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Effect of different concentrations of plant growth regulators on micropropagation of *Lantana camara*

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***Lantana camara* is a plant with numerous medicinal properties and belongs to Verbenaceae family. Too few studies on micropropagation of this plant exist in the literature. This study was designed and conducted to investigate the effective factors on *in vitro* shoot proliferation of *L. camara*. This experiment was conducted in Isfahan University of Technology, Isfahan, Iran. In this study, nodal segments of *L. camara* were used as explants and woody plant medium (WPM) as culture medium. The explants were placed in culture medium of WPM containing 6-benzyladenine (BA) and thidiazuron (TDZ) in various concentrations for shoot proliferation and MS medium containing different concentrations of indole-3-acetic acid (IAA) for rooting. After four weeks, different indices of shoot proliferation and rooting were investigated. The experiments' data were analyzed by Statistical Analysis System (SAS) software and the means were compared using Duncan's multiple range test (DMRT) at 5% probability level. The results indicated that the highest shoot and internode length, fresh and dry weight and the maximum number of leaves were obtained in control treatment. The maximum number of shoots was observed in 8 mg/L BA and the highest fresh weight was in 4 mg/L BA. Cytokinins, particularly BA, in high concentrations cause apical dominance to be overcome through declining auxin effect, and proliferation was increased. Therefore, shoot and internode length was decreased with increasing BA concentration. The most number of roots was also obtained in treatment with 0.5 mg/l IAA. The increase in roots' number occurs because of hydrolysis of foods and their transfer to the sprouts.**

Key words: Nodal segments, shoot proliferation, thidiazuron, woody plant medium.

INTRODUCTION

Lantana camara is an evergreen shrub and has heart shaped leaves with rough surface and serrated leaves. Tropical and subtropical regions are suitable for *L. camara* growth. In traditional medicine, it is used for treating leprosy, influenza, asthma, bronchitis and some other diseases. Antifungal, antibacterial, insecticidal and

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anticancer activity of *L*. *camara* secondary metabolites has been demonstrated by different researchers (Srivastava et al., 2011; Affonso et al., 2007; Passos et al., 2009). The germination percentage of *L. camara* seeds is very low. Layering and cutting are other ways of propagation of *L. camara* which have their own problems; such that a few plants were produced with these propagules. Micropropagation is one of the ways to mass propagate of plants and much study about *L. camara* has not been done. This experiment was conducted to study the effects of various concentrations of growth regulators to increase proliferation and rooting ability of *L. camara* for medicinal use.

Affonso et al. (2007) used Murashige and Skoog (MS) medium for *L*. *camara* micropropagation and reported 4.4 µmol/L 6-benzyladenine (BA), accompanied with 0.44 µmol/L thidiazuron (TDZ), which caused a significant decrease in shoot length and root formation, but 4.4 µmol/L BA alone caused increase in shoot and nodes number of each explant. In a research, woody plant medium (WPM) was used for *Ulmus parvifolia* Jacq. And it was reported that using 0.5 mg/L BA in this medium was suitable for growing and propagating the shoots obtained from nodal segments of this plant. Also, 0.5 mg/L TDZ and 2 mg/L N-(2-chloro-4-pyridyl)-N-phenylurea (4-CPPU) caused increase in nodal development in this plant. WPM containing 1 mg/L 1-naphthalene acetic acid and sucrose is the best treatment for rooting of this plant (Thakur and Karnosky, 2007).

A research on *in vivo* culture of a guava species offered woody plant medium (WPM) supplemented with 2 mg/L BA as the best medium for shoot induction while WPM with 1 mg/L BA was suitable for proliferation and shoot length, and the effect of MS, WPM, 1/2 MS and B5, as culture media, was found to be significant on shoot quality (Meghwal et al., 2003). Babu et al. (2003) used WPM culture containing active charcoal and various auxin and cytokinin hormones for *in vitro* proliferation of *Cinnamomum camphora*.

Direct shoot regeneration from nodal segments, internode, hypocotyl and embryo of *Withania somnifera* was investigated in MS medium. The required concentration and the type of cytokinin is different and dependent on the type of the explant: nodal segments in BA (0.1 to 5.0 mg/L) and TDZ (0.2 and 0.3 mg/L), internode explants in BA (1 and 5 mg/L), hypocotyl explants in BA (0.5 mg/L) and embryonic explants in TDZ (0.2 and 0.3 mg/L) generated shoot. The generated shoots, rooted in MS medium containing BA (0.01 mg/L) or 1/2 MS with no plant growth regulators (Kulkarni et al., 2000).

