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In vitro shoot proliferation and rooting of *Lallemantia iberica* under Thidiazuron exertion

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ABSTRACT: An *in vitro* culture of *Lallemantia iberica* was established for determination of optimum period for shoot production; for this purpose, the effect of thidiazuron (TDZ), as a cytokinin-like phenylurea compound, was investigated on *in vitro* shoot production and growth of *L. iberica* during every two weeks until six weeks of culture. TDZ in different concentrations (0.22-2 mg/l) was used as a supplement to the MS medium and nodal segments were employed as explants. Based on second and forth weeks investigation, low concentrations of TDZ could improve *in vitro* plant growth and 0.88 mg/l TDZ was found to be best productive for shoot (5.71±0.291), node (10.46±1.310) and leave (21.63±2.339) within four weeks, but in the third stage (6th weeks), TDZ had no effective function in comparison to control treatments. Based on the results obtained, increasing TDZ concentration decreased rooting percentage for *Lallemantia iberica.* The best rooting percentage (83.3±16.6%) was observed for free PGRs medium (control).

***Keywords*:** Growth parameters, *Lallemantia iberica*, Shoot culture, TDZ, MS medium

INTRODUCTION

Lallemantia iberica member of Lamiaceae family, commonly known as Iberican dragons head or lions head, is a high oil (~ 30%) producing plant (Samadi et al., 2007) and also it contains a high content of the valuable omega 3 fatty acids (Gunstone and harwood, 2007). In many Asian regions, *Lallemantia iberica* seeds have traditional uses as a stimulant, reconstitute, expectorant and diuretic (Samadi et al., 2007). Seed mucilage of *L. iberica* is using in treatment of several diseases such as nervous, hepatica and renal diseases (Amanzadeh et al., 2011). This plant grows well in dry areas and requires a light (sandy) or medium (loamy) soils and can not grow in shade (Strasil and Kas, 2005). According to literature, environmental conditions can affect productivity and yield of *Lallemantia iberica* (Badawy et al., 2013; Strasil and Kas, 2005).

Plant cell, tissue and organ culture is a suitable alternative method to conservation of many valuable medicinal plant species and prepare sufficient amount of these plants within short time. Thus this technique is often an effective system for plant cloning in controlled conditions (Zuzart et al., 2010). With these systems, *in vitro* cultured plants can be used for a variety of researches (Mendes et al., 2012).

During the last few decades, thidiazuron (N-phenyl-1, 2, 3-thidiazol-5-yl urea) has been used as a plant growth regulator to induce organogenesis in many plant species (Cuenca et al., 2000; Corredoira et al., 2008; Sriskandaray and Lundquist, 2009; Sefasi et al., 2013). The stimulatory effect of TDZ on shoot induction and multiple shooting has been reported by researchers in several plant species (Mukhtar et al., 2012). Thidiazuron (TDZ) as a well-known compound for regeneration of plants has cytokinin-like activity or it may induce the

endogenous cytokinin activity in optimal levels but its application as over exposure can have negative effects (Huetteman and Preece, 1993).

In vitro shoot regeneration of *Lallemantia iberica* has been carried out using different concentrations of 6-Benzylaminopurine by Ozdemir et al., (2014) for the first time; also, the effects of different plant growth regulators on secondary metabolites production in *L. iberica* were reported in our recent article (Pourebad et al., 2015). Because of some problems to in vivo culture of *L. iberica* as described above, it is necessary to develop an efficient and improved protocol for prepare sufficient amount of productivity and yield of this plant species. Hence, the present study was undertaken to note the effect of different concentrations of TDZ as a potent and alternative cytokinin source on shoot production and growth behavior of *L. iberica* in three periods to determine the optimum period for maximum shoot production. No report is available on the study of in vitro growth of *L. iberica* in different period's influence of TDZ concentrations.

MATERIALS AND METHODS

Plant material and in vitro culture

The seeds of *Lallemantia iberica* were obtained from the collection of medicinal plants seeds in Department of Plant Eco-Physiology (Faculty of Agriculture, University of Tabriz, Iran). The seeds were surface sterilized by immersion in 70% ethanol for three minutes and 20% sodium hypochlorite for 15 minutes. Following rinsing in sterile distilled water, the seeds were cultured on hormone-free MS medium (Murashige and Skoog, 1962). The pH of all media was adjusted to 5.6-5.8 with NaOH prior autoclaving at 121°C for 20 minute. Cultures were maintained in a growth chamber at 24±1°C under a 16h photoperiod, with light provided by cool fluorescent lamps.

