IN VITRO REGENERATION STUDIES IN KALLEVI (Gloriosa superba L.)

Dr. Sharad Pawar[1] and Dr. Vikram Jambhale[2]

[1-2]Mahatma Phule Krishi Vidyapeeth, Rahuri 413 722(M.S.)

ABSTRACT*: Gloriosa superba L. is declared as critically vulnerable in India and an alarming stage for Gloriosa superba L. So there is need of essential efforts for in vitro as well as ex vivo conservation of this plant. In present study, for micropropagation of an endangered medicinal plant Gloriosa superba L. Murashige and Skoog (MS) medium was used for initiation of explants with different concentrations of cytokinin (0.0, 0.5, 2.0, 4.0 or 6.0 mg L^{-1} BAP) for shooting and auxin (0.0, 0.5, 1.0, 2.0 and 4.0 mg L^{-1} IBA and NAA) for rooting. Different parts of plants such as apical shoot tips, nodal segments and meristematic regions of underground tubers were used as explants for culture initiation.*

The maximum number of shoots per explant in case of apical shoot explant obtained by using MS media supplemented with 0.5 mg L^{-1} BAP and in case of underground tuber explants best shooting results were obtained with 2.0 mg L^{-1} BAP. Similarly roots to the shooted explant under in vitro condition successfully obtained in case of underground tuber explant on MS supplemented with 2.0 mg L^{-1} IBA but aerial explant not able to produce roots under any auxin concentration level on MS media successfully. However, no shooting as well as rooting were obtained in case nodal explants with any concentrations of plant growth regulators. Present study also shown that hardening mixture sand: soil: vermicompost (1:2:1) gave better results for plant survival and plant growth.

INTRODUCTION:

Gloriosa superba L. is very important medicinal plant. "National Medicinal Plant Board of India" has included *Gloriosa superba* L. into the list of 32 nationally prioritized medicinal plants due to the presence of commercially important alkaloids - colchicine and colchicoside. Due to over exploitation and unscientific collection, *Gloriosa superba* L. is included in the world record of endangered plants i.e. Red Data Book by International Union for Conservation of Nature (IUCN) (Sivakumar and Krishnamurthy, 2000). Similarly due to highly unscientific collection of tubers from natural habitat for commercial exploitation, lead to scarcity of the plant in Indian scrub forests (Nautiyal, 2011).

It is generally used for curing asthma (Patil *et al.,* 2008), have antimicrobial activities (Budhiraja *et al.,* 2012). Some, more important properties observed in *Gloriosa superba* L. by various scientist like as a general tonic (Kala *et al.,* 2006), gout (Pal and Palit, 2011), antiparasitic, insecticidal and larvicidal properties (Madhumitha *et al.*, 2012).Conventional approaches of propagation of *Gloriosa superba* L. are carried out by tuber and seed, since poor seed germination are major drawback. Therefore, there is a need for better *in vitro* micropropagation technique for the high rate of plantlets development and conservation.

Tissue culture approaches of the plants are essential for mass multiplication rate, promotes *in vitro* production of secondary metabolites as well as a good approaches for conservation. The main advantage of this techniques is the year round availability of plant materials for colchicine production without geographical restriction, the products can be free from any pesticide contamination and the production cost can be reduced (Sivakumar *et al.,* 2004).

REVIEW OF LITERATURE:

Gloriosa superba L. is a herbaceous climber belonging to family Colchicaceae (monocotyledon). *Gloriosa superba* L. is an important medicinal plant, due to presence of colchicine. Eddleston and Persson (2003) studied about plant poisoning, antitoxin and antibodies activity of *Gloriosa superba* L.

Chakraborty and Bhattacharjee (2006) reported some general ethnomedicinal uses of *Gloriosa superba* L. tubers curing various diseases i.e. small pox and leprosy in Purulia district, West Bengal. Yineger *et al*. (2008) presented related information of ethnomedicinal plant and their practice by the Oromo ethnic group in south western Ethiopia. Khan *et al.* (2008) worked on antimicrobial activities of *Gloriosa superba* L. (Colchicaceae) extracts. Hemaiswarya *et al.* (2009) worked on antimicrobial and mutagenic properties of the root tubers of *Gloriosa superba* L. Pawar *et al.* (2010) studied anthelmintic activity of *Gloriosa superba* L.

Kavina *et al.* (2012) examined difenoconozole and propiconazole effects on antioxidant potentials of *Gloriosa superba* L. Bhide and Acharya reported (2012) use of langali (*Gloriosa superba* L.) as an ethnomedicinal

perspective. Budhiraja *et al.* (2012) worked on antimicrobial and cytotoxic activities of fungal isolates of medicinal plant *Gloriosa superba* L. Rehana and Nagarjan (2012) performed phytochemical screening for active compounds of leaves and tubers of *Gloriosa superba* L.

