

## HIGH FREQUENCY EMBRYOGENESIS AND ORGANOGENESIS IN *GLORIOSA SUPERBA* L. - A PLANT IN NEED OF CONSERVATION



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**Abstract:** *Gloriosa superba* L. is an important medicinal herb belongs to the family Liliaceae distributed in Asia and Africa. It is used to cure ulcers, piles, dyspepsia, cancer, gout, scrofula and act as antihelmintic, antipyretic, antileprotic and antiabortive. A number of compounds such as colchicine, chelidonic acid, colchicoside, gloriosine, Xasitosterol, luteolin etc. have been reported from *Gloriosa superba*. Due to its excessive usage in medicinal purposes, the species is on the verge of extinction and included in Red Data Book. The main aim of the present study was to evaluate the effect of different growth media with different hormonal combinations on somatic embryogenesis and organogenesis in *Gloriosa superba*. The results indicated that MS medium supplemented with NAA (0.15mg/l) + BAP (0.25mg/l) induced the callusing, with 2, 4-D (0.5mg/l) + Kinetin (0.25mg/l) induced the somatic embryogenesis, NAA (0.5 mg/l) and BAP (0.25 mg/l) promoted the formation of the maximum number of shooting and NAA (0.25mg/l)+ BAP( 0.15 mg/l) induced the rooting.

**Key words:** Colchicine, Colchicoside, Callus induction, Shoot induction, Root induction.

### INTRODUCTION

*Gloriosa superba* L. is one of the endangered perennial climbing herb among the medicinal plants (Ashok *et al.*, 2011 and Baloda *et al.*, 2002) with brilliant wavy edged yellow and red flowers (Rajak *et al.*, 1990). Among the seven upavishas in the Indian medicine, *Gloriosa* is an important one and which cure many ailments but may prove fatal on misuse (Joshi, 1993). It is one of the important medicinal herbs to cure various respiratory disorders. The leaf sap is used as smoothening agent for pimples and skin diseases. The medicinal property of *Gloriosa superba* is due to the presence of some active compounds in all parts of the plant. Several secondary metabolites have been isolated from tubers, leaves and seeds. The V- shaped tuber is used for the treatment of haemorrhoids, cancer, chronic ulcers, leprosy and also for inducing labour pains. The tuber is also used as abortifacient, and in smaller doses it acts as a tonic, stomachic, anti-inflammatory and anthelmintic. Colchicine is an important alkaloid present in both seeds and tubers. Because of the presence of colchicine tuber is used for the treatment of gout. Paste of the tuber can also cure parasitic skin diseases (Ghani,

1998).The conventional method for the cultivation of *Gloriosa superba* is through corms. The seed has poor germination capacity that restricts the multiplication of this plant. The cultivation using corm is not enough for the production of maximum number of *Gloriosa superba* for commercial purposes. So the tissue culture is the only way to conserve this highly medicinal and ornamental endangered plant.

Plant tissue culture is the technique of isolation of explants and grown them aseptically for indefinite period on a nutrient medium (Ignacimuthu, 1997). The commonly used nutrient medium is Murashige and Skoog's medium (Murashige and Skoog, 1962). Hence, the present study is planned to develop an effective protocol for propagation of this valuable medicinal plant *Gloriosa superba* L. in *in vitro* condition and transplant the commercially useful plant from laboratory to field condition.

### MATERIALS AND METHODS

**Plant material:** *Gloriosa superba* L. plant was collected from the garden of University College,

Thiruvananthapuram. Explants used were leaves and internode. The explants were cut into small pieces and washed in running water before rinsing in double distilled water. After that explants were surface sterilized in mercuric chloride solution for ten minutes. Later explants were rinsed with double distilled water for removing all remnants of mercuric chloride solution and used for *in vitro* culture.

**Nutritional medium:** The Murashige and Skoog's medium was used for *in vitro* culture of *Gloriosa superba* (Murashige and Skoog, 1962). The nutrient medium for most plant tissue culture is comprised of five groups of ingredients- inorganic nutrients, carbon source, vitamins, growth regulators and organic supplements. To solidify the medium Agar is used. Separate stock solutions were prepared for macronutrients, micronutrients, iron and vitamins (Shirugumbi *et al.*, 2009). MS medium supplemented with IAA + BAP, IBA + Kinetin, IBA + BAP, 2, 4 D + Kinetin, NAA +BAP and 2,4 D + BAP were used to induce callusing and organogenesis .

