International Journal of Botany Studies
www.botanyjournals.com
ISSN: 2455-541X
Received: 01-06-2021, Accepted: 16-06-2021, Published: 01-07-2021
Volume 6, Issue 4, 2021, Page No. 01-04

In vitro callus induction and organogenesis studies in medicinal plant *Gloriosa superba* L

Jyoti Ghodke, Narayan Pandhure
Department of Botany, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad, Maharashtra, India

Abstract
Gloriosa superba L. is an important Ayurvedic medicinal plants belongs to the family Colchicaceae. Due to high amount of colchicine and gloriosine it is used against number of diseases such as cancer, gout, piles, ulcer and dyspesia etc. It is cultivated in southern India but it is occurred in all over the country. As a result of over exploitation and slow propagation the plant is tern to an endangered category. So there is urgent need for rapid multiplication of this plant. Therefore in the present research work was carried out to evaluate the response of different concentration and combination of growth hormones in MS media for the regeneration of *Gloriosa superba* L. The highest growth of callus was obtained in MS medium with 2, 4-D (1.5 mg/l) + BAP (1.0 mg/l) and KIN (0.5 mg/l). Whereas BAP (2.0 mg/l) + KIN (1.0 mg/l) + GA3 (0.5 mg/l) induced the shooting and 2, 4-D (0.5 mg/l) + NAA (1.0 mg/l) + IBA (0.5 mg/l) induced the rooting from shoot tip as explants.

Keywords: *Gloriosa superba* L., ayurvedic, colchicines, gloriosine, etc

Introduction
Medicinal plants are playing a vital role to treat a number of diseases since time of immemorial and still today. Ayurveda and Unani have been recognized as one of the oldest remedial systems in India and also all over the world. The plants or plant parts used in natural medicines are keenly easily reached in rural region at comparatively cheaper than modern medicines (Mann *et. al.*, 2008) [6]. *Gloriosa superba* L. is very unique medicinal plants which contains number of alkaloids, largely colchicines and colchico side as well as non-alkaloidal curative compounds are also presence including β-sitosterol, stigmasterol, chelidonic acid, luteolin, etc. (Nautiyal, 2011) [10]. Ethnomedicinaly it is very important plant used by various Indian tribes against different diseases. It is treat almost twenty-nine diseases such as gout, ulcers, cancer, leprosy, sores, lice, rheumatism, skin diseases, abdominal pain, scorpion bite, snakebite, impotency, etc. (Bhide and Acharya, 2012) [1]. It is also useful in expulsion of placenta, promoting labor pain and muscle relaxant (Nadkarni, 2002) [8]. The plant also showed anti-microbial, anti-helmintic, Anti-venom, anti-inflammatory, anti-coagulant and anti-cancer activity (Ramakrishnan *et. al.*, 2017) [13]. Due to presence of colchicines the plant also plays significant role for having anti-abortive, anti-microbial and anti-cancer activity (Reuter *et al.*, 2010; Nikhila *et al.*, 2014; Mahajan, 2015) [14, 12, 15]. Slow propagation, low seed germination percent, much susceptible to pest and over exploitation for the medicinal purpose pushed this taxon to endanger category (Sivakumar and Krishnamurthy, 2000) [16]. If the condition goes similar for few years plant can extinct, therefore, *in vitro* propagation is very important alternative way for rapid multiplication and conservation of healthy plants. The frequently used nutrient medium is MS medium (Murashige and Skoog, 1962). Many researchers were previously reported the *in vitro* micropropagation methods of *Gloriosa superba* L. (Custers and Bergervoet, 1994; Sivakumar and Krishnamurthy, 2004; Hassan and Roy, 2005) [3, 17, 4]. But their methods having some limitations for large scale propagation of plantlets taking this consideration the present work is aimed to develop an efficient protocol for mass propagation of this precious medicinal plant *Gloriosa superba* L. in *in vitro* condition.

