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In vitro shoot proliferation of *Passiflora caerulea* L. via cotyledonary node and shoot tip explants

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Abstract

Passiflora caerulea L. is a herbaceous climber that belongs to the Passifloraceae family. One of the most important techniques used in plant biotechnology is tissue culture, which allows for the mass production of pathogen-free plants. Cotyledonary nodes have a great potential for shoot proliferation; however, to the best of our knowledge there are no reports regarding plant regeneration from cotyledonary nodes of *P. caerulea.* Therefore, this study aimed to evaluate the potential of two different types of explants (shoot tips and cotyledonary nodes) to obtain shoot multiplication of *P. caerulea*. Various concentrations of 6-benzylaminopurine (BAP) (0.5, 1, and 1.5 mg/l), 6-furfurylaminopurine (kinetin, KIN) (1 and 2 mg/l), and thidiazuron (TDZ) (0.25, 0.5, and 1 mg/l) in combination with indole butyric acid (IBA) were used in a completely randomized design, in three replications. The results showed that the highest percentage of regeneration frequency (90%) and a maximum number of shoots (8.86) in cotyledonary node explants were obtained on MS medium supplemented with 1.5 mg/l BAP along with 0.15 mg/l IBA. Furthermore, in the shoot tip explants, the percentage of regeneration rate (96.66%) and the highest number of shoots (9.86) were obtained in the above-mentioned medium. In rooting experiments, the maximum rooting percentage (90%) was obtained on MS medium containing 1 mg/l IBA. *In vitro*-raised plantlets were placed in pots and were stored in soil under room temperature for 20 to 30 days before planting, and it showed more than 90% survival rate. Based on our results, the protocol described in this study has a high potential to be used in the micropropagation of this valuable plant.

Key words: acclimatization, *Passiflora caerulea*, plant growth regulator, regeneration, rooting

Introduction

In vitro propagation of plants is a useful and efficient technique that allows for the mass production of virus-free plants. Tissue and organ culture has been widely used for mass production (Hesami and Daneshvar, 2016). With respect to the application of tissue culture techniques in the propagation of ornamental and medicinal plants, most studies have focused on two goals: mass production of plants and the production of disease-free plants (Yang et al., 2010). Indirect shoot organogenesis, as one of the methods of plant tissue culture, has some disadvantages: it is a time-consuming process, and it creates wide somaclonal varieties with morphological abnormalities. However, direct shoot organogenesis is an efficient method for the micropropagation because of its high potentiality of production of a large number of plants that are genetically identical to the mother plant, without any genetic instability, in a short time, (Lee and Phillips, 1987; Siwach and Gill, 2011). Shoot multiplication is affected by many factors such as plant species, growth medium, minerals, organic matter, carbohydrates, plant growth regulators (PGRs), and environmental conditions. One of the most important factors is the effect of PGRs, for example, the type and concentration of auxins and cytokinins (Pati et al., 2006).

Passion flower (*Passiflora caerulea* L.) is a climbing, evergreen shrub or ivy (Montanher et al., 2007) that belongs to Passifloraceae family. *Passiflora* species are

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spread in tropical and warm temperate regions of the world (Jafari et al., 2016). They are also grown in the northern parts of Iran, but unfortunately, there is little information about the history of this plant in Iran (Mozaffarian, 1996). Passion flower propagation can be achieved through seeds, which may limit its large-scale production because of low seed germination and plant's susceptibility to viral pathogens such as passion fruit woodiness virus and cowpea aphid-borne mosaic virus (Nascimento et al., 2012). Tissue culture is an alternative technique to control viral diseases and produce plants with high genetic uniformity (Gioria et al., 2000). *In vitro* propagation of *P. caerulea* has been performed through direct organogenesis by some researchers (Ragavendran et al., 2012; Anand et al., 2012; Vieira et al., 2014). Direct organogenesis of other *Passiflora* species has also been reported by several researchers. For example, Anand and coworkers (2012) investigated direct organogenesis of *Passiflora foetida* using node explants. They reported that the highest percentage of shoot regeneration was obtained on Murashige and Skoog (MS) medium containing 2 mg/l 6-benzylaminopurine (BAP) along with 1 mg/l 6-furfurylaminopurine (kinetin, KIN). In another study, Shekhawat and coworkers (2015), reported that 100% regeneration of *Passiflora edulis* was achieved on MS medium containing 2 mg/l of BAP for node explants.