Mahajan (2016) reported that the plant tubers are toxic due to presence of rich alkaloid, colchicine which plays an important role in cytology by inducing polyploidy. The alkaloid colchicine also plays important role for having antiabortive, antimicrobial and anticancer activity.Ashokkumar *et al*. (2017) conducted the experiment which shows that all the parts of *G. superba* L. *viz*., seeds, rhizomes, rind, leaves and flowers exhibited antifungal activity against *Fusarium oxysporum* by significantly reducing the mycelial growth *in vitro.*

The plant is used to cure arthritis, gout, rheumatism, inflammation, ulcers, bleeding piles, skin diseases, leprosy, impotency, snakebites, etc.Mahajan *et al.* (2016) concluded that there is threat to genetic diversity of *Gloriosa superba* L. due to overharvesting, so there is an urgent need to conserve this plant using biotechnological approaches.

Paudel and Pant (2013) establish a successful protocol for *in vitro* multiplication of a plant species is species-specific and depends upon several factors, including culture media, hydroponic systems (Lopes da Silva, 2006) and concentration of agar and sucrose in the medium.

MATERIAL AND METHODS:

The present study, "Micropropagation studies in Kallevi (*Gloriosa superba* L.)" was carried out at State Level Biotechnology Centre, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Explant material required (apical shoot tip, nodal segment and underground tuber) for micropropagation studies collected from Medicinal and Aromatic Plant Project, Mahatma Phule Krishi Vidyapeeth, Rahuri.

Media used for the present investigation was Murashige and Skoog (MS) medium as a basal media.

MS medium was used as establishment medium, supplemented with the different concentration of BAP (0.0, 0.5, 2, 4 and 6 mg L^{-1}). These cultures were incubated in culture room with 2000 lux light intensity and at 25^{o}C ± 2 temperatures; with 16 hrs light and 8 hrs dark regime. The observations were recorded after 1 month; for bud break %, shoot number, shoot length and node number. The effects of various concentrations of cytokinin (BAP) on MS medium were studied on shoot initiation from apical shoot tip, nodal segment and underground tuber explants.

Established cultures, after the growth of four weeks, were sub cultured on fresh medium for further shoot multiplication. For the purpose, different plant growth regulators were tested in MS medium, for shoot proliferation.

In vitro grown shoots from apical meristems were subjected to auxin treatment for production to complete plant. For rooting, half strength MS medium with different auxins were used. Percentage of the shoots that developed to root was recorded after 4-6 weeks of culture. The following treatments were tested:

1. MS medium supplemented with NAA (00, 0.5, 1.0, 2.0 and 4.0 mg L^{-1}).
2. MS medium supplemented with IBA (00, 0.5, 1.0, 2.0 and 4.0 mg L^{-1}).

Plantlets of *Gloriosa superba* L. with well developed shoots and roots were taken out from the culture vessels, washed gently with tap water and transferred to one of three different planting substrates, *viz*. sterile soil: sand (2:1), sand: soil: farm yard manure (FYM) (1:2:1) and sand: soil: vermicompost (1:2:1), for acclimatization. Potted plantlets were covered with transparent polyethylene bags to maintain humidity and watered every 2 days with half strength.

RESULTS AND DISCUSSION:

Three different explants-apical shoot tips, nodal segments and meristematic zone of underground tubers were used to initiate culture on MS medium containing different concentration of PGRs. Results were analysed by percent of bud break, number and length of shoots per explants, number of roots per plantlets, height of plantlets and number of plantlets survived during hardening.

Table 1: Surface disinfestation of underground tuber explant of *Gloriosa Superba* L.for culture establishment. Observations recorded after 4 weeks of culture

Procedure		Steps	Disinfectants	Concentration (%)	Treatment time (min)	Contamination (%)
In Explants Preparati -on Room	1	Washing in running tap water	-	-	3-4 times	-
	2	Treatment with liquid detergent	Tween-80 liquid detergent	0.1	10-15	-
	3	Fungicides and antibiotic treatment	Bavistin+ Dithane M-45 + Streptocycline (Antibiotic)	0.1+0.25 + 0.1	15-20	-
In LAF	4	Alcoholic dip Treatment	Ethyl alcohol	70	1	-
			Mercuric chloride ($HgCl_2$)	0.1	5	60
					10	40
					15	20
				0.2	5	40
					10	20
					15	00

The best result of zero contamination was recorded with the 15 min. duration of 0.1% $HgCl_2$ treatment

In the present study, there were 3 types of explants: apical shoot tips, nodal segments and meristematic regions of underground tubers were inoculated on MS medium which was supplemented with different concentration of BAP (0.0, 0.5, 2, 4 and 6 mg L^{-1}).