Materials and Methods
Collection of Plant Material and Surface Sterilization
Fresh and healthy plant parts (Leaf, shoot tip and nodal segment) of *Gloriosa superba* L. were collected in the month of August 2019 from different location of Jalna district of Maharashtra. Identification of plant species were confirmed using Flora of Marathwada by Naik *et al*., 1998. [11]. The collected explants were washed with running tap water then surface sterilized with 70% ethyl-alcohol followed by 0.1% $HgCl_2$ for 5 minutes after that wash with sterile water to remove the stresses of $HgCl_2$.

Culture Media and Culture Conditions
The MS (Murashige and Skoog, 1962) media was prepared fortified with 3% Sucrose and various concentrations and combinations of growth hormones such as 2, 4-D, BAP, KIN, NAA, GA3, NAA and IBA. Clerigar 2.5 gm was added to the medium as solidifying agent. Then pH was maintained 5.8 with NaOH/HCl and transfer the media in conical flask plug it and subjected to sterilization by autoclaving at 121^0C for 20 minutes. Surface sterilized explants were inoculated on MS medium in aseptic condition and the cultures were incubated in culture room at 25 ± 2^oC at 14 hrs photo-period provided by cool-white fluorescent light. The inoculated explants were regularly observed for further contamination and *in vitro* response.

Callus Induction and Organogenesis
For induction of callus, explants were inoculated on MS media with different concentrations of 2, 4-D (0.5 -2.5 mg/l), BAP (0.5-2.5 mg/l) and KIN (0.5-2.5 mg/l). The inoculated cultures were kept in culture room. After four weeks the induced callus was sub-cultured on fresh MS medium with different concentrations and combinations of

BAP (0.5-2.5 mg/l), KIN (0.5-2.5 mg/l) and GA3 (0.5 mg/l) for shoot induction and NAA (0.5-2.5 mg/l), IBA (0.5-2.5 mg/l) and 2, 4-D (0.5 mg/l) for rhizogenesis. After six weeks the shoots were counted and measured the length per explants.

Results and Discussion

Induction of Callus

For callus induction of different explants (leaf, shoot tip and nodal segment) were inoculated on MS medium supplemented with various concentration and combination of 2, 4-D, BAP and KIN were taken. The best results were obtained at the concentration of 2, 4-D (1.5 mg/l) + BAP (1.0 mg/l) and KIN (0.5 mg/l) with shoot tip and nodal segment as explants. PGR concentration viz. 2, 4-D (1.0 mg/l) + BAP (0.5 mg/l) and KIN (0.5 mg/l) along with MS for leaf explants. Whereas, the callus initiation was also recorded in MS medium with 2, 4-D (2.0 mg/l) + BAP (1.5 mg/l) and KIN (0.5 mg/l), (Table 1- Fig. 1). Ngomuo *et. al.*, (2014) [9] revealed that the various explants used for *in vitro* culture have a very efficient on callus induction when different concentrations and combinations of plant growth hormones are used.

Table 1: Effect of different concentration of growth hormones in MS medium on callus induction

Source of Explants	Growthhormone concentration (mg/L)	No. of days for callus initiation	Colours of Callus	Frequency of callus induction
Leaf	0.5 2,4-D +0.5 BAP +0.5 KIN	23	Yellowish green	+++
	1.0 2,4-D +0.5 BAP +0.5 KIN	22	Yellowish green	++++
	1.5 2,4-D +1.0 BAP +0.5 KIN	25	Yellowish green	++
	2.0 2,4-D +1.0 BAP +0.5 KIN	25	Yellowish green	+
	2.5 2,4-D +1.5 BAP +0.5 KIN	-	-	-
Shoot tip	0.5 2,4-D +0.5 BAP +0.5 KIN	27	Yellowish green	+
	1.0 2,4-D +0.5 BAP +0.5 KIN	24	Yellowish green	++
	1.5 2,4-D +1.0 BAP +0.5 KIN	26	Yellowish green	++++
	2.0 2,4-D +1.0 BAP +0.5 KIN	30	Yellowish green	+
	2.5 2,4-D +1.5 BAP +0.5 KIN	28	Yellowish green	+
Nodal segment	0.5 2,4-D +0.5 BAP +1.0 KIN	32	Yellowish white	+
	1.0 2,4-D +0.5 BAP +1.0 KIN	29	Yellowish white	++
	1.5 2,4-D +1.0 BAP +1.0 KIN	28	Yellowish white	++++
	2.0 2,4-D +1.0 BAP +1.0 KIN	32	Yellowish white	+
	2.5 2,4-D +1.5 BAP +1.0 KIN	31	Yellowish white	+

