Indian J.Sci.Res. 20(2): 01-05, 2018

ISSN: 0976-2876 (Print)
ISSN: 2250-0138(Online)

CALLUS INDUCTION FROM TUBERS IN *Gloriosa superba* L.

J. NAGA LAKSHMI[1]

Cytogenetics, Tissue Culture and Molecular Biology Lab, Department of Botany, Osmania University, Hyderabad, Telangana, India

ABSTRACT

***Gloriosa superba* L. is an important medicinal plant belonging to the family Colchicaceae. It is one of the important medicinal plant now in endangered list. In present study an efficient protocal was developed for induction of callus derived from tuber and nodal regions of *Gloriosa.* MS medium produced profuse, white, friable callus within two to three weeks in the combination of BAP + Kn induced friable embryonic callus from nodal and tuber explants. 2,4-D + Kn produced profuse, white, friable callus in all the cultured tuber explants. Highest callus formation was observed in tuber explants.**

KEYWORDS: *Gloriosa superba*, Organogenesis, Clochicine, Friable Callus, Ornamental

Gloriosa superba diversity still offers opportunities for further selection either for chemical constituents or as an ornamental. It is a semi- woody herbaceous branched climber attaining about 5 meter height, with brilliant wavy – edged yellow & red flowers. (Rajak and Rai, 1990).*Gloriosa superba* from seed requires more time.As seed germination is poor and vegetative propagation is slow, *In vitro* culture based micro propagation technique has been successfully used for rapid & mass propagation of many medicinal plants. In order to provide enough plant material for commercial exploitation, mass multiplication through tissue culture is urgently needed not only to conceive. Therapeutically properties of medicinal plants are very useful in healing various diseases and the advantage of medicinal plants are natural (Kalemba and Kunicka, 2003). Worldwide, medicinal plants have been used for their antibacterial, antifungal and antiviral activities (Ali *et al*. 1998; Barbour *et al.* 2004; Yasunaka *et al.* 2005). Researchers have focused their attention to natural products to develop better drugs against cancerand viral and microbial infections (Ibrahim, 1997; Towers *et al.* 2001; Koshy *et al.* 2009). Tubers of *Gloriosa* are sold in Indian market as an important source of alkaloid. Colchicine reduces the inflammation & relieves the pain associated with acute gout (Insel, 1996).

MATERIALS AND METHODS

Explants Sterilization and Inoculation

The leaves and nodal explants of *Gloriosa superba* were thoroughly washed under running tap water for 10 min and carried to laminar airflow bench. They were first rinsed with sterile distilled water, then 2 drops of Tween-20 were added to sterile water and once again rinsed for 10 min with intermittent shaking. Then explants were again rinsed with 0.01% (w/v) mercuric chloride for 5 minutes followed by rinsing them with 0.1% bavistin for 2 min, washed with sterile distilled water and again rinsed with 10% sodium hypo chlorite for 2 min, then finally these surface sterilized explants were washed 3-4 times with sterile distilled water and inoculated on to culture medium with sterile forceps into appropriate culture tubes.

Temperature and Light

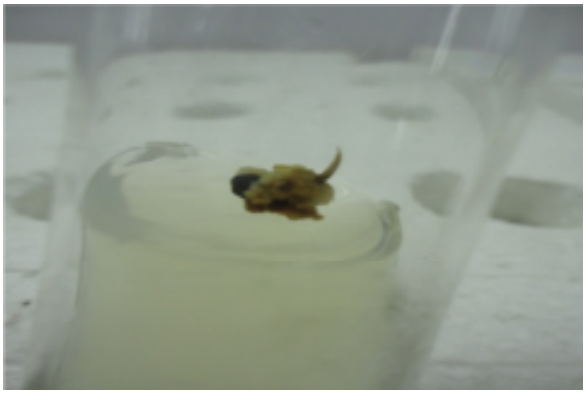
All the cultures were maintained at a temperature of 25+ 2^0C under illumination provided by cool white, fluorescent light with 16 hours photoperiod at with light intensity of (30µE m-2 S-1).