Leaf and internodal segments were used as the explants. Calli formed during the first culture were used as inoculation material for sub culture. The callus was cut into pieces and inoculated into the corresponding fresh media. After sub culture the tubes were placed in the incubation room maintained at 25 +/- 2 °C and 12 hours light and dark period.

The surface sterilized explants were placed on MS medium supplemented with different concentrations of NAA and BAP (0.1 - 0.5 mg/l) for callus induction. Different concentrations of Kinetin, 2, 4-D and IBA (0.1 - 0.5 mg/l) was used for inducing somatic embryogenesis. The leaf and internodes were inoculated in MS medium supplemented with different concentrations of BAP alone and BAP in combination with NAA, 2, 4-D and NAA (0.1 - 0.5 mg/l) for multiple shooting. Regenerated shoots were transferred to medium supplemented with different concentration of NAA+ BAP, IBA+ BAP and IAA+ BAP (0.1 - 0.5 mg/l) for root induction. Healthy shoots with well developed roots were transferred to plastic cups containing sand and soil in the ratio 1:2. Polyethylene covers were placed over the

plantlets for the first 2 weeks for acclimatization and then transferred to green house and finally acclimatized in the field.

## RESULTS AND DISCUSSION

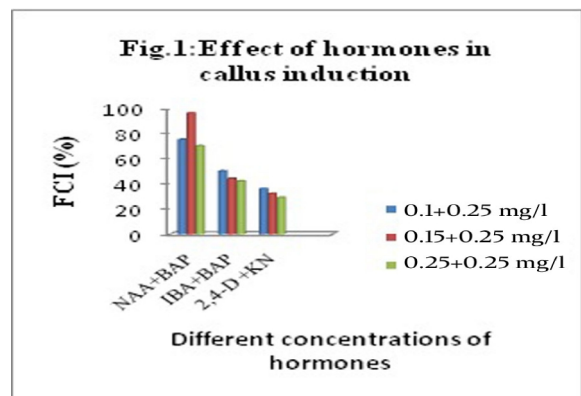
Different explants showed different response in MS media with various hormonal combinations.

**Callus induction:** The results indicated that MS medium supplemented with NAA (0.15 mg/l) + BAP (0.25mg/l) induced 96% callus proliferation from internode within 12 days of inoculation. *In Vitro* callus induction and regeneration of healthy plants of *Gloriosa superba* L. was done by Anirudha Rishi (2011). The results showed that the maximum callus proliferation was induced in B5 medium supplemented with NAA (2mg/l) and kinetin (0.5mg/l) after 5 weeks of inoculation. In the present study maximum callus proliferations were obtained on MS medium supplemented with NAA (0.15 mg/l) + BAP (0.25mg/l) from internode within 12 days of inoculation (Table 1 and Fig. 1).

**Table1.** Effect of PGRs on Callusing from internode (after 12 days)

S.No.	PGRs	PGRconc.	FCI(%)
1	NAA+BAP	0.1+0.25	75
		<b>0.15+0.25</b>	<b>96</b>
		0.25+0.25	70
2	IBA+BAP	0.1+0.25	50
		0.15+0.25	44
		0.25+0.25	42
3	2,4-D+KN	0.1+0.25	36
		0.15+0.25	32
		0.25+0.25	29

