BIHAREAN BIOLOGIST 6 (2): pp.73-76
Article No.: 121109

http://biozoojournals.3x.ro/bihbiol/index.html

The effects of explant type and phytohormones on African violet (*Saintpaulia ionantha*) micropropagation efficiency

Yousef GHASEMI, Ghorban Ali NEMATZADEH,
Valiollah Ghasemi OMRAN*, Ali DEHESTANI and Soghra HOSSEINI

Agricultural Genetics and Biotechnology Institute of Tabarestan, Sari Agricultural Sciences and Natural Resources University, Km 9 Darya Road, P.O. Box 578, Sari, Iran.
* Corresponding author, V.G. Omran, Telefax: +981513822715, E-mail: ghasemiomran@yahoo.com

Received: 18. September 2011 / Accepted: 24. April 2012 / Available online: 6. May 2012 / Printed: December 2012

Abstract. This research was conducted to study the effects of two explant types (leaf disc and petiole) and phytohormones including 5 levels of Benzyl Adenine (BA) (0, 0.2, 0.5, 1, 2 mgL^{-1}) and 2 levels of Indole Butyric Acid (IBA) (0 and 0.5 mgL^{-1}) onin vitro micropropagation of African violet (*Saintpaulia ionantha*). Based on primary data, experiments were performed with Murashige and Skoog`s basal media in factorial design was arranged in complete randomized blocks with four replications. Results showed that the number and length of adventitious shoots were higher in leaf discs compared with leaf petioles. It was also revealed that elimination of IBA as an auxin source decreased the regeneration efficiency and prolonged the time required for shoot regeneration. Generally, the highest numbers of adventitious shoots (80 shoots per explant) were observed on a medium containing 0.5 mgL^{-1} BA along with 0.5 mgL^{-1} IBA. Among the different hormonal combinations, the medium supplied with 0.2 mgL^{-1} Gibberellic Acid (GA_3) caused the maximum shoot elongation with an average length of 19.9 mm. The half strength MS medium containing 1 mgL^{-1} IBA lead to formation of normal plants with longer roots in micropropagated shoots. The rooted shoots were then acclimatized under high humidity (80-90% relative humidity) in a mixture of peat moss and perlite (3:1) for three weeks with a survival rate of about 95 %.

Key words: African violet, micropropagation efficiency, phytohormones, explant type.

Introduction

The industry of ornamental plants is growing worldwide and its monetary value has significantly increased over the last two decades promising a great potential for continued further growth in both domestic and international markets (Jain 2002). Major pot plants such as *Begonia*, *Ficus*, *Anthurium*, *Chrysanthemum*, *Rosa*, *Saintpaulia*, and *Spathiphyllum* are being produced in developed countries (Rout et al. 2006). Many commercial ornamental plants are being propagated by in vitro culture on the culture media containing auxins and cytokinins (Preil 2003, Rout & Jain 2004). Several different explants have been used for direct shoot formation from *Begonia*, *Cyclamen*, *Anthurium*, *Dracaena* and *Saintpaulia* (Rout et al. 2006). *Saintpaulia ionantha,* commonly known as African violet, is one of the most important commercial flowers that belong to the family Gesneriaceae. Some characteristics such as variation in color and shape and tolerance to shade, made it a popular household ornamental flower in the world (Grout 1990). Leaf cutting has been the traditional method for African violet propagation and propagated new plantlets have been produced with many difficulties like emergence of asymmetrical plants, high growing space requirements and disease development (Torres 1988). Species belonging to Gesneriaceae are mainly micropropagated from buds or adventitious shoots which are directly regenerated on leaf sections (Sameera et al. 2006). African violet has been propagated successfully on tissue culture media by many researchers e.g., from vegetative parts, i.e., leaf segments (Redway 1991, Lo 1997) and from floral parts (Molgaard et al. 1991). Different factors such as growth regulators, explant type, explant orientation and growth conditions (light and temperature) affect the regeneration frequency. Jain (1997) micropropagated *Saintpaulia ionantha* by culturing leaf disks on MS medium containing 0.22– 0.50 µM BA. They suggested that Addition of auxins with cytokinins is essential for shoot induction and multiplication depending on the explant type. The optimization of in vitro conditions for organogenesis and somatic embryogenesis decreased the related expenses and improved the commercial production of *Saintpaulia ionantha* (Rout et al. 2006). Despite the availability of the sufficient literature on the micropropagation of African violet, most of the successful methodologies have not been published due to commercial aspects. This paper describes a highly efficient protocol for shoot induction, plant regeneration and acclimatization of rooted plantlets of African violet.