RESULTS

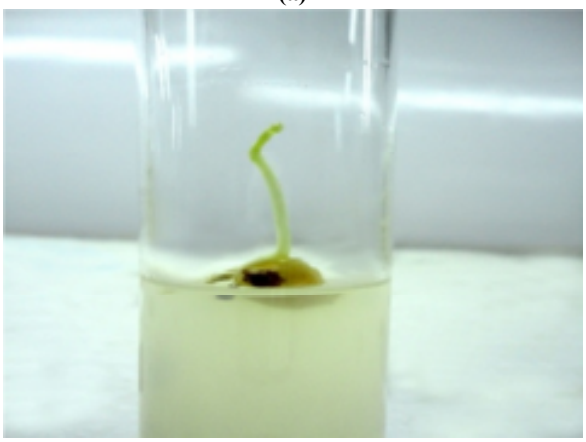
Callus Induction

The explants (tuber, nodal) were inoculated on basal medium supplemented with the combination of 2, 4-D + Kn and BAP + Kn. Callus was obtained in various conc. of 2, 4-D (Dichlorophenoxy acetic acid) (1-4 mg L^{-1}), BAP (6' – benzyladenine purine) (1-4 mg L^{-1}), Kn (Kinetin) (0.25-0.5 mg L^{-1}). MSMedium produced profuse, white, friable callus within two to three weeks. The best results were obtained at conc. of BAP (3.5 mg L^{-1}) + Kn (0.5 mg L^{-1}) induced friable embryonic callus from nodal and tuber explants. 2,4-D (3.5 mg L^{-1}) + Kn (0.5 mg L^{-1}) produced profuse, white, friable callus in all the cultured tuber explants. Highest callus formation was observed in tuber explants. (Table-1, Figure-1).

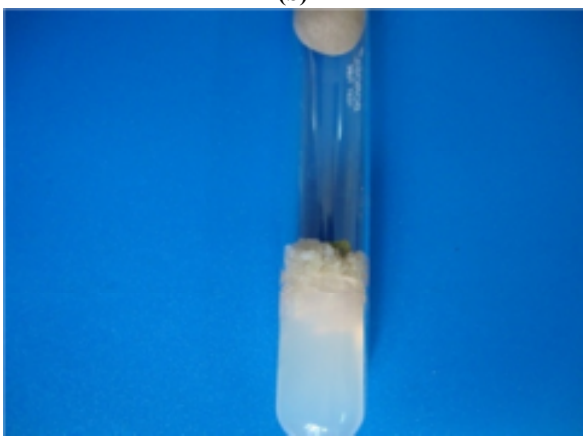
[1]Corresponding Author



(a)

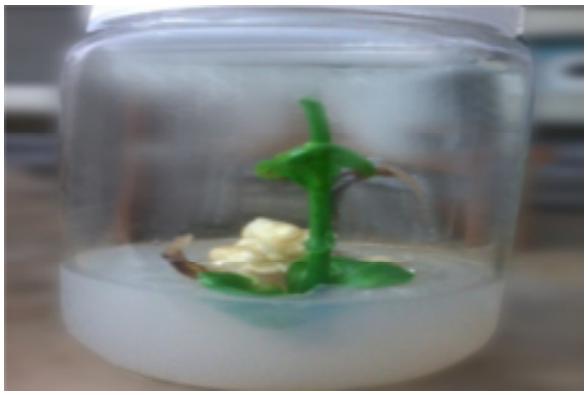


(b)

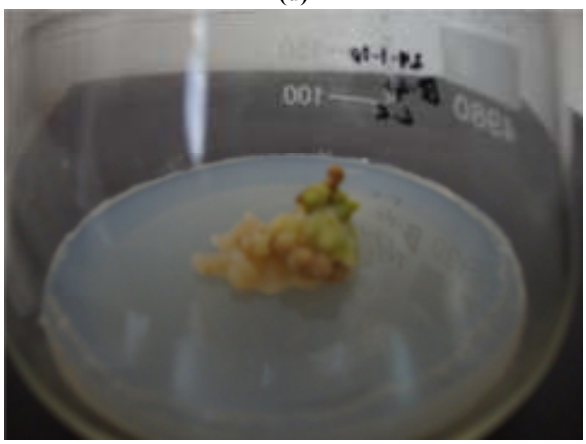


(c)

