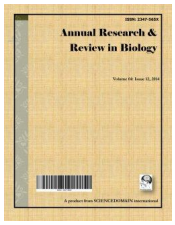

Annual Research & Review in Biology
7(2): 84-90, 2015, Article no.ARRB.2015.110
ISSN: 2347-565X

SCIENCEDOMAIN *international*
www.sciencedomain.org

Study the Effect of Growth Regulators on Micropropagation of *Gloriosa superba* L. from Seeds and Their Acclimatization

Dharmendra Singh[1*], Manish Mishra[2] and Anirudha Singh Yadav[1]

[1]Department of Botany, Government Motilal Vigyan Mahavidyalaya, Barkatullah University, M.P., India.
[2]Department of Ecosystem Management and Tech Forestry, IIFM, Bhopal, India.

Authors' contributions

This work was carried out in collaboration between all authors. Author DS designed the study, wrote the protocol and interpreted the data. Author MM anchored the field study, gathered the initial data and performed preliminary data analysis. Author DS managed the literature searches and produced the initial draft. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/ARRB/2015/12975
Editor(s):
(1) George Perry, Dean and Professor of Biology, University of Texas at San Antonio, USA.
Reviewers:
(1) Christophe Bernard Gandonou, Department of Plant Biology, University of Abomey-Calavi, Republic of Benin.
(2) Anonymous, Singapore.
(3) Anonymous, Brazil.
(4) Shahril Efzueni Rozali, Institute of Biological Sciences, Faculty of Science, University of Malaya, Kuala Lumpur, Malaysia.
(5) P. Giridhar, Plant Cell Biotechnology Department, CSIR-CFTRI, Mysore, India.
Complete Peer review History: http://www.sciencedomain.org/review-history.php?iid=974&id=32&aid=9036

Original Research Article

Received 26th July 2014
Accepted 7th January 2015
Published 29th April 2015

ABSTRACT

Background: *G. superb* L. is an important medicinal plant of Ayurvedic system of medicine. It is distributed naturally widely throughout the tropics, and worldwide as a pot plant. Due the poor germination and low viability of seeds plant required more time for growing. As seed germination is poor and vegetative propagation is slow, The methods of rapid micropropagation are required. Procedure for seed germination and micropropagation were established.
Results: Fresh and healthy seeds were cultured on Murashige & Skoog medium supplemented with different concentration of BAP and GA_3. Best seed germination was observed at 1.0 mg/l BAP and 0.5 mg/l GA_3 at minimum time of 49 days. MS medium supplemented with 1.5 mg/l BAP+ 0.5 mg/l IAA and 0.5 mg/l kinetin recorded the highest response of shoot multiplication (3.0±0.3) with

**Corresponding author: E-mail: dharmendrasingh036@gmail.com;*

best shoot length (3.2±0.24 cm). The regenerated shoots were cultured on MS medium. 1.5 IBA and 0.5 NAA mg/l was found to be the best concentration for maximum percent of root initiation. The rooted plantlets were successfully transferred into plastic cups containing sand, peat and soil in the ratio of 1:1:1 and subsequently established in the greenhouse. Thereafter they were reared for three weeks. The survival rate of regenerants was found to be 60%.

Conclusion: According to the observations suitable combination of cytokinin and GA_3 proved to be effective in the case of seed germination. In shoot cultures of *G. superb* L. BAP proved to be the effective cytokinin too.

Keywords: Gloriosa superba L. seed; micropropagation; rooting; acclimatization.

