

# A comparative phytochemical composition of *Gloriosa superba* L. callus tissues and its natural leaf and fresh tuber roots extracts

S.V.AHIRE

P.G. Department of Botany,

Nowrosjee Wadia College, Pune, (M.S.) INDIA.411001.

## ABSTRACT

*Gloriosa superba* L. also known as Malabar glory lily is a commercially important medicinal plant which has diverse medicinal applications and eventually due to over-exploitation this plant is facing local extinction. Plant tissue culture technology including micro propagation has much potential in facilitating experimental studies to gain a better understanding of the biology of endangered plants. In the present research work, the phytochemical composition of Callus, leaves and tuber roots was investigated using different phytochemical methods. The work is divided in two parts: *in vitro* propagation and phytochemical analysis. The explants used for *in vitro* propagation are leaf and fresh tuber roots. Maximum callus was obtained in M.S medium supplemented with BAP (1.5-3 mg/l) in combination with NAA (0.2-0.5 mg/l). Organogenesis responses better in M.S. medium supplemented with N.A.A. (0.5 mg/l) and BAP (1.5 mg/l). Obtained callus, fresh tuber roots and leaves are further used for phytochemical analysis which includes the qualitative and quantitative analysis of reducing sugar, alkaloids, proteins, and nucleic acid (DNA and RNA).

**Key words:** *Gloriosa superba* L., Phytochemical, *In Vitro* propagation.

## INTRODUCTION

*Gloriosa superba* L. also known as Malabar glory lily. Glory lily (*Gloriosa superba* L.) is a medicinal plant belonging to the family Liliaceae is a semi-woody herbaceous branched climber reaching approximately 5 meters height, with brilliant wavy-edged yellow and red flowers (Rajak & Rai, 1990). One to four stems arise from a single V-shaped fleshy cylindrical tuber. *Gloriosa superba* L. is one of the endangered species among the medicinal plants (Badola, 2002) commonly known as Kalihari in Hindi, Kal-lavi in Marathi, Manthorikhizangu in Malayalam and Kazhappaikizhangu in Tamil. Studies reveal that all parts of the plant especially the tubers & seeds contain alkaloids such as colchicines and Gloriosine (Trease and Evans, 1983). In the Ayurveda, the tubers are used as tonic, antiperiodic, antihelmenthic, and also against snake bites (Gupta *et al.*, 2005). *Gloriosa superba* L. so used in wounds, skin related problems, Fever, Inflammation, piles, blood disorders, Uterine contractions, General body toner, Poisoning (Haroon *et al.*, 2008). *Gloriosa superba* L. has gained the importance in medicine in recent years & is indicated promising drug for the production of colchicine on commercial scale (Kokate *et al.*, 2004). *G. superba* L. is an important medicinal plant, which contains many valuable secondary metabolites. Due to heavy market demand of different useful parts of plant, the availability is continuously decreasing in wilderness. Plant tissue culture, particularly micropropagation techniques can be applied to clone a large number of an endangered plant with minimal damage to the natural populations.

## RESEARCH METHODOLOGY

### Collection and identification of plant material

The plant material was collected from Botanical garden, Department of Botany, Sir Parashurambhau College, Pune. Efforts were made to collect the plant in flowering condition for the correct botanical identification and authentication. It was identified with help of Flora of Presidency of Bombay (Cooke, T.; 1967). Fresh tuber roots and shoots of *Gloriosa superba* L. were obtained from selected donor plant collected from S.P. college garden

### *In Vitro* propagation

The explants were washed thoroughly under running tap water, presoaked in 0.1% liquid detergent for about 30 min, wiped with cotton and dipped in 70% (v/v) ethanol for 1 min. They were then surface-sterilized with a 0.1% (w/v) mercuric chloride for 5 min, followed by three to five rinses with sterile distilled water inside a laminar air flow cabinet. The surface-sterilized fresh root tuber explants were sized to 1 - 1.5 cm length.

The explants were inoculated on different concentration of M.S. basal media supplemented with different concentration of BAP and NAA. The pH of all the concentrations was adjusted to 5.7.

### Callus and Organ development (Root and Shoot)

In the initial stage of callusing and organ development, cultures were kept in dark at 25°C and 90% humidity, in environmental test chamber, for 4-5 days. Then the cultures were transferred to culture room, where they were maintained at

25°C ±2°C and 16/8 hours (light/dark) photoperiod provided through white fluorescent tubes with light intensity of 3000 lux. The effect of treatments toward callusing and organogenesis was weekly recorded and also kept in daily observation.