Materials and Methods:

Plant material and explant sterilization

The plant materials were obtained from commercial providers and propagated at GABIT (Genetics and Agricultural Biotechnology Institute of Tabarestan) greenhouse. The plants were washed under running tap water and then sterilized by 1% sodium hypochlorite solution containing 0.5 % Tween-20 for 15 minutes. After that, the sterilized plants were rinsed with distilled water for three times, then leaf discs (about 1 cm^2) and petioles (1 cm) were used as explants for in vitro micropropagation on different MS media (Murashige & Skoog 1962) supplemented with different phytohormones.

Adventitious shoot induction

Shoot induction media (SIM) contained MS salts fortified with B5 vitamins, 30 grL^{-1} sucrose, 0.7% agar and 6-benzylaminopurine (BA) as cytokinin source at different concentrations (0, 0.2, 0.5, 1 and 2 mgL^{-1}) and indole-3-butyric acid (IBA) as auxin source (0 and 0.5 mgL^{-1}). The pH of media was adjusted to 5.8 before autoclaving. All cultures were maintained in glass jars for 4 weeks in a growth room at 25 ± 1 ^{0}C with 16 h light daily (Approximately 2500 Lux).

Shoot elongation

The shoots from the most appropriate SIM were subcultured for further growth in shoot elongation media (SEM). These media consisted of MS basal salts with 5 hormonal treatments (control (hormone free), 0.2 mgL^{-1} BA, 0.2 mgL^{-1} Kin, GA_3 at 0.2 and 0.5 mgL^{-1}). Shoots were cultured and maintained at the same growth conditions mentioned for shoot induction, and were subcultured after 4 weeks.

Rooting medium (RM)

After 1 month, elongated shoots were transferred to 1⁄2 strength of Murashige and Skoog`s basal medium (BM) supplemented with 3 different concentrations (0.1, 0.5 and 1 mgL^{-1}) of indole-3-butyric acid (IBA) and Naphthalene Acetic Acid (NAA) phytohormones.

Acclimatization

The rooted plantlets were transferred to pots containing peat moss and perlite with a ratio of 2:1 and covered with transparent plastic cover. The pots were incubated under high humidity at 25°C with 16 h of light per day.

Experiment design and Data analysis

All investigations were performed as factorial experiments in a completely randomized design with four replications (glass jars) per treatment and 4 explants per replication. Data of each experiment were subjected to analysis of variance (ANOVA) by the General Linear Models procedure using SAS software. Means were compared using the Least Significant Difference (LSD) method at $P \leq 0.05$.

Results and Discussion

Shoot induction

The results have shown the significant effect of explant type and hormonal combinations on shoot primordia induction after 2-3 weeks, in other words, compared to the petiole explants; the leaf explants are more efficient for shoot induction (Table 1).

Both explants produced green shoot primordia after 2-3 weeks of culture which were developed to adventitious shoots after 4-5 weeks. Similar results were reported by Sunpui and Kanchanapoom (2002). They reported a higher rate of shoot induction from petioles compared to leaf discs, while the regenerated shoots from leaf discs were longer than the petiole derived shoots. Sharifi et al. (2010) investigated different solidifying agents (potato starch, agar and liquid media) on in vitro regeneration of African violet and concluded that agar is the best agent for media preparation for Saintpaulia micropropagation.

Compared to explants type, hormonal combinations had a more remarkable effect on shoot induction. Primary investigations showed that IBA is essential for rapid induction of shoots and in the lack of IBA, the shoot induction was postponed. Number of induced shoots was higher in medium with 0.5 mgL^{-1} IBA compared with hormone free media (Fig. 1).