For *Vitex trifolia* treated with BA, Kin, 2-iP, TDZ and adenine, the highest number of shoots in each explants (nodal segments) was reported for treatment with 5 mg/L BA in MS medium (Hiregoudar et al., 2006). For *Tectona grandis*, the best regeneration of calli was obtained from internodes in MS medium containing 10 mg/L BA and 1 mg/L gibberellic acid. For proliferation of the generated shoots, they were subcultured in MS medium containing 10 mg/L BAP (Widiyanto et al., 2005). In a study, the effect of indole-3-acetic acid (IAA) and indole-3-butyric acid (IBA) was investigated on root propagation of Gisela 5' from *Prunus* genus, reporting that IBA inhibited callus formation in initial days of root formation and produced stronger roots in larger numbers. Both growth regulators caused the increase in rooting percentage as compared to the control treatment (Stefanicic et al., 2005). The treatment with 0.5 mg/L IAA has been offered as an appropriate treatment for *Digitalis lamarckii* rooting (Verma et al., 2011). Rathore et al. (2008) reported that 1 mg/L IBA was the most appropriate treatment for rooting of *Terminalia bellerica*.

MATERIALS AND METHODS

This experiment was conducted in the Horticultural Sciences Department of Isfahan University of Technology, Isfahan, Iran. Plant material was supplied from Shahre Golha greenhouse of Isfahan. The nodal segments (10 mm in length) of the current year's shoots were excised and washed using dishwashing liquid in 2/1000 ratio and placed under running water for four hours. For disinfection of plant materials, 20% sodium hypochlorite for twenty minutes and 70% alcohol for one minute were used and then explants were rinsed three to six times using sterile distilled water. After cutting the damaged parts caused by the disinfectant solution using sterile scalpel, the single buds were cultured in the medium as explants. To identify the best medium for proliferation, WPM containing different concentrations of TDZ and BA (0, 2, 4 and 8 mg/L) and to find the best medium for rooting, MS containing different concentrations of IAA (0, 0.25 and 0.5 mg/L) was used. The culture was kept at 24°C and 1000-lux light for 30 days. To assess proliferation in different treatments, the number and length of shoots, number of nodes, internode length, number of leaves, fresh and dry weight of shoots were measured at the end of period; also, the number of primary and secondary roots, root length, primary root diameter, fresh and dry weight of roots were measured at the end of period to assess rooting. The proliferation experiments were based on a factorial design with 2 factors: type of cytokinin with 2 levels (BA and TDZ) and concentration of cytokinin with 4 levels (0, 2, 4 and 8 mg/L) and the rooting experiments were conducted on a completely randomized design. These experiments were conducted with 3 replications and 3 observations for each replication. The experiments' data were analyzed by SAS (version 9.1) software and the means were compared using Duncan's multiple range test (DMRT) at 5% probability level.

RESULTS AND DISCUSSION

Shoot proliferation

As shown in Table 1, BA caused increase in number of shoots and some shoot proliferation factors, but TDZ acted as an inhibitory agent in all factors. The most number

Table 1. Comparison of mean effect of different BA concentrations on proliferation indices.

PGRs type	Concentration of PGRs (mg/l)	Number of shoots	Shoot length	Number of nodes	Internode length	Number of leaves	Fresh weight	Dry weight
BAP	0	2.22[c*]	1.04[a]	3.11[a]	0.33[a]	7.52[a]	0.46[b]	0.08[a]
	2	4.22[b]	0. 8[b]	2.90[b]	0.27[b]	4.46[c]	0.47[b]	0.05[c]
	4	4.44[b]	0.61[c]	2.57[c]	0.23[c]	4.80[b]	0.50[a]	0.06[b]
	8	4.77[a]	0.60[c]	3.13[a]	0.19[e]	7.45[a]	0.41[c]	0.04[d]
TDZ	0	2.22[c]	1.04[a]	3.11[a]	0.33[a]	7.52[a]	0.46[b]	0.08[a]
	2	1.00[d]	0.25[d]	1.16[d]	0.21[d]	3.33[d]	0.02[d]	0.002[e]
	4	0.46[e]	0.14[e]	0.91[e]	0.13[f]	1.63[f]	0.01[d]	0.001[f]
	8	0.45[e]	0.13[e]	0.83[f]	0.12[f]	2.31[e]	0.01[d]	0.001[f]

*In each column dissimilar letters indicate significant difference at 5% level.

of shoots and nodes was noted in 8 mg/L BA treatment, the highest shoot length, internode length, the highest dry weight and the most number of leaves was observed in control treatment and the highest fresh weight in 4 mg/L BA treatment. The least value of the indices was seen in 8 mg/L TDZ treatment. Cytokinins in appropriate concentration caused a considerable increase in DNA and RNA and subsequently increase in protein synthesis (Mok and Mok, 2001). Elimination of lateral bud dormancy, induction of adventitious bud formation, lateral bud growth and control of cell division cycle are some other functions of cytokinins inside the plant (Gaspar et al., 2003). Cytokinins, particularly BA, in high concentrations cause apical dominance to be overcome through declining auxin effect. This could be one of the reasons for decreased length of shoot and internode through increasing in BA concentration in the present study. On the other hand, the competition among shoots for nutrients could be the reason for decreased shoot growth as BA concentration increases.

