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Micropropagation of *Lantana camara* Through Axillary Shoots Proliferation

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Abstract

In vitro bud induction and shoot regeneration of *Lantana camara* L. was investigated using axillary nodal explants. Multiple shoot buds were induced on Murashige and Skoog (MS) media supplemented with 0, 2, 4 and 8 mg/l of 6-benzylaminopurine (BAP) or thidiazuron (TDZ) for proliferation and 0, 0.25 and 0.5 mg/l indole-3 butyric acid (IBA) for rooting. After 4 weeks, a maximum number of shoot buds was achieved on MS media containing 8 mg/l BAP and most buds were resulted in 8 mg/l TDZ. The results showed that the maximum length of shoots, internode length and weight were observed in MS media without any growth regulators. Suitable concentrations of cytokinins significantly increased RNA, DNA and protein synthesis and shoot induction. Difference in concentrations of BAP can be under the influence of genetic factors and experimental condition. Very low concentrations of TDZ stimulated the proliferation of lateral shoots in woody plants. Receptor molecules of TDZ had high affinity with this growth regulator. In this research, the highest number of roots was induced in MS media with 0.25 mg/l IBA. IBA is one of the strong PGRs in culture medium for stimulating and increasing the number of roots. IBA increases internal IAA, which is necessary for normal growth of root meristems and final development of roots. One of the effects of auxins is elongation of cells, but root cells need lower auxin for growth because auxin induces ethylene which is a root growth inhibitory.

Keywords: Axillary bud explants, Bud induction, *Lantana camara*, Rooting, Shoot proliferation.

INTRODUCTION

Lantana camara L. is native to tropical and subtropical regions of America. This plant is an evergreen shrub with heart-shaped leaves whose serrated leaf edges are rough surface. Flowers are formed as a rounded cup and are come out of axis of the leaves. Although *L. camara* is toxic for mammals, it is a traditional medicinal plant used in some parts of the world for the treatment of fever and flu, bronchitis and other diseases. Study of the chemical compositions of secondary metabolites of this plant has shown different compounds such as sesquiterpen, alpha kapayin, alpha flandren, trans karyofilin, beta gurjunen, etc. Secondary metabolites of this plant have shown antifungal and anti-bacterial activity. Also, these effective materials have an insecticide effect on the *Sitophilus zemais* from Coleoptera family (Affonso *et al*., 2007; Passos *et al*., 2009). Anti-cancer properties of this plant have been proved by Srivastava *et al*. (2011).

The germination percentage of the seeds of this plant is very low. Layering and cutting are other ways of propagation of *L. camara* which have their own problems so that a few plants are produced with these propagules (Day *et al.,* 2003). Micropropagation is another way for mass propagation of plants but just a few studies have been carried out on it.

Affonso *et al*. (2007) reported that the addition of 4.4 µM BA and 0.44 µM TDZ to MS culture medium significantly decreased longwise shoots and formation of the roots in *L. camara.* Although the addition of 4.4 µM BA alone increased the number of shoots per explants, it also increased the number of nodes per explant. Micropropagation of *Salix tarraconensis* under different concentrations of auxins and cytokinins were studied in MS and WPM media. The best results of shoot formation were observed in MS medium containing 4.9 µM BAP (Amo-Marco and Lledo, 1995). Explants of nodal segments and leaves were compared in regeneration of *Vernonia cinerea* and the results showed the most number of shoots were obtained in leaf explants (8.1) and nodal segments (11) in MS medium containing 2.5-3 mg/l BAP (Seetharma *et al.*, 2007). Direct shoot regeneration of *Withania somnifera* from node, internode, hypocotyl and embryo were studied in MS culture medium. Concentration and type of cytokinins used for proliferation depend on the type of the used explants. Shoot proliferation of nodal segments was observed in 0.1-5 mg/l BA and 0.2-0.3 mg/l TDZ, for internodes in 1 and 5 mg/l BA, for hypocotyls in 0.5 mg/l BA and for embryo in 0.2 and 0.3 mg/l TDZ (Kulkarni *et al.*, 2000). It has been reported that in *Vitex trifolia* among different treatments of TDZ, 2-ip, Kin, BA and adenine, the highest number of shoots per explant was obtained from nodal segments in MS medium treated with 5 mg/l BA (Hiregoudar *et al.,* 2006). In *Tectona grandis*, the best regeneration has been obtained from internode in MS medium containing 10 mg/l BA and 1 mg/l GA_3. To proliferate the regenerated shoots, these shoots were cultured in MS medium containing 10 mg/l BA (Widiyanto *et al.*, 2005). In a survey on proliferation of *Rauvolfia tetraphylla* by TDZ (0.5- 10 µM) in MS medium, it was revealed that the highest percentage of proliferation (90%) and the highest number of shoots per explant (18.5) were obtained from 5 µM TDZ (Ahmad and Anis, 2005). In *Terminalia bellirica* at auxin concentrations of more than 0.5 mg/l (IAA, IBA and NAA), the growth of callus was seen and IBA (1 mg/l) was introduced as the best rooting treatment (Rathore *et al.*, 2008).