As indicated in Fig. 2, the length of produced shoots in IBA was longer than those generated on hormone free media. Based on reports from Rout et al. (2006), the shoot number significantly increased by auxin hormone treatment in African violet. Assessment of BA effect on shoot induction (without considering the IBA level) revealed considerable changes in shoot numbers and their lengths with variations in BA concentrations. The highest number of shoots was observed on media containing 1 mgL^{-1} BA, while the longest shoots were developed on media supplemented with 0.2 mgL^{-1} BA (Fig 3). Kushikawa et al. (2001), reported that the highest shoot regeneration from hypocotyls after inoculation with Agrobacterium was achieved in a medium supplemented with 1 mgL^{-1} BA. Daud and Taha (2008), reported the highest shoot regeneration from floral explants in a medium fortified with 1 mgL^{-1} BA and 2 mgL^{-1} NAA.

As indicated in Table 1, there are significant reciprocal effects between phytohormones` levels and explants type on days to shoot formation, number of shoots and shoot length. Observation of shoot induction in all media revealed the high potential of the Saintpaulia for adventitious shoot formation. The media containing 0.5 or 1 mgL^{-1} BA along with 0.5 mgL^{-1} IBA was more capable for shoots formation rather than the other media (17-18 days after culture). As seen in Table 1, addition of IBA in media caused rapid shoot induction.

Mithila et al., (2008) reported that application of Thidiazuron at concentrations below 0.5 mgL-1 increased shoot induction from Saintpaulia leaf and petiole explants, while higher concentrations promoted somatic embryogenesis.

The highest number of shoot was induced from petiole explants on media supplemented with 0.5 mgL^{-1} BA and 0.5 mgL^{-1} IBA, with an average of 80 shoots per explant. The longest shoots belonged to media containing 0.2 or 0.5 mgL^{-1} BA along with 0.5 mgL^{-1} IBA from leaf disc (with an average of 7.6 mm), while shorter belong to the media containing

Table 1. Effects of combination of explant type, IBA and BA on shoot number, length and time required for shoot regeneration in MS medium after 4 weeks.

Explant	IBA (mg/l)	BAP (mg/l)	Days to shoot induction	Num of shoot /explant	Length of shoot /explants (mm)
Leaf disc	0	0.2	24-25	50^{c}	6.3^{bc}
Leaf disc	0	0.5	24-25	34.3^{ef}	4.6^{ef}
Leaf disc	0	1	20-22	45^{cd}	4.3^{efg}
Leaf disc	0	2	20-22	28.3^{fg}	7^{abc}
petiole	0	0.2	24-25	28.3^{fg}	3.6^{fgh}
petiole	0	0.5	24-25	24.6^{g}	2.8^{h}
petiole	0	1	20-22	33.3^{ef}	3.3^{gh}
petiole	0	2	20-22	30^{fg}	5^{df}
Leaf disc	0.5	0.2	19-20	43.3^{cd}	7.6^{a}
Leaf disc	0.5	0.5	17-18	80^{a}	7.3^{ab}
Leaf disc	0.5	1	17-18	63.3^{b}	7.6^{a}
Leaf disc	0.5	2	22-23	58.3^{b}	4.3^{efg}
petiole	0.5	0.2	19-20	33.3^{ef}	6^{cd}
petiole	0.5	0.5	17-18	46.6^{c}	4.6^{ef}
petiole	0.5	1	17-18	46.6^{c}	4^{efg}
petiole	0.5	2	22-23	38.3^{de}	4^{efg}
LSD				8.1	1.1

*Means with the same letter do not have significant differences at $P \leq 0.05$.