The findings of this study are consistent with those of others, indicating that the difference in BA concentration could be due to genetic factors and laboratory conditions. Throughout some research conducted by Affonso et al. (2007) on *L*. *camara*, introduction of 4.4 µmol/L BA caused increase in the number of shoots and nodes per explant. On the other hand, addition of 4.4 µmol/L BA and 0.44 µmol/L TDZ into MS culture medium caused a significant decrease in length of shoots and formation of the roots, indicating the importance of BA in inducing proliferation and cellular division in the cultured explants and formation of limbs in BA-treated tissues. 5 and 10 mg/L BA caused increase in number of shoots in *Adhatoda vasica* after two weeks but inhibited the growth of explants (Abhyankar and Reddy, 2007).

Dziedzic (2008) employed lateral buds of *Lonicera caerulea in vivo* and reported that the fresh weight obtained from the growth of explants and the number of formed shoots in culture medium depended on the plant genotype, the type of culture medium and BA concentration. One of the effects by cytokinins is cellular division and the young, proliferated cells have a large vacuole and contain lots of water (Opik and Rolfe, 2005). Increase in BA concentration causes increase in cellular division and since these cells do not grow and do not possess the remaining thick wall, their water is evaporated throughout drying process and hence dry weight declines.

Several researchers have used very low concentrations of TDZ for woody plants' proliferation (Rai, 2002; Sharma and Shahzad, 2008). The type and condition of the explant, the domestic hormones' levels and growth regulators' application and interactions with healthy tissues are among the factors contributing to *in vivo* regeneration and shoot generation (Lakshmi et al., 2010; Kesari et al., 2012). Lateral buds naturally have large amounts of cytokinin (because of the cytokinin accumulation made in the root and moving upward) (Zulfiqar et al., 2009). On the other hand, TDZ effect could be attributed to its ability to induce cytokinin accumulation inside the tissue (Victor et al., 1999). Large amounts of endogenous cytokinin in lateral buds of *L*. *camara* and its interaction with TDZ could explain the TDZ inhibitory effect on *L*. *camara* proliferation in the present work. According to the reports by some researchers, TDZ is not appropriate in some plants for rooting and causes highly decreased length in shoots in high concentrations. Use of TDZ in guava micro-propagation caused short, ungrown shoots accompanied with yellow leaves, exacerbated as TDZ concentration increased (Ning et al., 2007; Techado and Lim, 2000). The results of the present study are in agreement with these reports.

Rooting

In this experiment, all IAA concentrations caused increase in rooting index, although this was significant in

Table 2. Comparison of mean effect of different IAA concentrations on proliferation indices.

IAA concentration (mg/l)	Number of primary roots	Number of secondary root	Primary root length	Primary root diameter	Fresh weight	Dry weight
0	0.33[c]	2.22[a]	3.42[b]	0.29[a]	0.038[a]	0.004[a]
0.25	1.00[b]	6.11[a]	3.76[b]	0.62[a]	0.079[a]	0.007[a]
0.5	1.66[a]	9.33[a]	9.39[a]	0.88[a]	0.088[a]	0.026[a]

In each column dissimilar letters indicate significant difference at 5% level.

only some of the indices (Table 2). IAA is necessary in central cylinder to initiate cellular proliferation in the surrounding circle. IAA is necessary to progress cellular proliferation and maintain biological ability of the cells in growing lateral roots (Taiz and Zeiger, 2010). Several reports have appeared on IAA effect on increased number of roots (Stefanicic et al., 2005; Janarthanam et al., 2012). As IAA concentration increased, the number and length of root increased in lemon. The increase in roots' number occurs because of hydrolysis of foods and transferring them to sprouts (Thayamini and Umadevi, 2011). This could be the reason for increased number of primary and secondary roots as IAA concentration increases. In *L*. *camara*, the highest percentage of rooting was obtained in 0.44 µmol/L concentration of IAA (Affonso et al., 2000).

Conclusion

The highest proliferation were obtained with 8 mg/L BA while the highest shoot and internode length, fresh and dry weight and the maximum number of leaves were obtained in control treatment and the highest fresh weight was in 4 mg/L BA. The studies revealed that high concentrations of BA cause plants to overcome the apical dominance by declining IAA effects; thus, proliferation was increased but shoot and internode length was decreased. In this study, the most number of roots was also obtained in MS medium supplemented with 0.5 mg/l IAA.

Conflict of interest

Authors declare that there are no conflicts of interests

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