1. INTRODUCTION

G. superba L., a member of the Liliaceae family is a perennial tuberous climbing herb that distributed in tropical and subtropical region of India [1]. *G. superba* L. is among some of the modern important medicinal plant, which actually facing local extinction. Different parts of the plant have a wide variety of uses especially within an Indian traditional medicine of the time in immemorial. Tubers and seeds of the *G. superba* L. are an expensive export commodity. All parts of the plant contain colchicines and related alkaloids [2]. Due to the medicinal value, this plant collected from the wild and used as raw material for large-scale medicinal industries, leading to over exploiting condition, proved to be 95% endangered medicinal plant becomes endangered plant species and included in red data book [3]. It usually multiplies by corm. Many researchers have already reported propagation of this plant by tubers [4,5], But due to the market pressure and immature harvesting of tubers, this plant facing endangered condition [6]. Therefore, in order to safeguard and preserve this important plant biotechnological approach would be very useful. In this context, plant tissue culture through micropropagation makes the rapid multiplication of selected genotypes possible, allowing the useful metabolites to be collected in greater quantities, as well as providing an alternative means of propagation, given that in the field, the presence of seed tegument dormancy hinders the swift production of uniform plants [7]. In the present study, *G. superb* L. was chosen as an experimental plant because of its immense importance in natural medicine. This glorious herb was found in abundance once upon a time in Madhya Pradesh. Due to its terrific medicinal properties, the plant was cruelly exploited from the forest of Madhya Pradesh by local healers and its extensive use in modern medicines, leads to depletion of the species and has become endangered. This evoked us to conserve this plant through *in vitro* techniques. The present investigation was launched, to standardize the protocol for micropropagation of *G. superba* L. from seeds and the effect of different plant growth hormone concentration and their acclimatization.

2. MATERIALS AND METHODS

2.1 Sample Collection

The fresh seed samples of *G. superb* L. were collected from the forest area of Sarani, District Betul (M.P). It is constituted in the central part of the India. District Betul is one of the backward districts in Madhya Pradesh which is lying on the southern part of the Madhya Pradesh. The district extends between 21°22' and 22°24' North latitude and 77°04' and 78°33' East Longitude and forms a compact shape, almost a square with slight projections on the east and west. Betul is one of the richest district of Madhya Pradesh in respect of forest resources. In PBR the flora of Pachmarhi, Bori & Sarani forest ranges consists of 101 families consisting of 452 genera and 778 species. The species consists of 247 trees and shrubs as well as 531 herbs [8]. The collected seeds packed in poly beg for further study in the laboratory.

2.2 Preparation and Disinfection of Explants

Seeds of *G. superba* were collected and washed thoroughly in running tap water to remove the superficial dust, and then explants were washed with 2% (v/v) tween 20 for 10 min and then washed well by distilled water. The disinfected explants were dipped in 70% ethanol for 30 seconds. After pretreatment with ethanol, the explants were rinsed with distilled water twice for 5 min, so as to lower the toxic effect of ethanol. Then the seed surface was sterilized with 0.1% mercuric chloride ($HgCl_2$) for 5 min. This was followed by rinsing the explants with double

distilled sterile water thrice, so as to lower the toxic effects of $HgCl_2$. To remove the effect of endophytic fungal seeds was dipped in 1% solution of Bovistine antifungal agents for 2 min and then rinsed with distilled water.

2.3 Culture Condition

The explants were placed on MS [9] medium gelled with 0.8% (w/v) agar and supplemented with different concentrations and combinations of 6-Banzylaminopurine (0.5-1.5 mg/l) and Gibberellic acid (0.5-1.0 mg/l) for seed germination and BAP (0.5-2.5 mg/l), Indole -3-acetic acid (0.5 mg/l) or Kinetin (0.5 mg/l) for shoot proliferation and multiplication. For the rooting different concentration of Indole-3-butyric acid (0.5-2.5 mg/l) and 1-Naphthaleneacetic acid (0.5 mg/l) were used. The pH of medium was adjusted to 5.6-5.8 using 0.1 N NaOH or 0.1 N HCl before autoclaving (121°C, 20 min). All the cultures were incubated at 25±2°C under a 16 hrs photoperiod with cool and white fluorescent lamps (3000 lux). Rooted explants were planted in pots containing a sterile mixture of soil and sand, kept in the green house for acclimatization. The plantlets were kept in a polychamber at 80% relative humidity, 32±2°C under a 12 hrs. Photoperiod for acclimation. The plants were given fertilizer with 1/8th MS macronutrients biweekly. Established plants were transplanted in earthen pots under natural conditions and the survival rate was recorded.