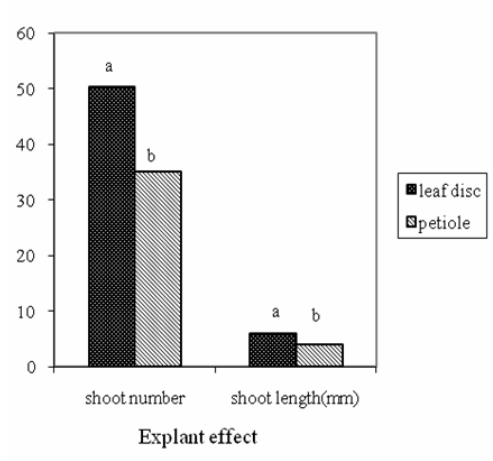

Figure 1. Effect of explant type on shoot number and shoot length of African violet on MS medium.

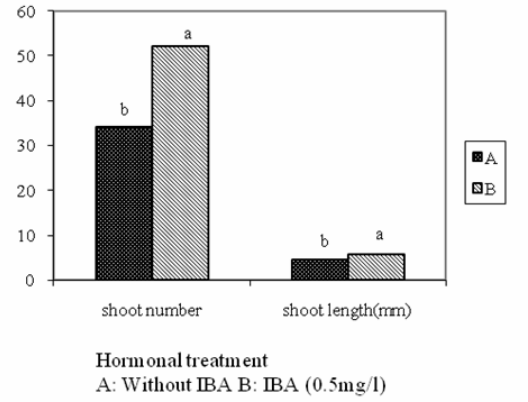

Figure 2. Effect of different levels of IBA on shoot number and shoot length of African violet on MS medium.

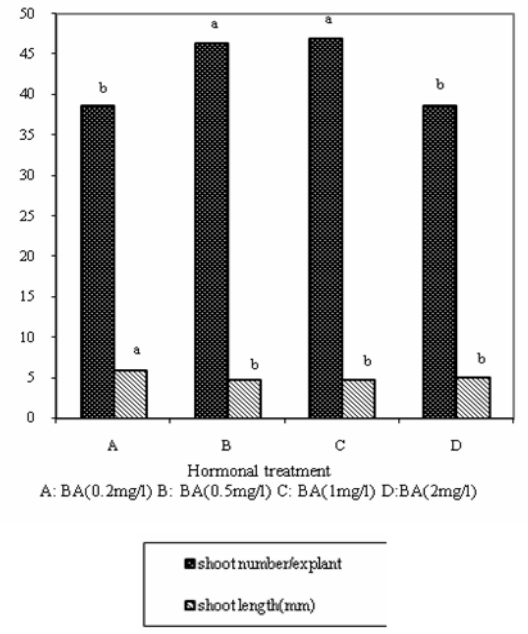

Figure 3. Effect of different levels of BA on the length of the adventitious shoots produced from African violet explants on MS medium.

only 0.5 mgL^{-1} BA, (with an average of 2.8 mm). These results indicated the crucial role of auxin (IBA) in promoting both number and length of the regenerated shoots.

Shoot elongation

Adventitious shoots produced after one month on media containing 0.5 mgL^{-1} BA and 0.5 mgL^{-1} IBA, were subcultured on SEM media for further growth. Results showed that the highest elongation rates related to the media supplemented with 0.2 mgL^{-1} GA_3 (Fig4), with an average shoots length of 19.9 mm after 3 weeks.

Shoots cultured on media with 0.2 mgL^{-1} BA and 0.2 mgL^{-1} Kinetin, produced new short shoots with proliferated small leaves (Picture 1). It appears that use of kinetin and benzyladenine decreased apical dominance resulting enhanced shoot proliferation with less growth. On the other hand, shoots cultured in media lacking phytohormones, had green broad leaves with roots emerging on some shoots (Fig. 4). The shoots on GA_3 containing medium had narrow leaf blades and longer petioles. Application of GA_3 produced longer distinct shoots which facilitated dividing and subsequent subculture of shoots for rooting. Jia-Tao (2006) reported that employing GA_3 promoted the number of normally regenerated shoots in African violet.

