BIOLOGIA PLANTARUM 51 (4): 787-790, 2007

BRIEF COMMUNICATION

Rapid micropropagation of *Ocimum basilicum* using shoot tip explants pre-cultured in thidiazuron supplemented liquid medium

I. SIDDIQUE and M. ANIS*

Plant Biotechnology Laboratory, Department of Botany, Aligarh Muslim University, Aligarh-202002, India

Abstract

An efficient protocol has been developed for rapid micropropagation of *Ocimum basilicum*. Multiple shoots were induced by culturing shoot tip explants excised from mature plants on a liquid Murashige and Skoog (MS) medium supplemented with 5 - 100 μM of thidiazuron (TDZ) for different treatment duration (4, 8, 12 and 16 d). The optimal level of TDZ supplementation to the culture medium was 50 μM for 8 d induction period followed by subculturing in MS medium devoid of TDZ as it produced maximum regeneration frequency (78 %), mean number of shoots (11.6 ± 1.16) and shoot length (4.8 ± 0.43 cm) per explant. A culture period longer than 8 d with TDZ resulted in the formation of fasciated or distorted shoots. The regenerated shoots rooted best on MS medium containing 1.0 μM indole-3-butyric acid (IBA). The micropropagated shoots with well developed roots were successfully established in pots containing garden soil and grown in greenhouse with 95 % survival rate. The regenerated plants were morphologically uniform and exhibited similar growth characteristics and vegetative morphology to the donor plants.

Additional key words: growth characteristics, indole-3-butyric acid, shoot multiplication, *Soilrite*, sweet basil

_____

Ocimum basilicum L. (*Lamiaceae*) commonly known as sweet basil is a native to tropical Asia. The leaves have numerous dot like oil glands, which secrete strongly scented volatile oils. The plant is used as food flavourant, in perfumery and cosmetics as well as in medicines.

The conventional method for propagation of *O. basilicum* is *via* seeds. However, poor germination potential restricts its multiplication. Moreover, seed derived progenies are not true to type due to cross pollination (Heywood 1978). *In vitro* micropropagation is an effective means for rapid multiplication of species in which conventional methods have limitations (Nehra and Kartha 1994, Sudha and Seeni 1994, Singh and Sehgal 1999, Martin 2002).

Thidiazuron (N-phenyl-1,2,3-thidiazol-5yl urea) has shown to possess potent activity as a cytokinin in the regulation of shoot organogenesis in several plant species (Huetteman and Preece 1993, Mithila *et al.* 2001, Faisal *et al.* 2005, Onofrio and Morini 2005, Radhika *et al.* 2006). There have been few previous reports on micropropagation of *O. basilicum via* nodal segment and shoot tip culture (Sahoo *et al.* 1997, Begum *et al.* 2002) but none of these reports have used TDZ for *in vitro* propagation. The present study assessed the potential of TDZ on the multiple shoot induction by culturing shoot tip explants of *O. basilicum* in liquid medium followed by their transfer to MS medium without TDZ.

Shoot tip measuring 0.5 - 0.8 cm in length were excised from 2-year-old plant grown in the Botanical garden of the University. These were washed first under running tap water for half an hour, then treated with a liquid detergent, *Teepol* 5 % (v/v) for 15 min and finally washed thoroughly in sterile double distilled water. The explants were surface sterilized in 0.1 % (m/v) $HgCl_2$ solution for 4 min followed by repeated washing with sterile double distilled water. The material was cut into

_____

Received 21 December 2005, *accepted* 22 July 2006.

Abbreviations: IBA - indole-3- butyric acid; MS - Murashige and Skoog medium; PGR - plant growth regulator; PPFD - photosynthetic photon flux density; TDZ - thidiazuron.

Acknowledgements: The authors are grateful to the Department of Science and Technology, New Delhi for partial assistance under FIST - DST Programme 2005.

* Corresponding author; fax: (+91) 571 2702214, e-mail: anism1@rediffmail.com

appropriate sizes and cultured on sterile Murashige and Skoog medium (1962). The basic nutrient medium consisted of MS salts and vitamins with 3 % (m/v) sucrose and 0.8 % (m/v) agar or 0.25 % (m/v) *Gelrite*. Liquid MS medium was supplemented with various concentration of TDZ (5, 25, 50, 75 and 100 μM). The pH of the medium was adjusted to 5.8 with 1 M NaOH prior to autoclaving at 121 °C at 1.06 kg cm^{-2} for 20 min. All cultures were maintained in a culture room at temperature of 24 ± 2 °C, 16-h photoperiod with a photosynthetic photon flux density (PPFD) of 50 μmol $m^{-2}s^{-1}$ provided by cool white fluorescent lamps (*Philips*, Mumbai, India) and relative humidity of 60 - 65 %.