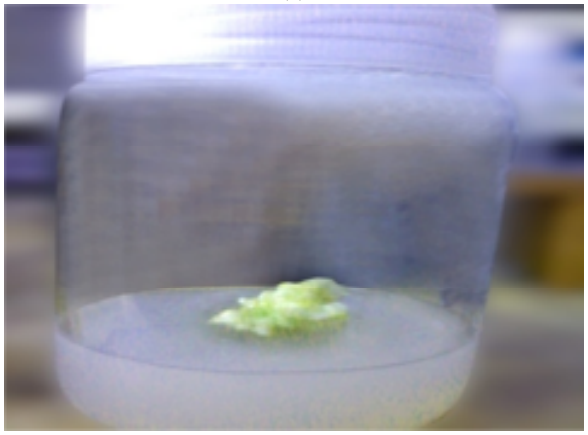
Figure (a-c): *Gloriosa superba*showing callus initiation from tuber in MS medium consisting of 2,4-D (3.5 mg L^{-1}) + Kn (0.5 mg L^{-1})



(d)



(e)



(f)

Figure (d-f): Callus initiation from node in MS medium consisting of (3.5 mg L^{-1}) + Kn (0.5 mg L^{-1})

Figure 1: Proliferated callus from tuber and nodal region of *Gloriosa*

Table 1: Effect of different plant hormones on callus induction in *G. superba*

Hormones (mg L^{-1})	Tuber	Nodal region	No. of days	Response
BAP+Kn, 1.0+0.25	+	+	35-40	slightly friable callus
BAP+Kn, 1.5+0.5	+	++	30-35	slightly friable embryogenic callus
BAP+Kn, 2.0+0.5	+	+++	20-25	Slightly, compact embryogenic callus
BAP+Kn, 2.5+0.5	+	+++	15-20	white proliferating callus
BAP+Kn,3.0+0.5	**++**	**+++**	**15-17***	**white proliferating callus**
BAP+Kn, 3.5+0.5	++	+++	20-25	white proliferating callus
BAP+Kn, 4.0+0.5	++	++	30-35	white proliferating callus
BAP+Kn, 4.5+0.5	+++	++	35-40	white friable callus
2 ,4-D+Kn, 1.0+0.25	+	+	15-17	white creamy callus
2,4-D+Kn, 1.5+0.5	++	+	17-20	white creamy callus
2,4-D+Kn, 2.0+0.5	**+++**	**+**	**20-25***	**white creamy callus**
2,4-D+Kn, 2.5+0.5	+++	+	25-30	white creamy callus
2,4-D+Kn, 3.0+0.5	+++	++	30-35	Slight green friable callus
2,4-D+Kn, 3.5+0.5	+++	++	35-37	Green callus turn to brown
2,4-D+Kn, 4.0+0.5	++	+++	37-40	Brown and death of the callus
2,4-D+Kn, 4.5+0.5	++	++	40-45	Brown and death of the callus

* Values of Mn ±SD of 3replicates.

** Plant regeneration after 4 weeks in culture

Table 2: Enhancement of tuber explants of *G. superba* on MS media combination of 2, 4-D and Kn.

Hormone conc.(mg L^{-1})	Callus induction (%)	Fresh weight(g)	Dry weight (g)
2,4-D+Kn,1.0+0.25	57.16	1.217±0.751	0.107±0.002
2,4-D+Kn, 1.5+0.5	60.39	1.267±0.097	0.119±0.004
2,4-D+Kn, 2.0+0.5	63.55	1.284±0.076	0.117±0.006
2,4-D+Kn, 2.5+0.5	65.76	1.472±0.0.21	0.164±0.049
2,4-D+Kn, 3.0+0.5	69.43	1.672±0.059	0.166±0.083
2,4-D+Kn, 3.5+0.5	**74.81**	**1.738±0.051**	**0.169±0.007**
2,4-D+Kn, 3.5+0.5	71.07	1.681±0.063	0.173±0.073
2,4-D+Kn, 4.0+2.5	68.49	1.572±0.24	0.162±0.003