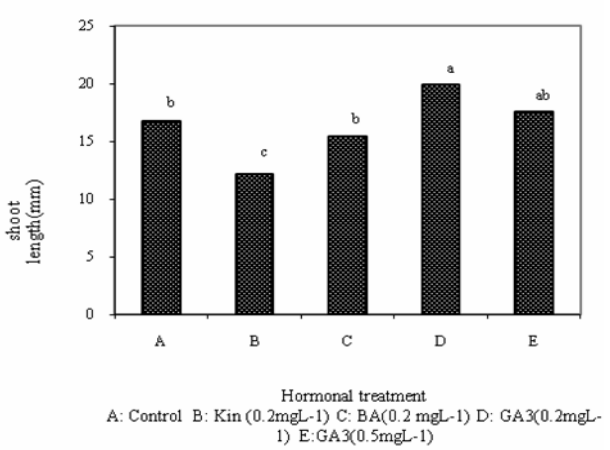

Figure 4. Effect of hormonal treatment on elongation of adventitious shoots of African violet produced on MS medium.

Rooting

The results showed that different treatments had significant effects on roots number, length and the morphology of the regenerated plantlets. Maximum and minimum root numbers were obtained in media containing 1 and 0.1 mgL^{-1} NAA, respectively (Fig. 5).

The primary results indicated that there is a positive correlation between NAA concentration and rooting percentage. Root length was also affected by hormonal alterations and maximum root length was obtained in media containing 1mgL^{-1} IBA and minimum root length was observed in media supplemented with 0.5 mgL^{-1} NAA (Fig. 6).

Auxin type and concentration affected the morphology of the regenerated plantlets. The use of 0.1 mgL^{-1} NAA produced normal plants, while increasing NAA concentration to 0.5 and 1mgL^{-1}, caused some abnormalities such as, callus formation at the shoot base and appearance of abnormal shoots with small leaves. In contrast, most of the plants

rooted in media supplemented with different levels of IBA showed normal morphology.

Therefore, despite of the higher root number in media containing NAA, the media with IBA were indicated as the most appropriate media for rooting of *Saintpaulia*. Similar results were also reported for some ornamental plants by different researchers as well (Hutchinson 1981, Lo 1997).

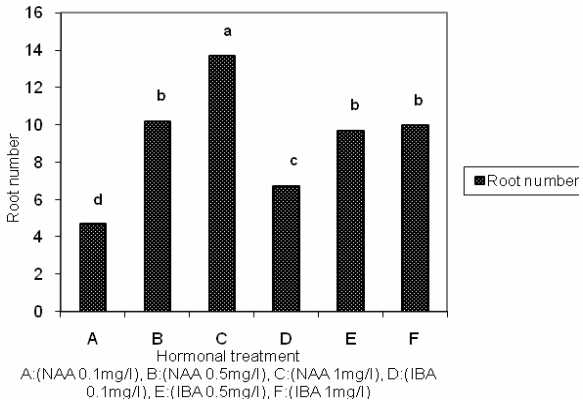

Figure 5. Effect of hormonal treatment on rooting of adventitious shoot number of African violet in MS medium.

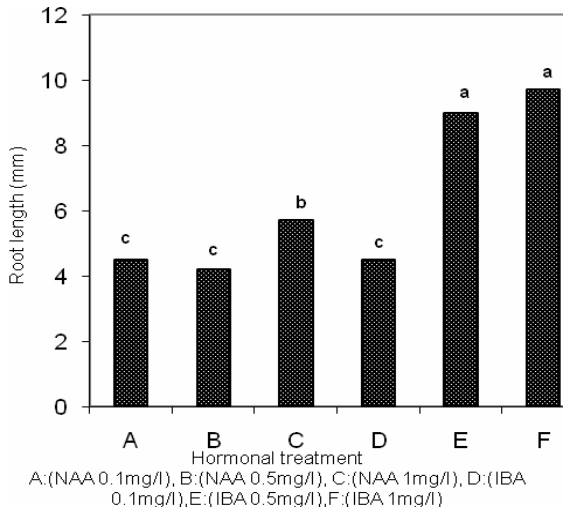

Figure 6. Effect of different concentrations of NAA and IBA on root length of African violet in MS medium.

Acclimatization

Results showed that when the rooted plantlets were transferred to a mixture of peat moss and perlite (3:1) under a light density of about 5000 Lux and a humidity between 80-90%, after 3 weeks more than 95% of plants survived and continued to develop new leaves.