The shoot tip explants were cultured in 50 cm^3 liquid MS media supplemented with TDZ (5 - 100 μM) on a rotatory shaker at 100 rpm. MS medium lacking growth regulator surved as control. For determining the optimal duration of exposure of the explants to the medium containing TDZ, the explants from each treatment were subcultured onto MS basal medium after 4, 8, 12 and 16 d of culture. All cultures were transferred to fresh medium after every 2 weeks. The percentage of explant forming shoot, number of differentiated shoots and shoot length per explant were recorded after 8 weeks of culture.

For root induction, excised shoots (> 4 cm) with four or more leaves were harvested and transferred to full strength MS medium supplemented with IBA (0.5, 1.0, 1.5, 2.0 and 5.0 μM). The percentage of rooting, mean number of roots and root length were recorded after 4 weeks of culture. Healthy plantlets with well developed shoot and roots were removed individually from culture, washed gently under running tap water and transferred to pots containing sterile *Soilrite* under 16-h photoperiod. Rooted plantlets were covered with transparent polythene bags punched with 4 - 5 holes to allow gaseous exchange and ensure high humidity. These were watered every 3 d for 2 weeks with half strength MS salt solution. Polythene bags were removed after 2 weeks and after 4 weeks, these plants were transferred to pots containing normal garden soil and maintained in greenhouse.

All the experiments were repeated thrice and twenty replicates were employed for each treatment. The effect of different treatments was quantified and data was analyzed using one way analysis of variance (*ANOVA*) and means were compared using Duncan's multiple range test at 5 % level of significance.

The shoot tips failed to respond morphogenetically to a growth regulator free MS medium. The explants cultured on the media containing different concentrations of TDZ for different length of incubation showed initial bud break after one week. The rate of shoot induction varied between 2 to 11 shoots per explants depending on the concentrations of TDZ, the duration of exposure in liquid medium followed by their transfer to MS gelled medium without TDZ (Table 1). The optimal concentration and duration of TDZ for induction of shoots was 50 μM for 8 d as it gave the highest shoot regeneration frequency (78 %), mean number of shoots (11.6 ± 1.16) and shoot length (4.8 ± 0.43 cm) after 8 weeks of transfer to TDZ free MS medium (Fig. 1*AB*). Although a low range of concentrations of TDZ, from 1 nM to 10 μM is recommended for shoot proliferation (Huetteman and Preece 1993), our results showed that a short term exposure to high concentrations of TDZ prior to culture in PGR free MS medium enhanced shoot multiplication in *O. basilicum*. The effectiveness of short term exposure

Table 1. Effect of different concentrations of TDZ and duration of culture on TDZ supplemented liquid MS medium on regeneration of shoot tip explants of *O. basilicum* 8 weeks after transfer to MS basal medium. Values represent means ± SE. Means followed by the same letter are not significantly different by the Duncan's multiple range test at 5 % probability level.

TDZ [μM]	Culture duration [d]	Regeneration [%]	Number of shoots [explant^{-1}]	Shoot length [cm]
5	4	43	2.1 ± 0.20^{k}	1.3 ± 0.17^{gh}
	8	52	4.7 ± 0.40^{ghi}	2.3 ± 0.23^{def}
	12	45	3.5 ± 0.30^{ijk}	1.8 ± 0.12^{fgh}
	16	39	2.8 ± 0.17^{jk}	1.0 ± 0.10^{h}
25	4	61	6.2 ± 0.61^{efgh}	2.4 ± 0.18^{def}
	8	72	8.6 ± 0.70^{bcd}	3.5 ± 0.23^{bc}
	12	65	7.6 ± 0.46^{cde}	2.7 ± 0.17^{cde}
	16	60	6.4 ± 0.37^{efg}	1.3 ± 0.34^{gh}
50	4	70	9.4 ± 0.86^{bc}	3.0 ± 0.31^{bcd}
	8	78	11.6 ± 1.16^{a}	4.8 ± 0.43^{a}
	12	72	10.2 ± 0.98^{ab}	3.7 ± 0.23^{b}
	16	67	8.3 ± 0.68^{cd}	2.4 ± 0.24^{def}
75	4	57	5.7 ± 0.46^{fgh}	2.5 ± 0.30^{def}
	8	68	7.1 ± 0.55^{def}	3.6 ± 0.37^{b}
	12	60	6.1 ± 0.44^{efgh}	2.6 ± 0.29^{def}
	16	55	4.6 ± 0.46^{ghi}	1.9 ± 0.26^{efg}
100	4	45	3.4 ± 0.30^{ijk}	2.0 ± 0.23^{def}
	8	59	4.2 ± 0.37^{hij}	2.6 ± 0.35^{def}
	12	52	3.0 ± 0.20^{jk}	1.9 ± 0.26^{efg}
	16	41	2.6 ± 0.19^{k}	1.3 ± 0.17^{gh}

Table 2. Effect of IBA concentration on root induction from *in vitro* raised shoots of *O. basilicum* after 4 weeks of culture. Values represent means ± SE. Means followed by the same letter are not significantly different by the Duncan's multiple range test at 5 % probability level.