Considering pharmaceutical and ornamental importance of this plant, the aim of this study was to examine the effective factors on *in vitro* production of this plant. In the present study, we investigated the effects of some plant growth regulators including various concentrations of cytokinins (BAP and TDZ) on proliferation and IBA on rooting of this species in order to obtain high shoot regeneration rate and high rooting frequency.

MATERIALS AND METHODS

Explant preparation

Nodal segments of *Lantana camara* (5 mm in length) were surface sterilized with 20% (v/v) sodium hypochlorite for 20 min. and 70% ethanol for 1 min. For the elimination of the disinfector residues, their drain was done using sterilized distilled water 3 times in 6 minutes.

Basal medium and culture conditions

In all experiments, MS (Murashige and Skoog, 1962) medium containing 3% (w/v) sucrose and 0.7% agar was used as basal medium. The pH of the media was adjusted to 5.8 before using agar. All the cultures were placed in growth chambers at 24±2°C under 16/8 h photoperiod and photosynthetic photon flux of 60 µmol m^{-2} s^{-1} provided by cool daylight fluorescent lamps.

Effect of plant growth regulators (PGRs) on bud induction and shoot regeneration

Different concentrations of TDZ or BAP (0, 2, 4, 8 mg/l) were used to find the best concentrations of them for bud induction and shoot regeneration of axillary nodal explants. Regenerated shoots were carefully excised and put on MS medium containing different concentrations (0, 0.25 and 0.5 mg/l) of IBA. Explants were cultured in glasses containing 30 ml of mentioned media.

The cultures were placed at 24±2° C under 1600 lux light for 30 days. For evaluation of the plant proliferation in different treatments, number of shoots, length of shoots, number of nodes, length of internodes, number of leaves and dry and fresh weight of shoots were measured. Evaluation of rooting, number of shoots with main roots, length of roots, thickness of main roots, number of lateral roots, dry and fresh weight were measured in the end of course.

Experimental design and data analysis

The experiments were based on a completely randomized design with three replications per treatment. Analysis of variance (ANOVA) was performed on the data with the General Linear Model procedure using SAS 9.1 software and the means were compared using Duncan's multiple range test (DMRT) at the 5% probability level. Graphs were plotted with MS-Excel program.

RESULTS AND DISCUSSION

Effect of plant growth regulators (PGRs) on bud induction and shoot regeneration

The number of induced buds and shoot regeneration per explant were directly proportional to the type and concentration of cytokinins. The highest frequency of shoots was observed in 8 mg/l BAP (8.66) and the lowest number of them was observed in 8 mg/l TDZ (1.77) (Table 1). The maximum length of the shoots was obtained in 8 mg/l BAP (1.09 cm). The highest number of nodes was observed in 2 mg/l TDZ (4.17) and the fewest nodes were observed in 2 mg/l BAP. The highest length of internode was observed in 2 mg/l TDZ (0.57 cm) and the lowest one in 8 mg/l BAP (0.31 cm). The highest fresh weight of shoots was obtained in control treatment (0.71 g) and the lowest fresh weight was observed in 8 mg/l TDZ (0.31 g). Whereas the highest dry weight of