Considering the important role of micropropagation technique in regeneration protocols, one of the goals of studies on tissue culture is to provide convenient and efficient methods for rapid organogenesis and mass production of plants under *in vitro* conditions, which can provide appropriate ground for other research such as genetic engineering. Previous studies on various plants such as *Sterculia urens* (Purohit and Dave, 1996), *Dalbergia sissoo* (Pradhan et al., 1998), *Cassia sophera* (Parveen and Shahzad, 2010), and *Stereospermum suaveolens* (Darshini et al., 2014) have shown that cotyledonary node explants have a great potential for shoot proliferation. However, based on the available literature, to date, there is no report on shoot proliferation of passion flower using cotyledonary node explants. Therefore, in this study, we aimed to evaluate the potential of cotyledonary nodes and shoot tip explants on shoot proliferation of *P. caerulea* by using different concentrations of growth regulators.

Materials and methods

Plant material

This study was conducted at the Department of Horticulture Science, Ramin Agriculture and Natural Resources University, Khuzestan, Iran. At first, to eliminate surface contamination, seeds were washed for 30 min under tap water. The seeds were then surface sterilized, with 70% ethyl alcohol for 40 s and then dipped in 10% sodium hypochlorite solution for 10 min (with 5% active chlorine) under a laminar air flow chamber. Then, the seeds were washed thrice in sterilized distilled water, for 3-5 min each time. In order to achieve seed germination and production of seedlings free from pollution and in order to prepare explants, seeds, after being surface sterilized, were cultured in a one-tenth strength MS medium, and all cultures were incubated in a culture room at 25 ± 2°C in light of 65 μmol $m^{-2}s^{-1}$. Explants including shoot tips and cotyledonary node segments were prepared from *in vitro*-grown seedlings (Fig. 1A).

Effects of PGRs on shoot regeneration

Explants (shoot tips and cotyledonary node segments) were cut into sections of 5-7 mm in length and were placed horizontally in MS medium containing 0.5, 1, 1.5 mg/l BAP; 1 and 2 mg/l KN; or 0.25, 0.5, and 1 mg/l thidiazuron (TDZ) in combination with indole butyric acid (IBA). Cytokinin to IBA ratio in the medium was 10:1. The basic culture medium consisted of MS medium (1962) fortified with 30 g/l sucrose as a source of carbohydrates and 7 g/l agar used to solidify the medium. Before adding agar, the pH of the medium was adjusted to 5.8. Glasswares containing the medium were disinfected using an autoclave at a temperature of 121.5°C and pressure of 15 psi for 20 min. After placing the explants in the medium, culture dishes were maintained in a growth chamber at 25 ± 2°C under 16 h photoperiod. The light intensity was 65 μmol $m^{-2}s^{-1}$. Parameters including regeneration rate, number, and length of shoots were recorded after 6 weeks of growth.

Root formation and acclimatization

After the stage of organogenesis, shoots formed from cotyledonary node explants (2 to 3 cm) were used for rooting stage. Modified MS medium with IBA,

1-naphthaleneacetic acid (NAA), or indole-3-acetic acid (IAA) at 0.5, 1, and 2 mg/l and medium without PGRs (control) were used for rooting the shoots. Parameters, including rooting frequency, number, and length of roots were recorded after 5 weeks of growth. Plantlets with desirable shoots and leaves as well as strong roots were transferred to sterile pots containing perlite mixed with cocopeat (ratio 1:1) for acclimatization purposes and were kept in a growth chamber for 20 to 30 days. They were finally transferred to a greenhouse and the survival rate of plantlets was recorded.