IBA [μM]	Rooting [%]	Number of roots [shoot^{-1}]	Root length [cm]
0.5	75	4.8 ± 0.17^{a}	2.5 ± 0.31^{b}
1.0	87	5.4 ± 0.30^{a}	4.3 ± 0.38^{a}
1.5	80	4.0 ± 0.23^{b}	3.6 ± 0.26^{a}
2.0	70	3.5 ± 0.20^{b}	2.4 ± 0.30^{bc}
5.0	63	2.7 ± 0.14^{c}	1.5 ± 0.23^{c}

and stimulating effect of TDZ on bud break and multiple shoot formation has been reported earlier for several aromatic and medicinal plant species including *Hypericum perforatum* (Murch *et al.* 2000), *Curcuma longa* (Prathanturarug *et al*. 2003), and *Arachis correntina* (Mroginski *et al*. 2004). It is suggested that TDZ may be needed as a trigger for initiating the proliferation of shoot meristems and further incubation on PGR free MS medium led the explants to further development (Khalafalla and Hattori 1999).

Explants which remained on the TDZ supplemented medium beyond 8 d of incubation, showed reduced number of shoots which appeared stunted and deformed. Similar deformities have been reported in several plant species by Pattnaik and Chand 1996, Murch *et al.* 2000, Faisal *et al.* 2005. Higher concentration of TDZ (100 µM) suppressed the regeneration frequency, mean number of shoots and shoot length as it induced callusing. Reduction in the number of shoots generated from each shoot apices at TDZ concentrations higher than the optimal level was also reported for several plants (Sudha and Seeni 1994, Hutchinson and Saxena 1996, Pattnaik and Chand 1996, Faisal *et al.* 2005). The shoots induced from different treatments of TDZ were subcultured 3 times to hormone free MS medium for shoot multiplication at an interval of 2 weeks.

The *in vitro* regenerated shoots induced roots when transferred to MS medium containing different concentrations (0.5 - 5.0 µM) of IBA (Table 2). Of the various concentrations tested, the maximum frequency of root formation (87 %), mean number of roots (5.4 ± 0.30) and root length (4.3 ± 0.38 cm) was achieved in a medium containing 1.0 µM IBA after 4 weeks of culture (Fig. 1*C*). Optimum rooting response using IBA has been reported for several plants including *Tylophora indica* (Faisal and Anis 2003), *Sesbania drummondii* (Cheepala *et al.* 2004), and in *Psoralea corylifolia* (Faisal and Anis 2006).

Fig. 1. Effect of the pretreatment of TDZ on shoot bud induction from shoot tip explants of *O. basilicum*. *A* - induction of shoot buds in PGR free MS medium after 8 d pretreatment of 50 µM TDZ; *B* - proliferation of shoots after 8 weeks in PGR free MS medium; *C* - rooted plantlet; *D* - an acclimatized plant.

Rooted plantlets with 5 - 6 fully expanded leaves and well developed roots were transferred to pots containing sterile *Soilrite* and hardened off in a growth chamber for 4 weeks. After one month, the micropropagated plants were planted in earthen pots containing garden soil and maintained in a greenhouse (Fig. 1*D*). The survival rate after transfer was 95 %. The regenerated plant exhibited morphological characteristics similar to those of the source plant.The present study describes an effective protocol for clonal propagation of *O. basilicum* using thidiazuron and the culture system produced masses of plant material that would be suitable for commercial applications. The protocol could also be helpful for the improvement of medicinal content by genetic engineering of this medicinally and pharmaceutically important plant species.

References

Begum, F., Amin, M.N., Azad, M.A.K.: *In vitro* rapid clonal propagation of *Ocimum basilicum* L. - Plant Tissue Cult. **12**: 27-35, 2002.

Cheepala, S.B., Sharma, N.C., Sahi, S.V.: Rapid *in vitro* regeneration of *Sesbania drummondii*. - Biol. Plant. **48**: 13-18, 2004.