Table 1. Comparison of the effects of different concentrations of BAP and TDZ on proliferation parameters in *Lantana camara*

PGR type	PGR concentration (mg/l)	Number of shoots per explant	Length of shoots (mm)	Number of nodes per explant	Length of internode (cm)	Fresh weight (mg)	Dry weight (mg)	Number of leaves per explant
BAP	0	2.11^{c}	2.2^{a}	4.17^{a}	0.51^{ab}	0.71^{a}	0.11^{a}	8.95^{a}
	2	5.88^{b}	1.34^{bc}	3.06^{b}	0.43^{cd}	0.61^{b}	0.09^{b}	7.05^{c}
	4	6.77^{b}	1.26^{bc}	3.26^{b}	0.38^{d}	0.57^{b}	0.08^{bc}	8.33^{b}
	8	8.66^{a}	1.09^{c}	3.43^{b}	0.31^{e}	0.69^{a}	0.07^{c}	7.14^{c}
TDZ	0	2.11^{c}	2.2^{a}	4.17^{a}	0.51^{ab}	0.71^{a}	0.11^{a}	8.95^{a}
	2	1.88^{c}	2.44^{a}	4.27^{a}	0.57^{a}	0.63^{ab}	0.11^{a}	8^{b}
	4	1.77^{c}	1.79^{ab}	4.38^{a}	0.41^{cd}	0.39^{c}	0.07^{c}	7.27^{c}
	8	1.77^{c}	2.08^{a}	4.49^{a}	0.47^{bc}	0.31^{d}	0.07^{c}	8.1^{b}

*In each column, dissimilar letter(s) indicates significant differences at P<0.05 by DMRT.

shoots was obtained in control treatment (0.11 g), the lowest one was observed jointly in 8 mg/l TDZ and 8 mg/l BAP (0.07 g) (Table 1). No significant differences were observed among different treatments in the number of the leaves. TDZ concentrations of higher than 2 mg/l led to vitrification of the explants which is an indicator of high and unsuitable concentration of this PGR.

Function of cytokinins in plants include stimulation of cell division, elimination of the lateral buds dormancy, induction of unsuitable buds formation, growth of lateral buds, and control of the cell division cycle (Gaspar *et al*., 2003). Cytokinins will overcome apical dominancy and break the dormancy of the lateral buds, and they are necessary for the induction of multiple shoots (Abdellatef and Khalafallah, 2007; Ashoc and Bashir, 2010). Suitable concentration of cytokinins increased RNA, DNA and protein synthesis and shoot induction significantly (Mok and Mok, 2001). Differences in concentrations of BAP can be associated with genetic factors and experimental conditions. In *Clitoria ternetea*, 5µM BA (Ismail *et al.*, 2011) and in *Adhatoda vasica*, 5-10 mg/l BA enhanced shoot induction (Abhyankar and Reddy, 2007). Affonso *et al.* (2007) reported that in *Lantana camara*, adding 4.4 µM BAP increased the number of shoots per explants (4.61). In *Litsea cubeba*, TDZ, 2iP and Kin did not produce new shoots in lateral bud and shoot tip explants and only BAP induced shoot elongation in these explants (Mao *et al.*, 2000). The effective rate of cytokinins is under the influence of proliferation PGRs receptors likeness (Verma *et al*., 2011). Very low concentrations of TDZ stimulate the proliferation of lateral shoots in woody plants. Receptor molecules of TDZ have high affinity with this growth regulator. In *Abelmoschus moschatus*, 0.01mg/l TDZ concentration was recognized as the best treatment for increasing the number of shoots (Sharma and Shahzad, 2008). A high proliferated shoots were obtained in *Nothapodytes foetida* in culture medium containing 2.2 µM TDZ (Rai, 2002). Several factors are responsible for *in vitro* organogenesis. Nature and status of explants, application of PGRs and their interaction with healthy tissue have a strong effect on regeneration (Lakshmi Prabha *et al.*, 2010). Type of explant, growth medium and the levels of internal PGRs affected the induction of shoots (Kesari *et al.,* 2012). Cytokinin is synthesized in roots and moves towards the aerial parts of plants. So, lateral buds are rich in cytokinin because of exposure to low portion of mother plants (Zulfiqar *et al.*, 2009), and on the other hand, the effect of TDZ may be made possible because of its ability in stimulation of cytokinin accumulation in interior tissue (Victor *et al.*, 1999). Likely, high concentration of indigenous cytokinin in lateral buds of this plants and its interaction with TDZ can be one of the factors inhibiting TDZ action in proliferation of *Lantana camara*.