2.4 Statistic Analysis

All investigations were based on three independent experiments. Every treatment was repeated at least three times and ten plants were investigated in one repetition. In relevant cases, the data were subjected to statistical analysis for computation of the standard error of the mean (SE). The analysis of variance (ANOVA) was performed using Graph pad prism 6.

3. RESULTS AND DISCUSSION

3.1 Seed Germination

In order to establish an efficient *in vitro* micropropagation system of *G. superb* L. for *in vitro* germination was achieved. To improve the germination capacity of seeds different concentrations of plant growth regulators were supplemented in MS media. The fresh seeds of *G. superba* were surface sterilized by different chemical substances and inoculated on seed germinating medium (MS medium) containing different concentrations of BAP with the combination of GA_3 (Fig. 1). Highest number of germination was observed in MS media containing 0.5 mg/l BAP with 0.5 GA_3 and 1.5 mg/l BAP with 0.5 GA mg/l (Table 1 & Fig. 1). Growth regulators not only induced the germination of seed but also reduce the time of germination. Poor and delayed seed germination in *G. superba* was reported and the germination was erratic which took three weeks to three months [10]. Comparative study of chemicals and growth regulators on seed germination of *G. superba* was already reported. They recorded that certain growth regulators like GA_3, kinetin and ethrel was show maximum result with the parameter of sprouting percentage (100%), an earlier sprouting of tubers (6.33 days), maximum plant height (99.32 cm) and a maximum number of leaves per plant (34.04) and plant girth (1.81 cm) [11]. More study was not carried out due to poor germination and low viability [12].