Conclusions

According to the results of the present study, it was revealed that the regeneration efficiency of the leaf blades is higher than that of petiole explants, which can be attributed to the higher nutritional and hormonal content of the tissue. Furthermore, it was showed that addition of IBA at a concentration of 0.5 mgL^{-1} in the shoot induction media enhanced the regeneration efficiency. The highest numbers of adventitious shoots were produced on media containing 0.5 mgL^{-1} BA and 0.5 mgL^{-1} IBA (80 shoots per explant). Addition of GA_3 in elongation media caused a significant increase in shoot growth. It was also revealed that compared to NAA, IBA was more appropriate for rooting process and produced more normal plantlets. The present study provides a comprehensive methodology for high efficiency micropropagation of African violet for commercial production.

Acknowledgements. This research was conducted with the financial support of the Genetics and Agricultural Biotechnology Institute of Tabarestan (GABIT), Sari, Iran.

References

Daud, N, Taha, RM. (2008): Plant regeneration and floral bud formation from intact floral parts of African violet (*Saintpaulia ionantha* H. Wendl.) cultured in vitro. Pakistan Journal of Biological Sciences 11: 1055-1058.

Grout, B.W.W. (1990): African violet. pp. 181-205. In: Ammirato, P.V., Evans, D.A., Sharp, W.R., Bajaj, Y.P.S. (eds), Handbook of Plant Cell Culture. McGraw-Hill, Inc.

Hutchinson, J.F. (1981): Tissue culture: propagation of fruit trees. Pp.12-18. In: Rao, A.N. (ed.), Proceedings of the COSTED Symposium of Economically Important Plants, ANBS Singapore.

Jain, S.M. (2002): Feeding the world with induced mutations and biotechnology. Proceedings of the International Nuclear Conference 2002: Global trends and Perspectives. Seminar 1: Agriculture and Bioscience. Bangi, Malaysia: MINT. pp.1–14.

Jia-Tao, H. (2006): Tissue Culture of Africa Violet. Tropical Agriculture Science and Technology 1: 10-12.

Kushikawa, S., Hoshino, Z., Masahiro, M. (2001): Agrobacterium-mediated transformation of *Saintpaulia ionantha* Wendl. Plant Science 161: 953-960.

Lo, K.H. (1997): Factor effecting shoot organogenesis in leaf disc culture of African violet. Scientia Horticulturae 72: 49-57.

Mithila, J., Hall, J., Victor, J. M.R., Saxena, P. (2008): Thidiazuron induces shoot organogenesis at low concentrations and somatic embryogenesis at high concentrations on leaf and petiole explants of African violet (*Saintpaulia ionantha* Wendl.). Plant Cell Reports 21: 408-414.

Molgaard, J. P., Roulund, N., Deichmann, V., Irgens- Moller, L., Andersen, S.B., Farestveit, B. (1991): In vitro multiplication of *Saintpaulia ionantha* Wendl. by homogenization of tissue cultures. Scientia Horticulturae 48: 285-292.

Murashige, T., Skoog, F. (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum 15: 473-497.

Redway, F.A. (1991): Histology and stereological analysis of shoot formation in leaf callus of *Saintpaulia ionantha* Wendl (African violet). Plant Science 2: 249-251.

Rout, G.R., Mohapatra, A., Mohan Jain, S. (2006): Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects. Biotechnology Advances 24: 531-560.

Sharifi, A., Moshtaghi, N., Bagheri, A. (2010): Agar alternatives for micropropagation of African violet (*Saintpaulia ionantha*). African Journal of Biotechnology 9: 9199-9203.

Sunpui, W., Kanchanapoom, K. (2002): Plant regeneration from petiole and leaf of African violet (Saintpaulia ionantha) cultured In vitro. Songklanakarin Journal of Science and Technology 24: 257-264.

Torres, K. (1988): In vitro Propagation of African Violets. pp.80-85. In: Torres, K. (ed.), Tissue Culture Techniques for Horticultural Crops. Van Nostrand Reinhold Co.