TDZ isn't suitable for proliferation of this plant and high concentration of this PGR results in a considerable loss of the length of the shoots (Ning *et al*., 2007). For the formation of shoot buds in mango leaf segment cultures and guava proliferation, it is necessary to remove TDZ from culture medium. Use of TDZ for guava micropropagation induced short and abortive shoots with yellow leaves and higher TDZ concentrations aggravated it (Te-chato and Lim, 2000). In this research, higher length of shoots and internodes in 2mg/l TDZ is linked with lower shoots and as a result of lower competition on nutrients.

Effect of IBA on rooting

The highest number of basic root was observed in medium containing 0.25 mg/l IBA (1.11) and the lowest one was in control (0.33) (Fig. 1). The highest number of lateral roots and thickness of basic roots were observed in 0.25 mg/l IBA with no significant differences to each other. On the other hand, the maximum root length, and fresh and dry weight of roots were observed in 0.5 mg/l IBA with no significant differences to each other.

IBA is one of the sustainable and strong PGRs in culture medium reported by different researchers for stimulating and increasing the number of roots (Stefancic *et al.*, 2005; Nissen and Sutter, 1990; Selvakumar *et al*., 2001; Ahmad and Anis, 2005; Ali Ahmad *et al*., 2005; Rathore *et al.*, 2008). IBA not only increases internal IAA, but also increases level of IAAsp (acid indole-3-acetyl-aspartic) which are necessary for normal growth of root meristems and final development

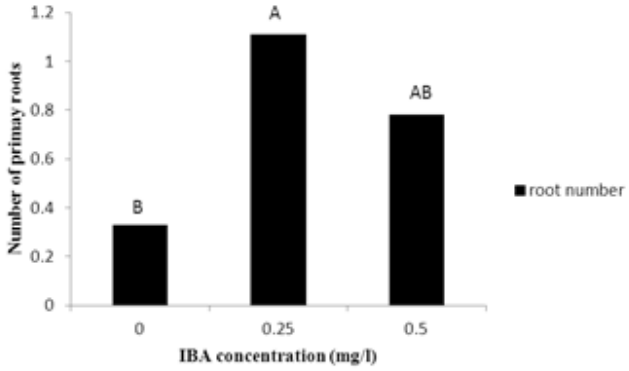

Fig. 1. Effect of different concentrations of IBA on root formation.
*Dissimilar letter(s) indicates significant differences at P< 0.05 by DMRT.

of roots. One of the effects of auxins is the elongation of cells, but cells of root need lower auxin for growing because auxin stimulates production of ethylene which is a root growth inhibitory (Ali *et al.*, 2009). Also, Singh and Tiwari (2010) found inhibitory effects of IBA in high concentrations (2 mg and more) in their research. According to Khalafalla *et al*. (2010), IBA is necessary only for root induction, probably in Lantana, and the concentration of indigenous auxin is enough for ensuring the continuation of the rooting process.

The authors did not find any reports in literature about thickness and fresh and dry weight of main root. Given the fact that the lowest thickness of roots, and their fresh and dry weight were obtained in control treatment and showed an increasing (yet insignificant) trend with increasing concentration of IBA. aThe report of Wahab *et al.* (2001) about the effect of IBA on increasing fresh weight of guava root confirms the present results.

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