Statistical analysis

All experiments were performed with completely randomized design in three replications (each replication consisted of 10 samples). Statistical analysis of these tests was performed using the SAS (9.3) software and mean comparison was tested with Duncan's multiple range test ($P < 0.05$).

Results and discussion

Effects of PGRs on shoot regeneration

Nowadays, tissue culture is considered by ornamental and medicinal plants growers as a technique for rapid and homogeneous production of high-quality plants as well as the production of pathogen-free plants at a commercial level (Sammaiah et al., 2011). Production of lateral shoots is one of the parameters that is important in tissue culture because lateral shoots can be separated in the subculture stage and each one can be cultured as a separate sample, thereby increasing the proliferation speed. Regeneration is an extremely complex process, affected by multiple qualitative and quantitative factors such as genotype, culture medium, PGRs (cytokinins and auxins), agar, type, size, age, the position of explants, and duration of light intensity (Gattuso et al., 2003). Exogenous PGRs can stimulate cell division, cell growth, and cell differentiation in the medium. So far, few studies have been reported in the field of direct regeneration and effects of different concentrations of PGRs on micropropagation of *P. caerulea* (Busilacchi et al., 2008; Ozarowski et al., 2013; Prithrivaj et al., 2015); there are also several reports on other species of this genus, such as *P. foetida* (Anand et al., 2012), *Passiflora setacea* (Vieira et al., 2014), *P. edulis*) Nhut et al., 2007) and *Passiflora cincinnata* (Da Silva et al., 2011). Shoot proliferation protocol via cotyledonary nodes and shoot tip segments as explants presented in this study, has important advantages such as large-scale production in a short period of time. In this study, type and concentration of cytokinins (BAP, KIN, and TDZ) in combination with an auxin (IBA) gave different effects on shoot proliferation of cotyledonary node and shoot tip explants (Table 1). After 6 weeks of culturing *P. caerulea* explants in different media, the explants showed different rate of regeneration. The presented results showed that the highest regeneration rate of cotyledonary node explants of *P. caerulea* (90%) and the highest number of shoots (8.86) were achieved in MS medium supplemented with 1.5 mg/l BAP along with 0.15 mg/l IBA (Fig. 1B), which showed a significant difference compared with other treatments. No regeneration response was recorded in the explants cultured on basal MS medium without PGRs (control treatment). Furthermore, the presented results of the effect of different concentrations of growth regulators on the regeneration of shoot tip explants of *P. caerulea* after 6 weeks of incubation showed that the highest regeneration rate (96.66%), as well as the highest number of shoots (9.86), was obtained on MS medium containing 1.5 mg/l BAP and 0.15 mg/l IBA (Fig. 1C), which showed a significant difference when compared with other treatment conditions. No response was observed in control medium (Table 1). This test showed that the number of shoots and the regeneration rate increased in the shoot tip and cotyledonary node explants with an increase in the BAP concentration from 0.5 to 1.5 mg/l. But the regeneration rate and the number of shoots were significantly reduced when the concentration of TDZ hormone increased from 0.25 to 1.0 mg/l (Table 1). Similar to our results, Ning and coworkers (2007) reported that the number of shoots per explant of *Prunus mume* decreased by increasing TDZ concentration. In this study, the MS medium containing BAP in combination with IBA was the best treatment for regenerating both explant types (cotyledonary nodes and shoot tip segments). These results are in line with the findings of Ragavendran and coworkers (2012) who showed that BAP compared with TDZ and KIN is more effective in explant regeneration. Furthermore, Anand and coworkers (2012) reported that the maximum shoot regeneration of *P. foetida* via nodal segments was observed in MS medium supplemented with 1.5 mg/l BAP and 0.5 mg/l NAA. In case of

Table 1. Effect of different plant growth regulators in MS medium on morphogenic response of *Passiflora caerulea* from shoot tip and cotyledonary node explants