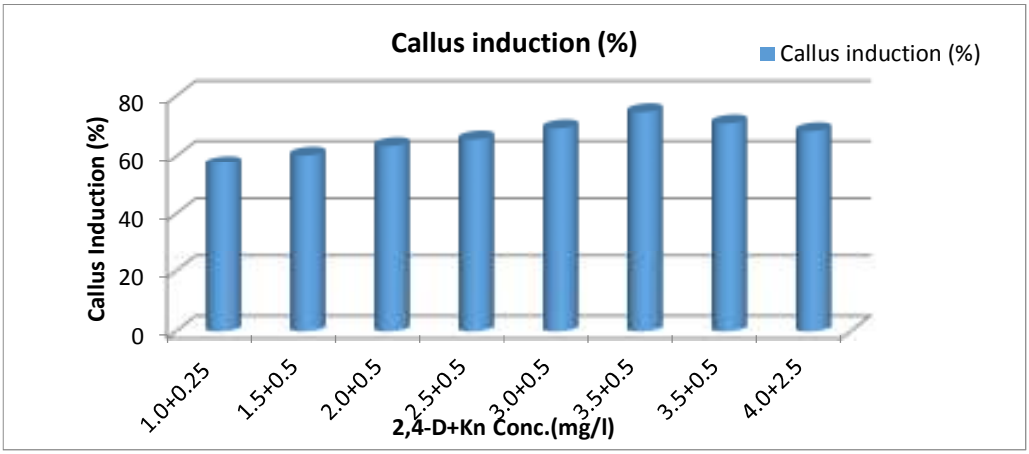


Figure 2: Enhancement of callus induction from tuber explants of *G. superba* on MS media combination of 2, 4-D and Kn.

Table 3: Enhancement of nodal region explants of *G. superba on* MS media combination of BAP and Kn.

Hormone conc. (mg/l)	Callus induction (%)	Fresh weight (g)	Dry weight (g)
BAP+Kn, 1.0+0.25	63.3	1.287±0.078	0.116±0.006
BAP+Kn, 1.5+0.5	67.42	1.435±0.026	0.167±0.003
BAP+Kn, 2.0+0.5	69.54	1.675±0.069	0.163±0.091
BAP+Kn, 2.5+0.5	74.71	1.751±0.055	0.171±0.006
BAP+Kn, 3.0+0.5	**77.86**	**1.932±0.062**	**1.193±0.009**
BAP+Kn, 3.5+0.5	71.27	1.615±0.510	1.134±0.025
BAP+Kn, 3.5+0.5	68.19	1.582±0.024	0.172±0.004
BAP+Kn, 4.0+2.5	65.04	1.708±0.034	0.168±0.049

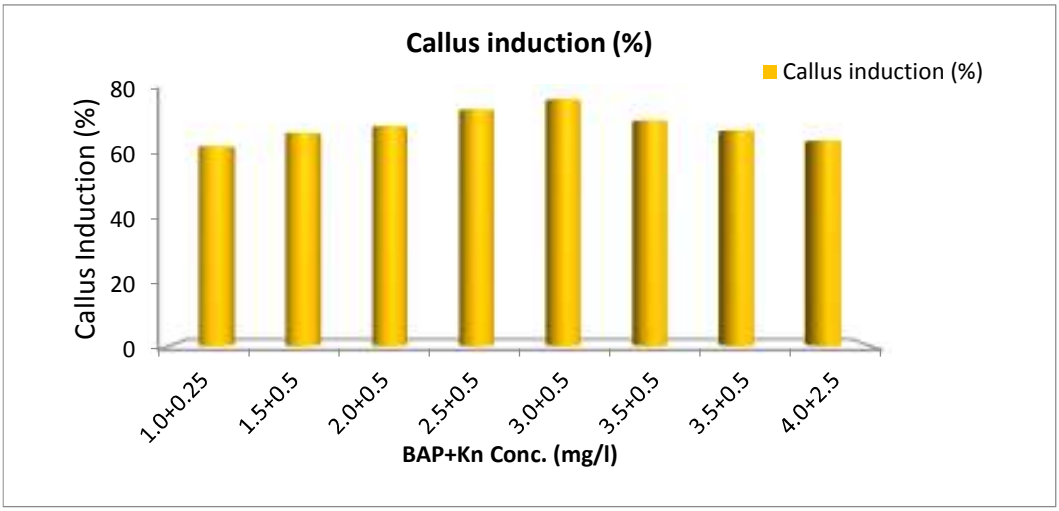


Figure 3: Enhancement of callus induction from nodal region explants of *G. superba o*n MS media combination of BAP and Kn

Callus obtained in the experiments was sub cultured in various organogenic media (i.e.,, MS supplemented with BAP and Kn). After 2-3 passages the fresh and dry weights were taken. BAP (3.0 mg/l + Kn 0.5 mg/l) proliferated callus as shown by fresh and dry weights. Further transfer onto organogenic medium did not show any shoot or root formation.