Shoot Induction

Small pieces of friable callus obtained from leaf explants taken for callus induction, the pieces of callus were inoculated on fresh MS medium with different concentration of BAP, KIN and GA3. After six weeks of inoculation average number of (7.8 ±0.2) shoots and maximum shoot regeneration (79.25%) was recorded in MS medium containing with BAP (2.0 mg/l), KIN (1.0 mg/l) and GA3 (0.5 mg/l) (Table 2- Fig. 2). While the concentration of BAP and KIN was increased to 3.0 and 1.0mg/l respectively the number of shoots decreased. Custers and Bergervoet (1994) [3] reported that MS medium with various concentrations of KIN and IBA were useful for regeneration of multiple shoot in *G. superba* L. while, several researchers reported combination of BAP and NAA promoted the shoot multiplication (Hassan and Roy, 2005) [4].

Table 2: Effect of different concentration of growth hormones in MS medium on Shoot induction from callus

Growth hormone concentration (mg/L)			Average number of shoot per culture	Percentage of shoot growth
BAP	KIN	GA3		
0.5	0.5	0.5	1.1 ±0.7	5.50
1.0	0.5	0.5	3.7 ±0.2	42.50
1.5	1.0	0.5	5.2 ±0.1	52.20
2.0	1.0	0.5	7.8 ±0.2	79.25
2.5	1.5	0.5	3.8 ±0.3	37.85

Data is from five replicates and is represented as mean ± SD

Root Induction

For root induction, the well-developed shoot were separated and transferred to fresh MS medium containing different concentrations and combinations of addition of Auxins such as 2, 4-D, NAA and IBA. Best rhizogenesis was observed in MS fortified with 1.5 mg/l 2, 4-D, 1.0 mg/l NAA and 0.5 mg/l IBA resulting 61.20% of rooting with average number of roots per explants 5.8±0.1. Less rhizogenesis was recorded on MS medium fortified with 2, 4-D, NAA and IBA at the concentration of 0.5 mg/l. Many researchers were already recorded the response of different combination and concentration of IAA and IBA on *in vitro* rooting (Chabukswar and Deodhar, 2005) [2]. Rocha *et. al*., (2005) [15] reported that the application of phytohormones and carbohydrates particularly auxins plays an important role to drive root development.

Table 3: Effect of different concentration of growth hormones in MS medium on Root induction from callus

Growth hormone concentration (mg/L)			Average number of root per culture	Percentage of root growth
2, 4-D	NAA	IBA		
0.5	0.5	0.5	0.2 ±0.7	03.50
1.0	0.5	0.5	2.6 ±0.5	25.50
1.5	1.0	0.5	5.8 ±0.1	61.20
2.0	1.0	0.5	2.8 ±0.4	29.75
2.5	1.5	0.5	0.7 ±0.1	11.85