Plant Growth Regulators [mg/l]				Shoot tips		Cotyledonary node	
BAP	TDZ	KIN	IBA	organogenesis frequency [%]	shoot number	organogenesis frequency [%]	shoot number
0	0	0	0	0.00 ^{h}	0.00 ^{g}	0.00 ^{g}	0.00 ^{i}
0.5	0	0	0.05	70.00 ^{c}	6.23 ^{c}	70.00 ^{b}	5.63 ^{c}
1.0	0	0	0.1	76.66 ^{b}	8.16 ^{b}	73.33 ^{b}	7.13 ^{b}
1.5	0	0	0.15	96.66 ^{a}	9.86 ^{a}	90.00 ^{a}	8.86 ^{a}
0	0.25	0	0.025	40.00 ^{e}	4.06 ^{e}	33.33 ^{d}	3.73 ^{f}
0	0.5	0	0.05	20.00 ^{g}	2.83 ^{f}	10.00 ^{f}	2.16 ^{h}
0	1.0	0	0.1	30.00 ^{f}	3.23 ^{f}	23.33 ^{e}	3.03 ^{g}
0	0	1.0	0.1	56.66 ^{d}	3.13 ^{f}	50.00 ^{c}	4.13 ^{e}
0	0	2.0	0.2	60.00 ^{d}	5.20 ^{d}	50.00 ^{c}	4.63 ^{d}

Note: MS – Murashige and Skoog; BAP – 6-aminobenzylpurine; KIN – 6-furfurylaminopurine; TDZ – thidiazuron; IBA – indole butyric acid. Mean comparison to the 5% level according to Duncan's multiple range test. Columns with common characters, no significant difference

studies performed by other researchers, BAP turned out to play an important role in the regeneration process, which is consistent with the results obtained in our experiments (Busilacchi et al., 2008; Ozarowski et al., 2013; Prithrivaj et al., 2015). The balance between auxins and cytokinins is an important morphogenic factor for the initial growth of shoots. The differentiation process is also regulated by the relative concentration of auxins and cytokinins in the growth medium (Murashige, 1980). Pasternak and coworkers (2000) reported that auxins are involved in DNA replication and cytokinins are necessary for plant cell division. In general, cytokinins play multiple roles in the control of plant development such as cell cycle in plant cells, initiation and lateral meristem activity, RNA and protein synthesis, and stimulation and activation of enzymes involved in the development of plants (Dobránszki and Da Silva, 2010). BAP can shift the apical dominance toward the growth of lateral buds, which in turn leads to cell division in the meristem cells and increased number of branches as well as increased cell division rate in lateral buds (Gomez-Leyva et al., 2008).

Effect of IBA, IAA, and NAA on root formation

Rooting is a crucial step in the success of micropropagation. Without effective root system, plant acclimatization would be difficult, and the rate of plant propagation might be severely affected. The process of root formation is influenced by a number of internal and external factors. Among the internal factors, the most important role is ascribed to phytohormones, especially the auxins (Techato and Lim, 2000). Auxin-type and concentration significantly influence the root formation. Auxins (IBA, NAA, and IAA) are needed only in the early stages of the emergence of new roots. They also affect the growth of newly formed roots (Dobránszki and Da Silva, 2010). In our experiments, individual shoots with 2-3 leaves, grown to 1-2 cm long, were separated from multiple shoots clump and inoculated to MS basal media containing different concentrations of IBA (0.5, 1, and 2 mg/l), NAA (0.5, 1, and 2 mg/l), IAA (0.5, 1, and 2 mg/l), and without growth regulators (control) for rooting. After 5 weeks of growth, the percentage of shoots forming roots, the number of roots per shoot, and the length of roots were recorded. The results of the effect of different PGRs on the formation of roots showed that the maximum percentage of rooting (90%) and the maximum number of roots (9.83) were observed in MS medium supplemented with 1.0 mg/l IBA (Fig. 1D). No rooting was observed on MS medium without PGRs (control) (Table 2). In this study, IAA or NAA were less effective than IBA in stimulating root formation. Likewise, Prithrivaj and coworkers (2015) reported that the highest percentage of root formation of *P. caerulea* was