Shoot multiplication

After 20 days of culture, nodal segments of the *in vitro* plantlets were cultured for shoot proliferation on MS medium containing different concentrations of Thidiazuron (0, 0.22, 0.44, 0.66, 0.88, 1.1, 2 mg/l). The cultures were placed in a growth chamber at 24±1°C. The number of shoot, node and leaves were determined every two weeks for a six-week period. In addition, the rooting percentage was investigated for all treatments after six weeks of culture.

Statistical analysis

This experiment was set up in a randomized complete block design for three replicates with 8 explants per treatment. One-way analysis of variance (ANOVA) was used to process the data. The significance level was fixed at $\alpha=0.05$

RESULTS AND DISCUSSION

The effect of TDZ on some growth parameters during shoot multiplication

We made an attempt to determine the optimum period for maximum shoot production by TDZ concentrations in different periods (2, 4 and 6 weeks). During the culture time, plantlets increased their shoot, node and leave numbers under all treatments. The quantities of these parameters were recorded in three different times for all treatments during six weeks. In the first phase (two weeks after culture) and second phase (four weeks after culture), low concentrations of TDZ were more efficient (Tables 1, 2 and 3). These results were supported by some other reports which showed that low levels of TDZ have been useful for shoot proliferation or organogenesis (Huettman and preece 1993; Upeti and Dhar 1996; Fasial et al., 2005; Grabkowska et al., 2014). The higher number of shoot, node and leaves were observed at TDZ concentrations of 0.22 and 0.88 mg/l in the first and second recorded times, but as TDZ concentrations increased (0.88-2 mg/l), the number of these growth parameters decreased which these results are in accordance with Faisal and Anis (2006); reports that high shoot number produced in low concentrations of TDZ. These results are according to Mukhtar et al., (2012) results for *Clitoria ternatea* and also can be in agreement with Verma and Bansal (2014); for *Hedychium coronarium*. In the present investigation, low concentrations of TDZ could increase *in vitro* response and 0.88 mg/l TDZ was found to be best productive for shoot (5.71±0.291), node (10.46±1.310) and leave (21.63±2.339) within four weeks. Thidiazuron (TDZ, N-phenyl-N'-1, 2, 3-thiadiazol-5-ylurea) with high cytokinin-like activity is more efficient for shoot proliferation in a number of plant species, especially the members of Lamiaceae family (Skala and Wysokinska, 2004). The stimulatory role of cytokinin on the morphogenesis might be due to the transport of endogenous metabolites (Liu et al., 2003). In the third time (six weeks after culture), TDZ treatments had no significant difference with control treatments for shoot, node and leave production, but in general the amount of theses parameters decreased for TDZ treatments in comparison with control. These results are in agreement with the

findings of Sajid and Aftab (2009); for *Solanum tubersum*. In this research, it was observed that the prolonged cultures on media containing TDZ resulted in low growth frequencies with low shoot, node and leave number. This is in accordance with Faisal et al., (2005) results for *Rauvolfia tetraphylla* that continued presence of TDZ in media culture can have deleterious effect on the growth. According to previous reports, TDZ has high stability in culture media and can resist to cytokinin oxidase (Mok et al., 1987; Murtiy et al., 1995), even though, long exposure to TDZ can disturb plant growth in media cultures (Lu, 1993).

Effect of TDZ on in vitro rooting

After sixth week of culture, rooting percentage was determined for all treatments. The best rooting percentage (83.3±16.6%) was obtained on free PGRs medium (control), while increasing TDZ concentration decreased this parameter (Fig 1). Our results on the negative effects of TDZ on rooting confirmed some other observations (Singh et al., 2001; Grabkowska et al., 2014). It has been suggested that thidiazuron can induce endogenous ethylene production (Suttle, 1985), which this may cause difficulty in rooting.

CONCLUSION

Our present investigation shows that low concentrations of TDZ can be useful for *in vitro* plant growth in first weeks of culture for *Lallemantia iberica*. However, prolonged exposure to TDZ has negative effects on morphological characteristics of plants specially shoot production and other growth parameters such as leave and root formation, which high stability of TDZ in culture media and resistance to cytokinin oxidase may cause difficulty in growth.