Table 2: Effect of BAP on shoot initiation from shoot tip explants of *Gloriosa superba* L., on MS medium; after 4 weeks

BAP mgL^{-1}	Bud Break (%)	No. of shoot per Explants (Mean ± SD)	Shoot length (Mean ± SD) (cm)	No. of node (Mean ± SD)
00	60	1 ± 0	3.7 ± 0.3	2.0 ± 0.8
0.5	90	2.18 ± 0.4	6.3 ± 0.1	3.6 ± 0.5
2.0	90	1.21 ± 0.4	3.9 ± 0.2	2.2 ± 0.7
4.0	80	1.18 ± 0.4	3.0 ± 0.2	1.6 ± 0.5
6.0	50	1.12 ± 0.3	3.0 ± 0.2	1.8 ± 0.5

Table 3 : Effect of BAP on shoot initiation from underground tuber explants of *Gloriosa superba* L. on MS medium; after 4 weeks

BAP mgL^{-1}	Bud break (%)	No. of shoots per explants (Mean ± SD)	Shoot length (cm) (Mean ± SD)	No. of nodes (Mean ± SD)
00	40	1 ± 0	2.2 ± 0.1	2.3 ± 0.5
0.5	60	1.75 ± 0.3	2.2 ± 0.2	2.4 ± 0.5
2.0	70	2.30 ± 0.3	3.2 ± 0.2	2.9 ± 0.4
4.0	50	1.81 ± 0.5	2.6 ± 0.2	2.2 ± 0.5
6.0	50	2.10 ± 0.4	2.6 ± 0.2	2.3 ± 0.4

Table 4 : Performance of all the explant types of *Gloriosa superba* L., during culture initiationon MS medium; after 4 weeks

Explant Types	BAP (mg L^{-1})	Bud break (%)	No. of shoots per explant (Mean ± SD)	Shoot length (cm) (Mean ± SD)
Shoot tip	0.5	90	2.18 ± 0.4	6.3 ± 0.1
Underground tuber	2.0	70	2.30 ± 0.3	3.2 ± 0.2

Table 5: Effect of different auxins on rooting of regenerated shoots from underground tuber explant *Gloriosa superba* L. on half strength on MS medium; after 4 weeks

Auxin	mgL^{-1}	Average no. of roots per explant (Mean ± SD)
NAA	00	00
	0.5	5.1 ± 0.6
	1.0	4.3 ± 0.5
	2.0	2.4 ± 0.4
	4.0	2.7 ± 0.5
IBA	00	00
	0.5	1.8 ± 0.4
	1.0	3.9 ± 0.4
	2.0	7.4 ± 0.5
	4.0	5.8 ± 0.5

Table 6 : Hardening of tissue culture plantlets of *Gloriosa superba* L.

S. No.	Hardening Media	Plantlets Survived (%)	Average plant height (cm) (After 120 days)
1	Sterile soil: sand (2:1)	77.40	14.2

2	Sand: soil: F.Y.M. (1:2:1)	85.00	17.4
3	Sand: soil: vermicompost (1:2:1).	90.00	23.2

SUMMARY AND CONCLUSIONS:

Three different explants of *Gloriosa superba* L. were used to initiate *in vitro* culture. In case of underground tuber explant, much higher treatment 0.2 % mercuric chloride for 15 min. was required for successful contamination free culture than the two aerial shoot explant-shoot tips and nodal segments: 0.1% mercuric chloride for 15 min. At culture establishment stage, all the 3 explant types behaved differently. The best bud break response and shoot elongation were recorded in presence of 0.5 mgL^{-1} BAP in MS medium from apical shoot tip explants.

On the other hand, 2nd explant type the nodal segments failed to produce shoots *in vitro,* in any case; even when the apical meristem was absent and also the exogenous cytokinin was present.

The third explant, underground tuber meristems exhibited best shoot production in the presence of 2 mgL^{-1} BAP in MS medium. Strong apical dominance in *Gloriosa superba* L. was also recorded during *in vitro* culture when only the apical bud was produced shoot; the nodal meristems were unable to produced shoots even when the apical shoot tip was absent and exogenous cytokinin was present in the medium.

Acclimatization of plantlets was carried out by using different hardening media among these hardening mixture sand: soil: vermicompost (1:2:1) gave better results for plant survival and plant growth, so this mixture is most suitable for the hardening of the *in vitro* generated plantlets of *Gloriosa superba* L.

Thus, the present report showed faster production of plantlets from underground tuber explants of *Gloriosa superba* L. than previous reports.

LITERATURE CITED

1) Ashokkumar, N., Shanthi, A., Sivakumar, M. and Rajamani, K. 2017. Studies on nutrient enhancement of Glory lily (Gloriosa superba L.), Rind J. Pharmacognosy and Phytochem.; 6(6): 1410-1412

2) Bhide, B. and Acharya, R. 2012. Uses of langali (Gloriosa superba L.): an ethnomedicinal perspective. Ayurpharm Int.J.Ayur.Alli. Sci., 1(3):65-72.