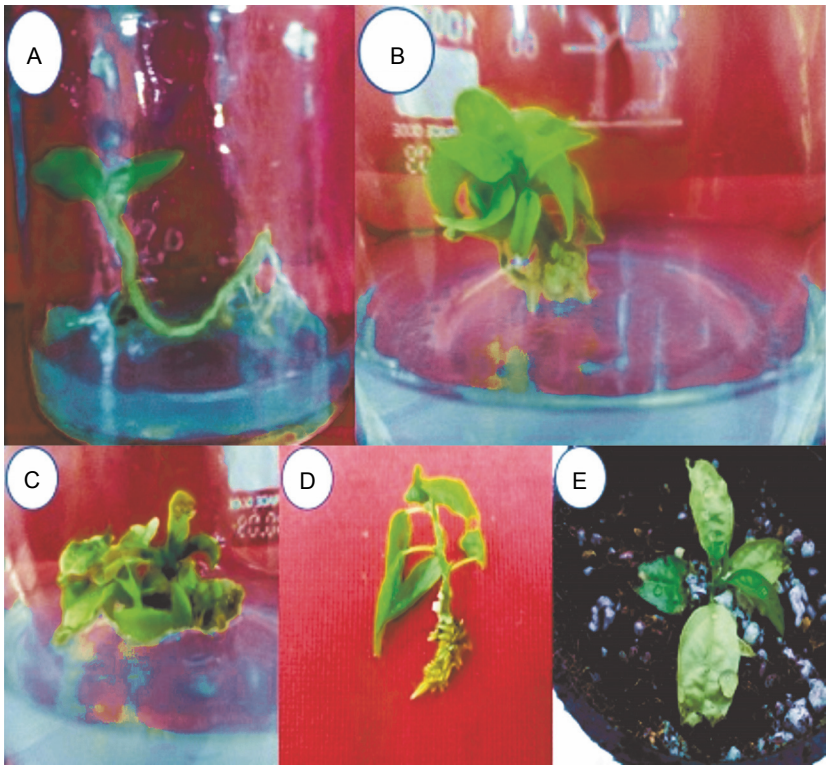

Fig. 1. Shoot proliferation of *P. caerulea* from shoot tips and nodal segments. A – *in vitro* seed germination of *P. caerulea*, B – induction of shoots in six weeks of culture from shoot tip explants, C – development and multiplication of shoots after six weeks of culture from cotyledonary node explant, D – induction of roots in two weeks of culture, E – acclimatized regenerated plants of 20 days old

observed on MS medium containing 0.5 mg/l IBA after 4 weeks of incubation. In other experiments, different concentrations of auxins were used in the root formation of *P. caerulea* (Busilacchi et al., 2008; Ozarowski et al., 2013; Prithrivaj et al., 2015). Ragavendran and coworkers (2012) and Anand and coworkers (2012) have reported that 1 and 0.5 mg/l IBA, respectively, were suitable for rooting of *P. foetida*. Bellamine and coworkers (1998) also reported that IBA is more appropriate than NAA and IAA in inducing the rooting process. IBA is more stable and less sensitive to the degradation and is slowly metabolized by the peroxidase enzyme; which can be one of possible causes for better effect of IBA compared to other auxins (Dobránszki and Da Silva, 2010). In general, auxin at low concentration favors root initiation, whereas at higher concentration induces callus formation (Kollmeier et al., 2000). Therefore, our study showed that the production of callus in the root formation stage can be increased exponentially by high concentrations of IBA, which in turn, can exert a negative impact on roots. Thus, high concentrations of IBA can weaken the roots and destroy a high percentage of plants in the acclimatization stage. Similar results were obtained by previous researchers. Kollemeier and coworkers (2000) reported that callus formation, at the end of the plantlet, can impair vascular connections between roots and shoots and can also prevent the absorption of water and nutrients. In order to sustain plant's life and increase its efficacy in various environmental conditions, it is necessary to gradually change rhizosphere in order for plants to withstand stresses (Gaspar et al., 1996; Ludwig-Muller, 2000). Thus, after 3 weeks, the plantlet was subcultured in the hormone-free medium for 2 weeks under the complete concentration of mineral elements. Subsequently, the newly formed roots were observed.