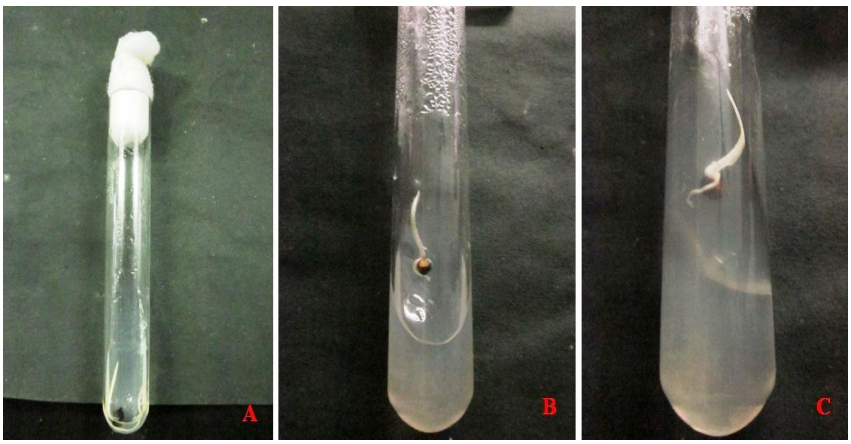

Fig. 1. Effect of growth regulators on seed germination of *G. superba*

3.2 Initiation and Multiplication of Shoots

Shoot induction was not found in basal medium even after two weeks of culture. The explants were found to be swollen and they produced two to three shoots within three - four weeks after inoculation in MS containing BAP alone but the number of shoots increased up to four when the explants were cultured in MS with 1.5 mg/l BAP + 0.5 mg/l IAA +0.5 mg/l kinetin (Table 2, Fig. 2). The nodal explants of *G. superb* L. were inoculated on MS medium fortified with different concentrations of BAP (0.5, 1.0, 1.5, 2.0 and 2.5 mg/l) with 0.5 mg/l IAA and 0.5 mg/l kinetin. After three weeks, multiple shoots emerged from the explants. The highest number (3.0±0.3) of multiple shoots was obtained when MS medium fortified with 1.5 mg/l BAP+0.5 mg/l IAA and 0.5 mg/l kinetin, was the ideal concentration for highest percentage of multiple shoot induction. At this concentration, produced shoots showed maximum length (3.2±0.24 cm). As it in the present study a method for *in vitro* shoot multiplication of *G. superb* L. from apical shoot buds and meristems was reported [13], Best shoot initiation response was achieved on MS Medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA on both apical shoot bud & meristem explants. Addition of BAP in combination of Kinetin or Kinetin alone failed to show a good shoot initiation response. Meristems showed more pronounced effect of shoot formation than apical shoot bud explants [14]. The positive effect of coconut water and activated charcoal on shoot multiplication was investigated by Hassan and Roy, 2005 [15], which increased the number of shoots up to 15 per culture and the survival rate of regenerants was found to be 85-90%. Similarly the effects of different growth regulators on shooting and rooting of *G. superba* L. by tubers was reported [16].

Fig. 2. Effect of plant hormones on shoot initiation and Multiplication

Table 1. Effect of growth regulators on seed germination

S. no.	Growth regulators concentration (mg/l)	No. of seed inoculated	No. of seed germinated	Time required for germination (Days)
	Control	10	1.3 ± 0.21^{c}	72.00 ± 0.42^{a}
1.	0.5 BAP	10	1.3 ± 0.42^{c}	70.33 ± 0.00^{a}
2.	1.0 BAP	10	2.6 ± 0.21^{b}	72.33 ± 0.42^{a}
3.	0.5BAP+0.5 GA_3	10	4.0 ± 0.21^{a}	62.66 ± 0.42^{b}
4.	1.0 BAP+0.5 GA_3	10	1.3 ± 0.85^{c}	45.66 ± 0.85^{c}
5.	1.5 BAP+0 .5 GA_3	10	4.3 ± 0.42^{a}	67.33 ± 0.42^{b}

Value represent mean ± SE (standard error), n=30, according to ANOVA the results are statistically significant (P<0.001), F=17.11, SS=238.5, MS=44.27 Tukey's comparison test (p<0.001) a>b>c

3.3 Rooting and Acclimatization

The shootlets obtained from the seeds was excised and transferred to the root induction medium. MS medium supplemented with various concentrations of IBA (0.5-2.5 mg/l) and NAA (0.5 mg/l) was used as root induction medium. Maximum value for response to rooting (22.3±2.7%) root length (3.6 cm) was observed in the MS medium supplemented with 1.5 mg/l IBA + 0.5 mg/l NAA at the minimum time of 18 days (Table 3 and Fig. 3a). Effect of different concentration of IAA and IBA on *in vitro* rooting have already been reported by many researchers [17,18]. The effect of different carbohydrates on *in vitro* rooting has already investigated on many plants by researchers. They found the type and concentration of sugars had a significant effect on rooting percentage, mean root number, mean root length, hyperhydricity, as well as survival rate [19]. The availability of carbohydrates is often considered exclusively as an energetic requirement to drive root development; the major regulatory role in the process is often attributed to phytohormones, particularly auxin [20]. Activated charcoal (0.22%) was supplemented in the rooting media for its beneficiary role in rooting *in vitro*. It reduces the light intensity at the base of the shoots providing an environment conducive to the accumulation of auxins or cofactors or both, thus providing a dark environment for the growth of the roots. It absorbs substances such as inhibitory phenolics and any excess auxin or cytokinin carried over from previous media. It enhances the lateral root formation and it is often effective in arresting intervening callus formation, thereby helping in establishment of vascular connection between roots and shoots [21]. Rooted shoots were dipped in 1% antifungal solution to provide a shield from fungal infection till it establishes anchorage in soil. After 15 days, small leaves in the rooted shoots broadened and turned dark green indicating robust functioning of the photosynthetic system. Usage of transparent plastic sheets allowed the light to penetrate while maintaining high humidity internally. The 10 plants transferred to sand+ peat+ soil (1:1:1) mixture, 60% plants survived and hardened in 3 weeks (Fig. 3b). All hardened plants survived on transfer to pots in the greenhouse. The best response for hardening was obtained with a mixture containing sand + peat + soil (1:1:1) at 95% humidity level under natural light conditions.