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Table 1. Effect of TDZ on shoot number in during three different recorded times

TDZ treatments (mg/l)	S1	S2	S3
Control	1.46±0.083^{c}	3.04±0.151^{c}	7.42±2.190^{a}
0.22	2.88±0.450^{a}	5.08±0.712^{ab}	5.25±0.782^{a}
0.44	2.13±0.000^{b}	3.84±0.400^{bc}	5.33±1.012^{a}
0.66	2.29±0.109^{ab}	3.79±0.479^{bc}	3.99±0.761^{a}
0.88	2.34±0.151^{ab}	5.71±0.291^{a}	4.79±1.432^{a}
1.1	2.00±0.072^{bc}	4.34±0.615^{abc}	4.50±0.761^{a}
2	2.08±0.083^{b}	3.80±0.795^{bc}	4.62±1.442^{a}

Values represent means ± SE. Means followed by the same letter within columns are not significantly different by the Duncan's comparison mean test at 5 % probability level. (S1: 2 weeks, S2: 4weeks, S3: 6 weeks)

Table 2. Effect of TDZ on leaves number in during three different recorded times

TDZ treatments (mg/l)	S1	S2	S3
Control	5.00±0.361^{d}	14.75±0.813^{ab}	35.00±8.663^{a}
0.22	11.42±1.235^{a}	17.88±1.772^{ab}	21.58±2.829^{ab}
0.44	8.17±0.357^{bc}	16.79±1.325^{ab}	27.17±0. 726^{ab}
0.66	8.17±0.220^{bc}	13.83±2.205^{b}	15.50±3.431^{b}
0.88	10.04±0.736^{ab}	21.63±2.339^{a}	20.83±5.703^{ab}
1.1	8.25±1.155^{bc}	16.88±2.292^{ab}	19.25±3.826^{ab}
2	7.08±0.464^{cd}	14.17±3.196^{b}	20.83±7.021^{ab}

Values represent means ± SE. Means followed by the same letter within columns are not significantly different by the Duncan's comparison mean test at 5 % probability level. (S1: 2 weeks, S2: 4weeks, S3: 6 weeks)

Table 3. Effect of TDZ on node number in during three different recorded times

TDZ treatments (mg/l)	S1	S2	S3
Control	2.59±0.183^{d}	7.29±0. 292^{a}	21.66±5.741^{a}
0.22	5.67±0.546^{a}	8.46±1.022^{a}	8.75±1.064^{a}
0.44	4.13±0.190^{bc}	8.42±0. 738^{a}	15.08±4.357^{a}
0.66	4.05±0.083^{bc}	6.79±1.099^{a}	11.37±4.719^{a}
0.88	5.00±0.440^{ab}	10.46±1.310^{a}	14.29±5.430^{a}
1.1	4.13±0.577^{bc}	8.05±1.294^{a}	10.16±1.924^{a}
2	3.54±0.231^{cd}	6.96±1.590^{a}	13.46±5.735^{a}

Values represent means ± SE. Means followed by the same letter within columns are not significantly different by the Duncan's comparison mean test at 5 % probability level. (S1: 2 weeks, S2: 4weeks, S3: 6 weeks)

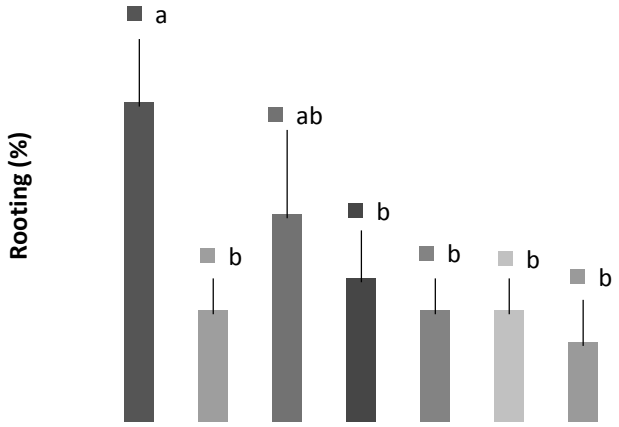

Fig 1 Effect of different concentrations of TDZ (mg/l) on *in vitro* rooting for *Lallemantia iberica*

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