Acclimatization

The rooted plants with expanded leaves and well-developed roots were transferred to the pots containing perlite and cocopeat (1:1). Normal growth for the potted plants was observed after 25 to 30 days from the

Table 2. Effect of IAA, IBA and NAA on root induction in regenerated shoots of *Passiflora caerulea* on MS medium

Growth regulator	Percent of shoots producing roots [%]	Number of roots/shoot
Control (Free of PGRs)	0.00 ^{h}	0.00 ^{i}
0.5 mg/l IBA	50.00 ^{c}	7.10 ^{b}
1.0 mg/l IBA	90.00 ^{a}	9.83 ^{a}
2.0 mg/l IBA	60.00 ^{b}	6.26 ^{c}
0.5 mg/l NAA	50.00 ^{c}	3.93 ^{d}
1.0 mg/l NAA	40.00 ^{d}	3.20 ^{e}
2.0 mg/l NAA	33.33 ^{e}	2.13 ^{f}
0.5 mg/l IAA	20.00 ^{f}	1.60 ^{g}
1.0 mg/l IAA	20.00 ^{f}	1.23 ^{h}
2.0 mg/l IAA	13.33 ^{g}	1.06 ^{h}

Note: MS – Murashige and Skoog; BAP – 6-aminobenzylpurine; NAA – 1-naphthaleneacetic acid; IBA – indole butyric acid; PGRs – plant growth regulators. Mean comparison to the 5% level according to Duncan's multiple range test. Columns with common characters, no significant difference

transfer. After this period, the pots were transferred to a greenhouse, and 90% of the rooted plantlets survived (Fig. 1E), which is somewhat in agreement with other studies (e.g., Busilacchi and coworkers (2008), Ozarowski and coworkers (2013), and Prithrivaj and coworkers (2015)), where the authors used this method with different planting substratum for acclimatization. Da Silva and coworkers (2011) acclimatized tissue culture plantlets of *P. edulis* on a mixture of coconut fiber and Plantmax® (1 : 1) and obtained healthy plants.

Conclusions

The results of this study revealed that cotyledonary nodes and shoot tip explants had the highest regeneration rate in modified MS medium with 1.5 mg/l BAP along with 0.15 mg/l IBA. Furthermore, our overall results showed that IBA was more effective in root formation of *P. caerulea* when compared with NAA and IAA. The described proliferation protocol has significant advantages such as large-scale production in a short period of time and is considered not only as an aspect of *in vitro* propagation of *P. caerulea* but also as a suitable technique for breeding and gene transfer purposes.

References

Anand S.P., Jayakumar E., Jeyachandran R., Nandagobalan V., Doss A. (2012) *Direct organogenesis of Passiflora foetida L. through Nodal Explants*. Plant Tiss. Cult. Biotechnol. 22(1): 87-91.

Bellamine J., Penel C., Greppin H., Gaspar T. (1998) *Confirmation of the role of auxin and calcium in the late phases of adventitious root formation*. Plant Growth Regul. 26(3): 191-194.

Busilacchi H., Severin C., Gattuso M., Aguirre A., Di Sapio O., Gattuso S. (2008) *Field culture of micropropagated Passiflora caerulea L. histological and chemical studies*. Bol. Latinoam. Caribe. 7: 257-263.