DISCUSSION

The best results were obtained at conc. of BAP (3.5 mg L^{-1}) + Kn (0.5 mg L^{-1}) induced friable embryonic callus from nodal and tuber explants (Table-1, Figure-1). 2, 4-D (3.5 mg L^{-1}) + Kn (0.5 mg L^{-1}) produced profuse, white, friable callus in all the cultured tuber explants (Table-16, Fig-18). Highest formation in tuber explants. The similar reports were obtained in *Helianthus annuus* Reddy *et al*. (1990). After 2-3 passages the fresh and dry weights were taken. BAP (3.0 mg L^{-1} + Kn 0.5 mg L) proliferated callus as shown by fresh and dry weights. Further transfer onto to organ genic medium did not show any shoot or root formation. But in the present studies somatic embryos were also induced from the callus developed from shoot primordial (Jadhav and Hedge, 2001) and directly from leaf explants (Manju and Joy, 2008). Histological marker to organogenesis calli of *Gloriosa superba* (Manju *et al*. 2010). SEM analysis were made to conform the growth and development of somatic embryos in Date palm (Rao *et al.*2001).

CONCLUSION

Gloriosa superba is a commercially imperative medicinal plant which has diverse medicinal applications and eventually due to over-exploitation this plant is facing local extinction. It has been affirmed as endangered plant by IUCN and hence there is a pressing need to conserve the plant by callus multiplication in micro propagation method so as to meet the ever increasing demand from the industries. Furthermore, responsiveness should be

generated among the common people concerning the importance of *G. superba* and its overexploitation by the people. Peoples participation in conservation of rare and endangered medicinal plants like *G. superba* will also be very useful.

REFERENCES

Murashige T.F. and Skoog F., 1962. A revise medium for rapid and bioassays with tobacco tissue culture. Physiologia Plantarum, **15**: 473-497.

Karnakar Reddy S., Talat T. Farook, Srinivas Charya K. and Farook S.A., 1990. Induction of callus from various explants in Sunflower (*Helianthus Annuus* L.). Mendel, **7**(3-4): 303-308.

Jadhav S.Y. and Hedge B.A., 2001. Somatic embryogenesis and plant regeneration in *Gloriosa superba* L. India J.Exp. BioL., **39**(9):943-946.

Madhavan M. and Joy Joseph P., 2008. Direct somatic embryogenesis in *Gloriosa superba* L., an endangered medicinal plant of India.

Madhavan M. and Joy Joseph P., 2010. Histological Marker to Differentiate Organogenic Calli fromNon Organogenic Calli of *Gloriosa superba* L. Plant Tissue Cult. & Biotech., **20**(1): 1-5.

Rao A.M., Reddy S.K., Rao V.R.M. and Bhalla J.K., 2001. Clonal propagation of Date palm through somatic embryogenesis. Indian Forestry, pp.463-465.

Yasunaka K., Abe F., Okabe H., Mumizi E.E., Aguilavi A. and Ryes-Chilps R., 2005. Antibacterial activity of crude extracts from Mexican medicinal plants and purified coumauries and vanthones. J. Ethnopharmacol., **97**: 293-299.

Ibrahim M.B., 1997. Anti-microbial effects of extract leaf, stem and root bark of *Anogeissus leiocarpus* on *Staphylococcus aureaus*, *Streptococcus pyogenes*, *Escherichia coli* and *Proteus vulgaris*. J. Pharma. Devpt., **2**: 20-30.

Insel P.A., 1996. Analgesic-antipyretic and anti inflammatory agents and drugs employed in the treatment of gout: colchicine. In: Goodman L.S., Limbird L.E., Milinoff P.B., Ruddon R.W., Goodman Gilman A. (eds) Goodman and Gilman's: The Pharmacological Basis of Therapeutics, 9th edn. Mc Graw-Hill, pp. 614–658.

Ali M.S., Yaghmour R.M. and Faidi Y.R., 1998. Antimicrobial activity of 20 plants used in folkloric medicine in the Palestinian area. J. Ethnopharmacol., **60**: 256-271.

Rajak R.C. and Rai M.K., 1990. Herbal Medicines Biodiversity and Conservation Strategies. International Book Distributors, pp. 75-79.

Kalemba D. and Kunicka A., 2003. Antibacterial and antifungal properties of essential oils. Current Medicinal Chemistry, **10**: 813-829, ISSN 0929-8673.