### Phytochemical analysis-

#### Phytochemical Study of *In Vitro* and *In vivo* plants–

The fresh root tubers and leaf of *Gloriosasuperba*L.(*In vitro*&*In vivo*) were analyzed for presence of various secondary metabolites using standard methods.

**Protein** was estimated by the Lowry's *et al.* Method (1951).

**Reducing sugar** estimated by sugar was done by Somogyi M. Method 1952

**Nucleic acid** was estimated by Witham *et al.* method (1971).

**Alkaloids** were estimated by Singh *et al.* (2004) .

## RESULTS AND DISCUSSION

### Characteristic of explant

The leaves and fresh root tubers of *Gloriosa superba* L. were used as explants that have greenish and brownish color respectively. The leaves have an average height of 6 to 7 cm long and up to 1.5 to 1.8 cm wide. The roots are basically long, fleshy tubers.

### Callus and Organ development

Tissue culture studies were made on Murashige and Skoog's (M.S.),(1962) medium supplemented with different growth regulators. The explants used as leaf and fresh root tubers of *Gloriosa superba* L. Maximum callus was obtained in M.S medium supplemented with BAP (1.5 mg/l) in combination with NAA (0.5 mg/l) Organogenesis responses better in M.S. medium supplemented with N.A.A. (0.5 mg/l) and BAP (1.5 mg/l)(Table 1).Somani *et al.* (1989) and Shivkumar and Krishnamurthy (2000) reported the similar results regarding micropropagation of said plant.

**Phytochemical studies** (Reducing sugars, total proteins, nucleic acids and alkaloids) of fresh leaf and fresh root tubers (*in vivo*) and callus obtained from root and leaf explants (*in vitro*) were carried out. It was observed that, maximum protein contains reported in leaf callus (1.541 µg/g) where as minimum proteins were observed in roots. More R.N.A. were present in leaf (1.090 µg/g) as compare to callus and fresh roots. Alkaloids were reported in fresh leaf, fresh roots as well as in leaf callus and root callus. Maximum alkaloids (2.012 µg/g) and reducing sugars(22.86 µg/g) were reported in root callus. The present results are in accordance with the Aiyer *et al.* (1973) who has worked thoroughly on phytochemical analysis of Indian medicinal plants. This finding will help to save this endangered medicinal plant *Gloriosa superba* L.by using callus to extract active principal of this plant. (Table- 2).

## CONCLUSIONS

*Gloriosa superba* L. 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 *insitu* and *ex situ* multiplication in general and micropropagation in particular so as to meet the everincreasing demand from the industries.

As the explant used for the *in vitro* propagation is responding a very good result it is concluded that the experiment was successfully achieved. The best medium for rooting and shooting of *Gloriosa superba* L.was MS medium with N.A.A and BAP as a growth regulators.. The plantlets of *Gloriosa superba* L. were resulted from these research more than 50 individuals. This study will be helpful for the conservation of plants and to meet the more demand of *Gloriosa superba* L. for the preparation of traditional and other pharmaceuticals remedies.

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**Table 1- In Vitro propagation of *Gloriosa superba* L.  
GROWTH RESPONSE ON M.S. MEDIUM**

Sr. No.	Growth Regulators (mg/l)	Explant	Callus	Root	Shoot
1.	2, 4-D (1) + B.A.P. (0.5)	ROOT & LEAF	+++	---	+++
2.	I.A.A. (1) + B.A.P. (1)	ROOT & LEAF	++	---	---
3.	N.A.A. (0.5) + B.A.P. (1)	ROOT & LEAF	---	+++	+++
4.	N.A.A. (0.5) + B.A.P. (0.5)	ROOT & LEAF	--	---	---
5.	N.A.A. (0.5) + B.A.P. (1)	ROOT & LEAF	+++	++	---
6.	N.A.A. (0.5) + I.A.A. (1)	ROOT & LEAF	--	---	---

- indicates no growth.

+ to +++ indicates weak to vigorous growth.

**Table 2 - Comparative Phytochemical composition of  
fresh root tubers, leaf and Callus in the *Gloriosa superba* L.**

Sr. no	Chemical constituent	Callus ( $\mu\text{g/g}$ )		Fresh Roots ( $\mu\text{g/g}$ )	Fresh Leaf ( $\mu\text{g/g}$ )
		Root	Leaf		
1	Proteins	1.312	1.541	1.029	1.256
2	Phenols	1.522	1.032	1.467	3.244
3	Alkaloid	2.012	1.723	1.306	1.230
4	Reducing sugar	22.86	18.74	14.40	16.26
5	DNA	0.368	0.472	0.408	1.324
6	RNA	0.109	0.623	0.223	1.090