Darshini R., Trivedi D.R., Joshi A.G. (2014) *In vitro shoot regeneration of Stereospermum suaveolens using cotyledonary node and nodal explants*. Plant Tissue Cult. Biotechnol. 24(2): 235-246.

Da Silva C.V., De Oliveira L.S., Loriato V.A.P., Da Silva L.C., De Campos J.M.S., Viccini L.F., Otoni W.C. (2011) *Organogenesis from root explants of commercial populations of Passiflora edulis Sims. and a wild passion fruit species, Passiflora cincinnata Masters*. Plant Cell Tiss. Organ. Cult. 107(3): 407-416.

Dobránszki J., Da Silva T.J.A. (2010) *Micropropagation of an apple a review*. Biotechnol. Adv. 28: 462-488.

Gaspar T.H., Kevers C., Penel C., Greppin H., Reid D.M., Thorpe T.A. (1996) *Plant hormones and plant growth*

regulators in plant tissue culture. In Vitro Cell. Dev. Biol. Plant. 32(4): 272-289.

Gattuso S., Severin C., Salinas A., Gattuso M., Giubileo G., Aguirre A., Busilacchi H. (2003) *Micropropagation of Passiflora caerulea L. and histological studies of tissue regeneration*. J. Trop. Medic. Plant. 4(2): 249-256.

Gioria R., Bosquę G.G., Rezende J.A.M., Amorim L., Kitajima E.W. (2000) *Incidencia de viroses de maracujazeiro na Alta Paulista – SP e danos causados pelo "Passion fruit woodiness virus"*. Fitopatol. Bras. 25: 182-189.

Gomez-Leyva J.F., Martinez-Acosta L.A., Lopez Muraira I.G., Silos-Espino H., Ramirez-Cervantes F., Andrade-Gonzalez I. (2008) *Multiple shoot regeneration of roselle (Hibiscus sabdariffa L.) from a shoot apex culture system*. Int. J. Bot. 4: 326-330.

Hesami M., Daneshvar M.H. (2016) *Development of a regeneration protocol through indirect organogenesis in Chenopodium quinoa Willd*. Indo-Am. J. Agric. Vet. Sci. 4(1): 25-32.

Jafari M., Daneshvar M.H., Lotfi A. (2016) *Control of in vitro contamination of Passiflora caerulea by using of sodium Hypochlorite*. Indo-Am. J. Agric. Vet. Sci. 4(2): 8-15.

Kollmeier M., Mafelle H.H., Horst W.J. (2000) *Is basipetal auxin flow Involved in inhibition of root elongation*. Plant Physiol. 122: 945-956.

Lee M., Phillips R.L. (1987) *Genomic rearrangements in maize induced by tissue culture*. Genome 29: 122-128.

Ludwig-Muller J. (2000) *Indole-3-butyric acid in plant growth and development*. Plant Growth Regul. 32: 219-230.

Montanher A.B., Zucolotto S.M., Schenkel E.P., Frode T.S. (2007) *Evidence of anti-inflammatory effects of Passiflora edulis in an inflammation model*. J. Ethnopharmacol. 109: 281-288.

Mozaffarian V.A. (1996) *Dictionary of Iranian Plant Names*. Tehran: Farhang Moaser Press.

Murashige T. (1980) *Plant growth substances in commercial uses of tissue culture*. [in:] *Growth Substances*. Ed. Skoog F., Berlin, Heidelberg: Springer-Verlag: 426-434.

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

Nascimento K.S., Ferreira K.T. (2012) *Estabelecimento in vitro tissue culture maracujá silvestre (Passiflora foetida L.)*. Rev. Bras. Plant. Med. 14: 138-142.

Nhut D.T., Khiet B.L.T., Thi N.N., Thuy D.T.T., Duy N., Hai N.T., Huyen P.X. (2007) *High-frequency shoot formation of yellow passion fruit (Passiflora edulis f. flavicarpa) via thin cell layer (TCL) technology*. [in:] *Protocols for Micropropagation of Woody Trees and Fruits*. Ed. Jain S.M., Häggman H., Berlin, Heidelberg: Springer-Verlag: 417-426.