Faisal, M., Anis, M.: Thidiazuron induced high frequency axillary shoot multiplication in *Psoralea corylifolia*. - Biol. Plant. **50**: 437- 440, 2006.

Faisal, M., Ahmad, N., Anis M.: Shoot multiplication in *Rauwolfia tetraphylla* L. using thidiazuron. - Plant Cell Tissue Organ Cult. **80**: 187-190, 2005.

Faisal, M., Anis, M.: Rapid mass propagation of *Tylophora indica* Merill via leaf callus culture. - Plant Cell Tissue Organ Cult. **75**: 125-129, 2003.

Heywood, V.H.: Flowering Plants of the World. - Oxford University Press, Oxford 1978.

Huetteman, C.A., Preece, J.E.: Thidiazuron: a potent cytokinin for woody plant tissue culture. - Plant Cell Tissue Organ Cult. **33**: 105-119, 1993.

Hutchinson, J.M., Saxena, P.K.: Acetylsalicylic acid enhances and synchronizes thidiazuron-induced somatic embryogenesis in geranium (*Pelargonium* × *hortorum* Bailey) tissue cultures. - Plant Cell Rep. **15**: 512-515, 1996.

Khalafalla, M.M., Hattori, K.: A combination of thidiazuron and benzyl adenine promotes multiple shoot production from cotyledonary node explants of faba bean (*Vicia faba* L.). - Plant Growth Regul. **27**: 145-148, 1999.

Martin, K.P.: Rapid propagation of *Holostemma ada-kodien* Schult., a rare medicinal plant through axillary bud multiplication and indirect organogenesis. - Plant Cell Rep. **21**: 112-117, 2002.

Mithila, J., Murch, S.J., Krishnaraj, S., Saxena, P.K.: Recent advances in *Pelargonium* in *in vitro* regeneration systems. - Plant Cell Tissue Organ Cult. **67**: 1-9, 2001.

Mroginski, E., Rey, H.Y., Gonzalez, A.M., Mroginski, L.A.: Thidiazuron promotes *in vitro* plant regeneration of *Arachis correntina* (Leguminosae) via organogenesis. - Plant Growth Regul. **23**: 129-134, 2004.

Murashige, T., Skoog, F.: A revised medium for rapid growth and bioassay with tobacco tissue culture. - Physiol. Plant. **15**: 473-497, 1962.

Murch, S.J., Choffe, K.L., Victor, J.M.R., Slimmon, T.Y., Krishnaraj, S., Saxena, P.K.: Thidiazuron induced plant regeneration from hypocotyl cultures of St. Johns wort (*Hypericum perforatum* cv. Anthos). - Plant Cell Rep. **19**: 576-581, 2000.

Nehra, N.S., Kartha, K.K.: Meristem and shoot tip culture: Requirements and Applications. - In: Vasil, I.K., Thorpe, T.A. (ed.): Plant Cell and Tissue Culture. Pp. 37-70. Kluwer Academic Publishers, Dordrecht 1994.

Onofio, C.D., Morini, S.: Development of adventitious shoots from *in vitro* grown *Cydonia oblonga* leaves as influenced by different cytokinins and treatment duration. - Biol. Plant. **49**: 17 -21, 2005.

Pattnaik, S.K., Chand, P.K.: *In vitro* propagation of the medicinal herbs *Ocimum americanum* L. syn. *O. canum* Sims. (hoary basil). - Plant Cell Rep. **15**: 846-850, 1996.

Prathanturarug, S., Soonthorn Chareonnon N., Chuakul, W., Phaidee, Y., Saralamp, P.: High frequency shoot multiplication in *Curcuma longa* L. using thidiazuron. - Plant Cell Rep. **21**: 1054-1059, 2003.

Radhika, K., Sujatha M., Nageswar Rao, T.: Thidiazuron stimulates adventitious shoot regeneration in different safflower explants. - Biol. Plant. **50**: 174-179, 2006.

Sahoo, Y., Pattnaik, S.K., Chand, P.K.: *In vitro* clonal propagation of a medicinal plant *Ocimum basilicum* L. (sweet basil) by axillary shoot proliferation. - *In vitro* cell. dev. Biol. Plant. **33**: 293- 296, 1997.

Singh, N.K., Sehgal, C.B.: Micropropagation of 'Holy Basil' (*Ocimum sanctum* Linn.) from young inflorescences of mature plants. - Plant Growth Regul. **29**: 161-166, 1999.

Sudha, G.C., Seeni, S.: *In vitro* multiplication and field establishment of *Adhatoda beddomei* CB Clarke, a rare medicinal plant. - Plant Cell Rep. **10**: 67-70, 1994.