Fig. 3a. Root induction at different concentration of rooting hormones

Table 2. Effect of growth regulators on shoot induction and multiplication

S. no.	Growth regulators concentration (mg/l)	No. of shoot	Shoot length (cm)
1	0.5BAP+0.5IAA	0.66 ± 0.17^{c}	0.76 ± 0.21^{c}
2	1.0 BAP+0.5 IAA+0.5 Kinetin	1.30 ± 0.17^{b}	1.60 ± 0.09^{c}
3	1.5 BAP+0.5 IAA+0.5 Kinetin	3.00 ± 0.30^{a}	3.20 ± 0.24^{a}
4	2.0 BAP+0.5 IAA+0.5 Kinetin	1.60 ± 0.17^{b}	2.50 ± 0.13^{b}
5	2.5 BAP+0.5 IAA+0.5 Kinetin	1.00 ± 0.30^{b}	1.60 ± 0.22^{c}

Value represent mean ± SE (standard error), Tukey's comparison test ($p<0.05$) a>b>c

Fig. 3b. Acclimatization of tissue cultured plant of *G. superba*

Table 3. Effects of different concentration of IBA and NAA on *in vitro* rooting

S. no.	Plant growth regulators concentration (mg/l)	Root length cm	Time (days)	% of rooting
1	0.5IBA+0.5NAA	0.83 ± 0.92^{c}	23.33 ± 0.75^{a}	5.00 ± 0.21^{c}
2	1IBA+0.5NAA	0.90 ± 0.16^{c}	21.33 ± 0.62^{a}	6.30 ± 0.92^{c}
3	1.5 IBA+0.5NAA	3.60 ± 0.25^{a}	18.66 ± 0.46^{b}	22.30 ± 2.07^{a}
4	2 IBA+0.5NAA	1.30 ± 0.10^{b}	19.33 ± 0.46^{b}	9.60 ± 2.00^{b}
5	2.5 IBA+0.5NAA	0.92 ± 0.02^{c}	23.00 ± 0.30^{a}	7.30 ± 0.04^{b}

Value represent mean ± SE (standard error), Tukey's comparison test ($p<0.05$) a>b>c

4. CONCLUSION

In this investigation an efficient protocol was developed for micropropagation of an endangered medicinal plant *G. superba* under aseptic controlled conditions by using seeds as explants. It was further proved that seed germination, induction of tissue growth and its differentiation was dependent on different concentrations of growth regulators and other supplements in the medium. According to the observations suitable combination of cytokinin and GA_3 proved to be effective in the case of seed germination. In shoot cultures of *G. superb* L. BAP proved to be the effective cytokinin too.

ACKNOWLEDGEMENTS

We wish to thank Principal of Govt. M.V.M, Bhopal for providing research facilities and also thank the Director of CMBT, Bhopal for their valuable guidance and encouragement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Ade R, Rai M. Multiple shoot formation in *Gloriosa superba*: A rare and endangered Indian medicinal plant. Bioscience. 2011; 3(2):68-72.
2. Madhavan M, Joseph JP. Histological marker to differentiate organogenic calli from non organogeni ccalli of *G. superba* L. Plant Tissue Culture & Biotechnology. 2010;20(1):1-5.
3. Arumugam A, Gopinath K. *In vitro* micropropagation using corm bud explants: an endangered medicinal plant of *Gloriosa superb* L. Asian Journal of Biotechnology. 2012;4(3):120-128.
4. Yadav K, Aggarwal A, Singh N. Evaluation of genetic fidelity among micropropagated plants of *Gloriosa superba* L. using DNA-based markers — a potential medicinal plant. Fitoterapia. 2013;89:265-270.
5. Selvarasu A and Kandhasamy R. *In vitro* tuberization of glory lily (*Gloriosa superba* L.). Journal of Horticulture and Forestry. 2012;4(5):81-84.
6. Singh D, Mishra M, Yadav AS. Callus induction from corms of *Gloriosa superba* Linn: An endangered medicinal plant. Biotechnology an Indian Journal. 2012; 6(2):53-55.
7. Nepomuceno G, Santana L. Micropropagation and acclimatization of *Bauhinia cheilantha* (an important medicinal plant. African Journal of Biotechnology. 2011;10(8):1353-1358.