Ning G., Bai S., Bao M., Liu L. (2007) *Factors affecting plantlet regeneration from in vitro cultured immature embryos and cotyledons of Prunus mume "Xue mei"*. In Vitro Cell. Dev. Biol. Plant. 43: 95-100.

Ozarowski M., Thiem B., Gryszczynska A., Budzianowski J. (2013) *Studies on in vitro seed germination and plant regeneration from the mature leaf, internodal and petiole explants of Passiflora caerulea L.* Bot. Sci. 23(6): 937-947.

Pasternak T., Miskolczi P., Ayaydin F., Mészáros T., Dudits D., Fehér A. (2000) *Exogenous auxin and cytokinin-dependent activation of CDKs and cell division in leaf protoplast-derived cells of alfalfa*. Plant Growth Regul. 32: 129-141.

Parveen S., Shahzad A. (2010) *TDZ-induced high-frequency shoot regeneration in Cassia sophera L. via cotyledonary node explants*. Physiol. Mol. Biol. Plants. 16(2): 201-206.

Pati P.K., Rath S.P., Sharma M., Sood A., Ahuja P.S. (2006) *In vitro propagation of rose – a review*. Biotechnol. Adv. 24(1): 94-114.

Purohit S.D., Dave A. (1996) *Micropropagation of Sterculia urens Roxb. An endangered tree species*. Plant Cell Rep. 15: 704-706.

Pradhan C., Kar S., Pattnaik S., Chand P.K. (1998) *Propagation of Dalbergia sissoo Roxb. through in vitro shoot proliferation from cotyledonary nodes*. Plant Cell Rep. 18: 122-126.

Prithviraj H.S., Hemanth K., Prakasha N.K., Shobha J. (2015) *An efficient in vitro regeneration of multiple shoots from leaf explant of passiflora caerulea L. an important medicinal plant*. Int. J. Recent. Sci. Res. 6(11): 7263-7265.

Ragavendran C., Kamalanathan D., Reena G., Natarajan D. (2012) *In vitro propagation of nodal and shoot tip explants of Passiflora foetida L. An exotic medicinal plant*. Asian. J. Plant. Sci. Res. 2: 707-711.

Sammaiah D., Shekar C., Goud M.J.P., Reddy K.J. (2011) *In vitro selection of Pesticide tolerance and regeneration of plantlets in Solanum melongena L.* J. Microbiol. Biotechnol. Res. 1(1): 66-70.

Shekhawat M.S., Manokari M., Ravindran C.P. (2015) *An improved micropropagation protocol by ex vitro rooting of Passiflora edulis sims. f. flavicarpa deg. through nodal segment culture*. Scientifica Doi/10.1155/2015/578676.

Siwach P., Gill A.R. (2011) *Enhanced shoot multiplication in Ficus religiosa L. in the presence of adenine sulfate, glutamine, and phloroglucinol*. Physiol. Mol. Biol. Plant. 17(3): 271-280.

Techato S., Lim M. (2000) *Improvement of mangosteen micropropagation through meristematic nodular callus formation from in vitro-derived leaf explants*. Sci. Hort. 86: 291-298.

Vieira L.M., Rocha D.I., Taquetti M.F., Da Silva L.C., De Campos J.M.S., Viccini L.F., Otoni, W.C. (2014) *In vitro plant regeneration of Passiflora setacea (Passifloraceae): the influence of explant type, growth regulators, and incubation conditions*. In Vitro Cell. Dev. Biol. Plant. 50(6): 738-745.

Yang J.L, Seong E.S, Kim M.J, Ghimire B.K, Kang W.H., Yu C.Y., Li C.H. (2010) *Direct somatic embryogenesis from pericycle cells of broccoli (Brassica oleracea L. var. italica) root explants*. Plant Cell Tiss. Organ. Cult. 100: 49-58.