Data is from five replicates and is represented as mean ± SD

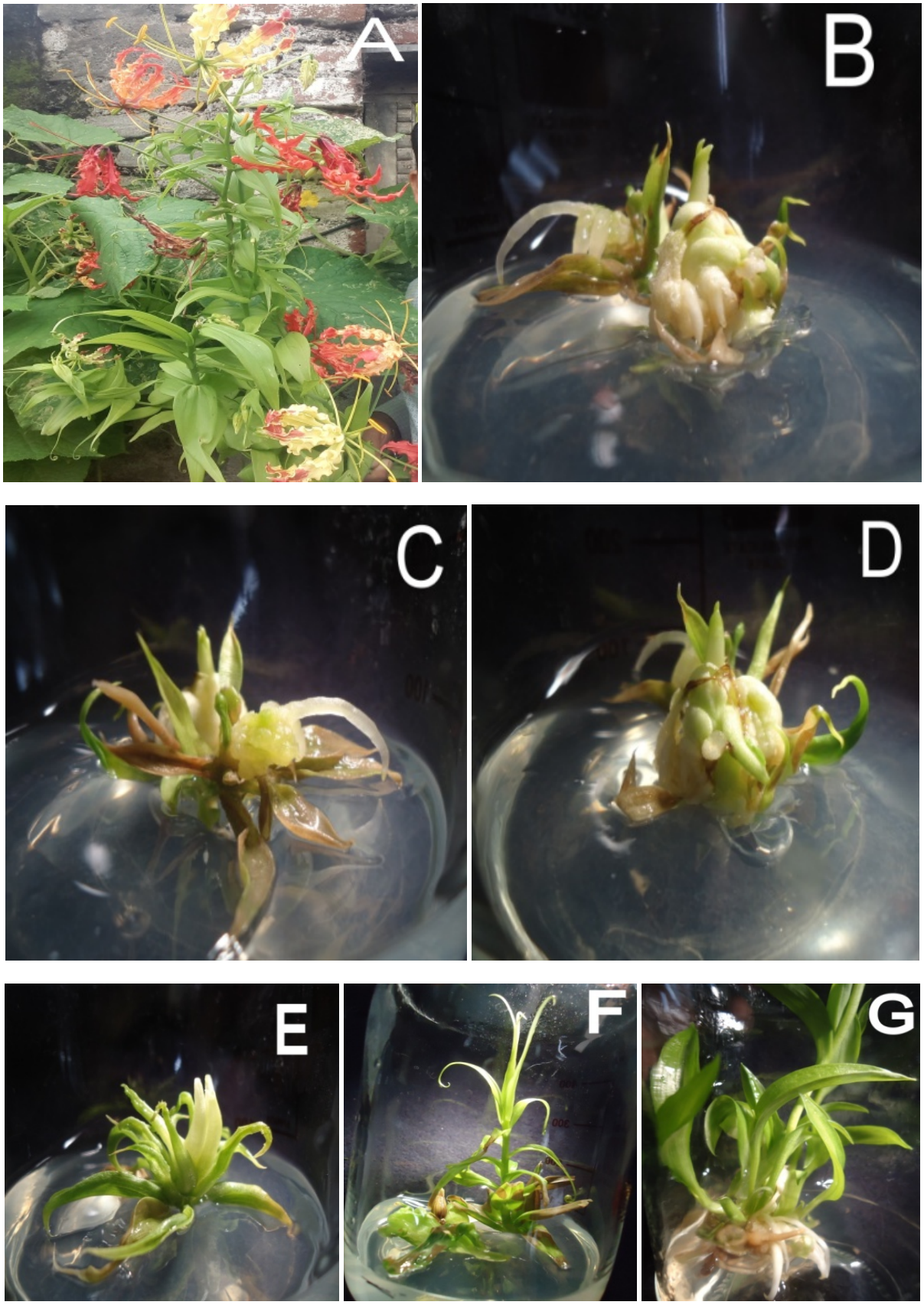

Plate 1: (A) flowering twig of *Gloriosa superba* L. (B) Callus induction by leaf (C) Callus induction by nodal segment (D) Callus induction by shoot tip (E) Multiple shoots (F) Shoot induction (G) Root induction.