8. Khandel AK, Ganguly S, Bajaj A, Khan S. New Records, Ethno-pharmacological Applications & Indigenous Uses of *Gloriosa superb* L. (Glory lily) Practices by Tribes of Pachmarhi Biosphere Reserve, Madhya Pradesh, Central India. Nature and Science. 2012;10(5):23-48
9. Murashige T, Skoog F. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 1962;15:473-497.
10. Anandhi SK, Rajamani M. Jawaharlal: Propagation studies on *Gloriosa superba*. Medicinal and Aromatic Plant Research Journal. 2013;1(1):1-4.
11. Anandhi S, Rajamani K. Studies on seed germination and growth in *Gloriosa Superba* L. Global J Res. Med. Plants & Indigen. Med. 2012;1(10):524–528.
12. Singh D, Mishra M, Yadav AS. G*loriosa superb* linn: An important endangered medicinal plant and their conservation strategies. IJBR. 2013;3(1):19-26.
13. Khandel AK, Khan S, Ganguly, Bajaj A. In vitro shoot initiation from Apical shoot buds & Meristems of *Gloriosa superba* L. An endangered medicinal herb of high commercial value. Researcher. 2011; 3(11):36-45.
14. Venkatachalam P, Ezhili N, Thiyagarajan M. *In vitro* shoot Multiplication of *Gloriosa Superba* L. An Important Anticancer Medicinal Herb. International Conference on Biotechnology, Biological and Biosystems Engineering (ICBBBE'2012) December 18-19, Phuket (Thailand).
15. Sayeed Hassan AKM, Roy SK. Micropropagation of *Gloriosa superba* L. through high frequency shoot proliferation. Plant Tissue Cultture. 2005; 15(1):67-74.
16. Yadav K, Aggarwal A, Singh N. Actions for *Ex situ* conservation of *Gloriosa superba* L. – an endangered ornamental cum medicinal plant. Journal of Crop Science & Biotechnology. 2012;15(4):297-303.
17. Ebrahim N, Shibli R, Makhadmeh I, Shatnawi M, Abu-Ein A. *In vitro* Propagation and *In vivo* acclimatization of three coffee cultivars (*Coffea arabica* L.) from Yemen. World Applied Science Journal. 2007;2(2):142-150.
18. Chabukswar MM, Deodhar MA: Rooting and hardening of *In vitro* plantlets of *Garcinia indica* chois. Indian Journal of Biotechnology. 2005;4:409-413.
19. Bahmani R, Karami O and Gholami M. Influence of Carbon Sources and Their Concentrations on Rooting and Hyperhydricity of Apple Rootstock MM.106. World Applied Science Journal. 2009;6(11):1513-1517.
20. Rocha Correa L, Paim DC, Schwambach J, Fett-Neto AG. Carbohydrates as regulatory factors on the rooting of *Eucalyptus saligna* Smith and *Eucalyptus globules* Labill. Plant Growth Regulators. 2005;45:63–73.
21. Kumar S: *In vitro* Regeneration of *Pongamia pinnata*. Pierre. Journal of Plant Biotechnology. 2006;33(4):263-270.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?iid=974&id=32&aid=9036