3) Budhiraja, A., Nepali, K., Kaul, S. andDhar, K. L. 2012. Antimicrobial and cytotoxic activities of fungal isolates of medicinal plant Gloriosa superba L. Int. J. Rec. Adv. Pharm. R., 2(1):37-45.

4) Chakraborty, M. K. and Bhattcharjee, A. 2006. Some common ethnomedicinal uses for various diseases in Purulia district, West Bengal. Ind. J. Trad. Knowledge, 5(4):554-558.

5) Eddleston, M. and Persson, H. 2003. Acute plant poisoning and antitoxin antibodies. J. Toxicol.Clinical Toxicol., 41(3):309-315.

6) Hemaiswarya, S., Rathinam R.,Chinnathambi, A. and Venkatesan, T. 2009. Antimicrobial and mutagenic properties of the root tubers of Gloriosa superba L. Pak. J. Bot., 41(1): 293- 299.

7) Kala, C. P. and Dubey, Y. 2006. Anthropogenic disturbances and status of forest and wildlife in the dry deciduous forests of Chhattisgarh state in India. J. Forestry R., 23(1): 45-52.

8) Kavina, J., Gopi, R. and Pannerrselvam, R. 2012.Difenoconozole and propiconazole‟s effects on antioxidant potentials of Gloriosa superba L. World J. Agril. Sci., 8(3):247- 252.

9) Khan, H., Khan, M. A., Mahamood, T. and Choudhary, M. I. 2008. Antimicrobial activities of Gloriosa superba L. (Colchicaceae) extracts. J. Enzyme Inhibition and Medi. Chem., 23(6): 855-859.

10) Lopes da Silva, A. L., Henz, F. E. T., Horbach, M.A. and Walter, J. M. 2006. Aclimatizacão demudas de Cattleya tigrina A. Rich. Ex Beer (Orchidaceae) emsistemahidroponico. Cuaderno de PesquisaSér Bio.18: 129–139.

11) Madhumitha, G., Govindasamy, R. K., Roopan, S. M., Rahuman, A. A., Priya, K. M., Saral, A.M., Khan, F. R. N. and Khanna, V. G. 2012.Acaricidal, insecticidal, and larvicidal efficacy of fruit peel aqueous extract of Annona squamosa and its compounds against blood-feeding parasites. Parasitol. Res., 111:2189-2199.

12) Mahajan, R. 2016. Gloriosa superba L.:An endangered medicinal plant. Hort. Flora Research Spectrum,4: 168-171.

13) Murashige, T. and Skoog, F. 1962.A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiol. Plant, 15: 473- 497.

14) Nautiyal, O. P. 2011. Isolation of 3-demethylcolchicine from Gloriosa superba L. sludge and coupling with α-acetobromoglucose to yield colchicoside and thio-colchicocide.J. Natural Products, 4: 87-93.

15) Pal, S. and Palit, D. 2011. Traditional knowledge and bioresource utilization among Lepcha in North Sikkim. NeBIO, 2(1):13-17.

16) Patil, G. G., Mali, P. Y. and Bhadane, V.V. 2008. Folk remedies used against respiratory disorders in Jalgaon district, Maharashtra. Natural Product Radiance, 7(4): 354-358.

17) Paudel, M. R. and Pant, B. 2013. A reliable protocol for micropropagation of Esmeralda clarkei (Orchidaceae). AsPac. J. Mol. Bio. Biotechnol.21: 114-120.

18) Pawar, B. M., Wavhal, V.P., Pawar, N.D., Agarwal, M. R., Shinde, P.B. and Kamble, H. 2010. Anthelmintic activity of Gloriosa superba L. (Colchicaceae). Int. J. Pharm. Tech. R., 2(2):1483-1487.

19) Rehana, B.H. and Nagarajan, N. 2012. Phytochemical screening for active compounds in Gloriosa superba leaves and tubers. Int.J. Pharmaco. Phytochem. R., 4(1):17-20.

20) Sivakumar, G. and Krishnamurthy, K.V. 2000. Micropropagation of Gloriosa superba L.-an endangered species of Asia and Africa. Curr. Sci., 78(1):30-32.

21) Sivakumar, G. and Krishnamurthy, K. V. 2004. In vitro organogenesis responses of Gloriosa Superba L. Rus. J. Plant Physiol., (51) 5:713-721.

22) Yineger, H., Yewhalaw, D. and Teketay, D. 2008. Ethnomedicinal plant knowledge and practice of the Oromo ethnic group in southwestern Ethiopia. Journal of Ethnobiology and Ethnomedicine, 4 (11): 1-10.