Conclusions

Low seed germination rate and overexploitation of *Gloriosa superba* L. tubers for their bioactive compounds threatens making the population of scarce. Therefore, an *in vitro technique helps* their rapid multiplication and conservation.

Through this study we developed an efficient protocol for *in vitro* propagation of this plant and the significant results were obtained by using various combination and concentration of growth hormones.

Acknowledgements
We are heartily thankful to SARTHI, Pune for their financial support. We are also thankful to Professor and Head, Department of Botany, Dr. Babasaheb Ambedkar Marathwada University Aurangabad for providing the basic facilities to carry out this research work.

References

1. Bhide B, Acharya R. Uses of langali (*Gloriosa superba* Linn.): An Ethno-medicinal perspective. Ayurpharm - International Journal of Ayurveda and Allied Sciences,2012:1(3):65-72.
2. Chabukswar MM, Deodhar MA. Rooting and hardening of *In vitro* plantlets of *Garcinia indica* chois. Indian Journal of Biotechnology,2005:4:409-413.
3. Custers JBM, Bergervoet JHW. Micropropagation of *Gloriosa Superba*, Towards a Practical Protocol, Scientia Horticult,1994:57:323-334.
4. Hassan SA, Roy SK. Micropropagation of *Gloriosa Superba* L. Through high Frequency Shoot Proliferation, Plant Tissue Cult.,2005:15(1):67-74.
5. Mahajan R. *Gloriosa superba L.*: An endangered medicinal plant. Hort Flora Research Spectrum,2015:4:168-171.
6. Mann A, Banso A, Clifford LC. An antifungal property of crude plant extracts from Anogeissus leiocarpus and Terminalia avicennioides. Tanzania J. Health Res.,2008:10(1):34-38.
7. Murashige T, Skoog FA. revised medium for rapid growth and bioassays with tobacco tissue cultures, Plant Physiology,1962:15:473-497.
8. Nadkarni AK. Indian Materia Medica. Mumbai: Popular Prakashan Limited, 2002:579-581.
9. Ngomuo M, Mneney E, Ndakidemi PA. The *in vitro* propagation techniques for producing banana using shoot tip cultures. American Journal of Plant Sciences,2014:5:1614-1622.
10. Nautiyal OP. Isolation of 3-demethylcolchicine from *Gloriosa superba* L. sludge and coupling with -acetobromoglucose to yield colchicoside and thiocolchicoside /Journal of Natural Products,2011:4:87-93.
11. Naik VN. and Associates. Flora of Marathwada. Amurt Prakashan, Aurangabad, India, 1998:1-2.
12. Nikhila GS, Sangeetha G, Swapna TS. Antimicrobial screening of a medicinally potent plant *Gloriosa superba*. CIBTech Journal of Microbiology,2014:3:48-57.
13. Ramakrishnan R, Periyasamy R, Lakkakula S, Subramani P, Arockiam SR, Manikandan R. *et al*. *In vitro* Propagation and Conservation of Useful Endangered Medicinal Plants with Anticancer Activity. J Mol Biol Biotech,2017:(2):3:8:1-8.
14. Reuter S, Prasad S, Phromnoi K, Ravindran J, Sung B, Yadav RV. *et al*. Thiocolchicoside Exhibits Anticancer Effects through Downregulation of NF-B Pathway and Its Regulated Gene Products Linked to Inflammation and Cancer. Cancer Prevention Research,2010:3:1462-1472.
15. Rocha CL, 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.
16. Sivakumar G, Krishnamurthy KV. Micropropagation of *Gloriosa superb*a L. and endangered species of Asia and Africa. Current Science,2000:78(1):30-32.
17. Sivakumar G, Krishnamurthy KV. *In vitro* Organogenetic Responses of *Gloriosa Superba* L, Russian J. Plant Physiol,2004:51:790-798.