** (1989) Micropropagation of *Cephaelis ipecacuanha*. Plant Cell Rep. **8** : 437 - 439.

Jha S and **Sen S** (1985) Regeneration and rapid multiplication of *Bowiea volubilis* Harv. in tissue culture. Plant Cell Rep. **4** : 12 - 14.

Kirtikar KR and **Basu BD** (1994) Indian Medicinal Plants. 2nd edition, Vol. III, Bishen Singh Mahendra Pal Singh, Dehra Dun, pp. 1884 - 1888.

Kumar S, Choudhary ML and **Raghava SPS** (1995) *In vitro* manipulation of *Rosa damascena* Mill for oil contents. 2nd Intl. Plant Tissue Cult. Conf., Dhaka, 10 - 12 Dec., pp. 47.

Lal N and **Ahuja PS** (1989) Propagation of Indian rhubarb (*Rheum emodi* Wall.) using shoot tip and leaf explants culture. Plant Cell Rep. **8** : 439 - 496.

Lundergan CA and **Janick J** (1980) Regulation of apple shoot proliferation and growth *in vitro*. Hort. Res. **20** : 19 - 24.

Nandwani D and **Ramawat KG** (1991) Callus culture and plantlets formation from nodal explants of *Prosopis julflora* (Swartz) DC. Indian J. Expt. Biol. **29** : 523 - 527.

Pramanik TK and **Datta SK** (1986) Plant regeneration and ploidy variation in culture derived plants of *Asclepias curassavica*. Plant Cell Rep. **5** : 219 - 222.

Roy SK, Rahman L and **Datta PC** (1987) Propagation of *Mitragyna parviflora* Korth. Plant Cell, Tissue and Organ Cult. **12** : 75 - 78.

Sen J, Islam MS, Roy SK and **Hadiuzzaman S** (1992) Micropropagation of juvenile and adult *Gmelina arborea*. Plant Tissue Cult. **2** : 89 - 95.

Wakhlu AK and **Barna KS** (1989) Callus initiation, growth and plant regeneration in *Plantago ovata* Forsk. cv. GI-2, Plant Cell, Tissue and Organ Cult. **17** : 235 - 241.

Xim SY and **Zhang ZZ** (1987) Explant tissue culture and plantlets regenertion of sweet potato. Inst. Acta. Botanica. Sinica. **23** : 114 - 116.