PGR- Plant Growth Regulator,  
FCI (%) - Frequency of Callus Induction

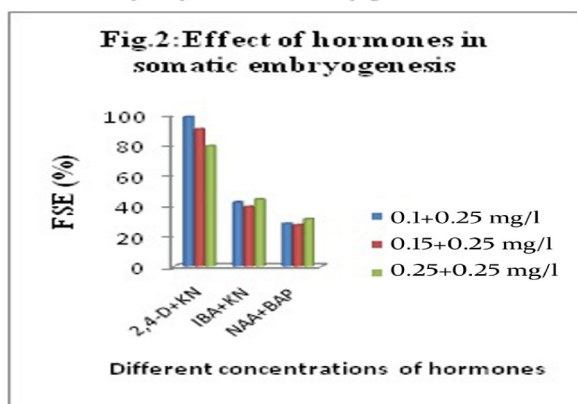


**Embryogenesis:** Maximum Somatic embryogenesis was observed (98%) on the medium supplemented with 2, 4-D (0.5 mg/l) + Kinetin (0.25 mg/l) after 17 days of inoculation from the internode (Table 2 and Fig. 2). Somatic embryogenesis and plant regeneration in *Gloriosa superba* L. were done by Jadhav et al. (2001). In this study friable callus was initiated from shoot apices of *Gloriosa superba* L. on basal MS medium supplemented with 2, 4-D (4mg / l) + Kinetin (5 mg /l) + CH(10 mg /l) + CW(20%). Subculture of callus on the same medium after 4-5 weeks showed induction of large number of somatic embryos. But in the present study maximum somatic embryogenesis was observed on the medium supplemented with 2, 4 -D (0.5 mg/l) + Kinetin (0.25 mg/l) without CH and CW after 17 days of inoculation without subculture.

**Table 2.** Effect of PGRs on Somatic embryogenesis from internode (after 17 days)

S.No.	PGRs	PGRconc.	FSE(%)
1	2,4-D+KN	0.5+0.25	98
		0.15+0.25	90
		0.25+0.25	79
2	IBA+KN	0.5+0.25	42
		0.15+0.25	39
		0.25+0.25	44
3	NAA+BAP	0.5+0.25	28
		0.15+0.25	27
		0.25+0.25	31

FSE(%) - Frequency of Somatic Embryogenesis



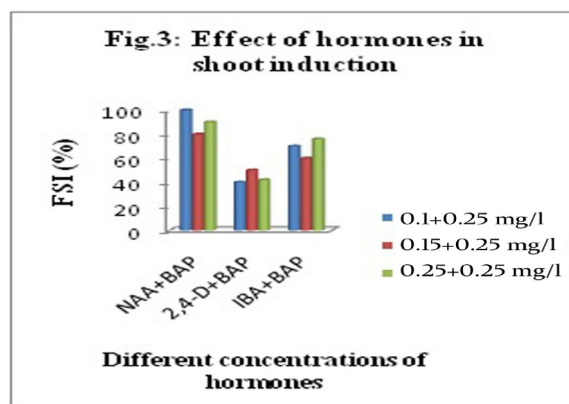
**Shoot induction:** The multiple shooting of *Gloriosa superba* L. were observed (100%) on the MS basal medium supplemented with NAA (0.5 mg/l) and BAP (0.25 mg/l) within 36 days from the leaf explants with mean shoot number 9 and shoot length of 7 cm (Table 3 and Fig. 3).

Multiple shoot formation in *Gloriosa superba* using tuber as explants were reported (Ravindra et al., 2011). In this study the maximum number of multiple shoots observed on basal MS medium supplemented with 2, 4- D and BAP shoots compared to IAA, IBA and with Gamborg B5 medium supplemented with kinetin, IBA and BAP.

**Table 3.** Effect of PGRs on regeneration responses from leaf (after 36 days)

No.	PGRs	PGRconc.	FSI (%)	MSN	MSL (cm)
1	NAA+BAP	0.5+0.25	100	9	7
		0.15+0.25	80	5	2.5
		0.25+0.25	90	11	10.2
2	2,4-D+BAP	0.5+0.25	40	3	3
		0.15+0.25	50	4	1.5
		0.25+0.25	42	7	2.1
3	IBA+BAP	0.5+0.25	70	1	2.9
		0.15+0.25	60	1	2.2
		0.25+0.25	76	2	3.3

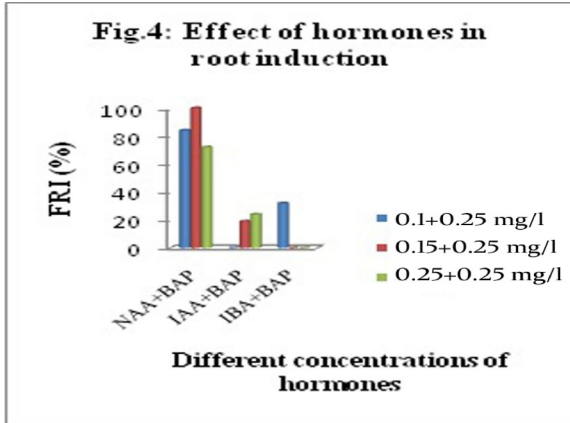
FSI- Frequency of Shoot Initiation, MSN- Mean Shoot Number, MSL- Mean Shoot Length



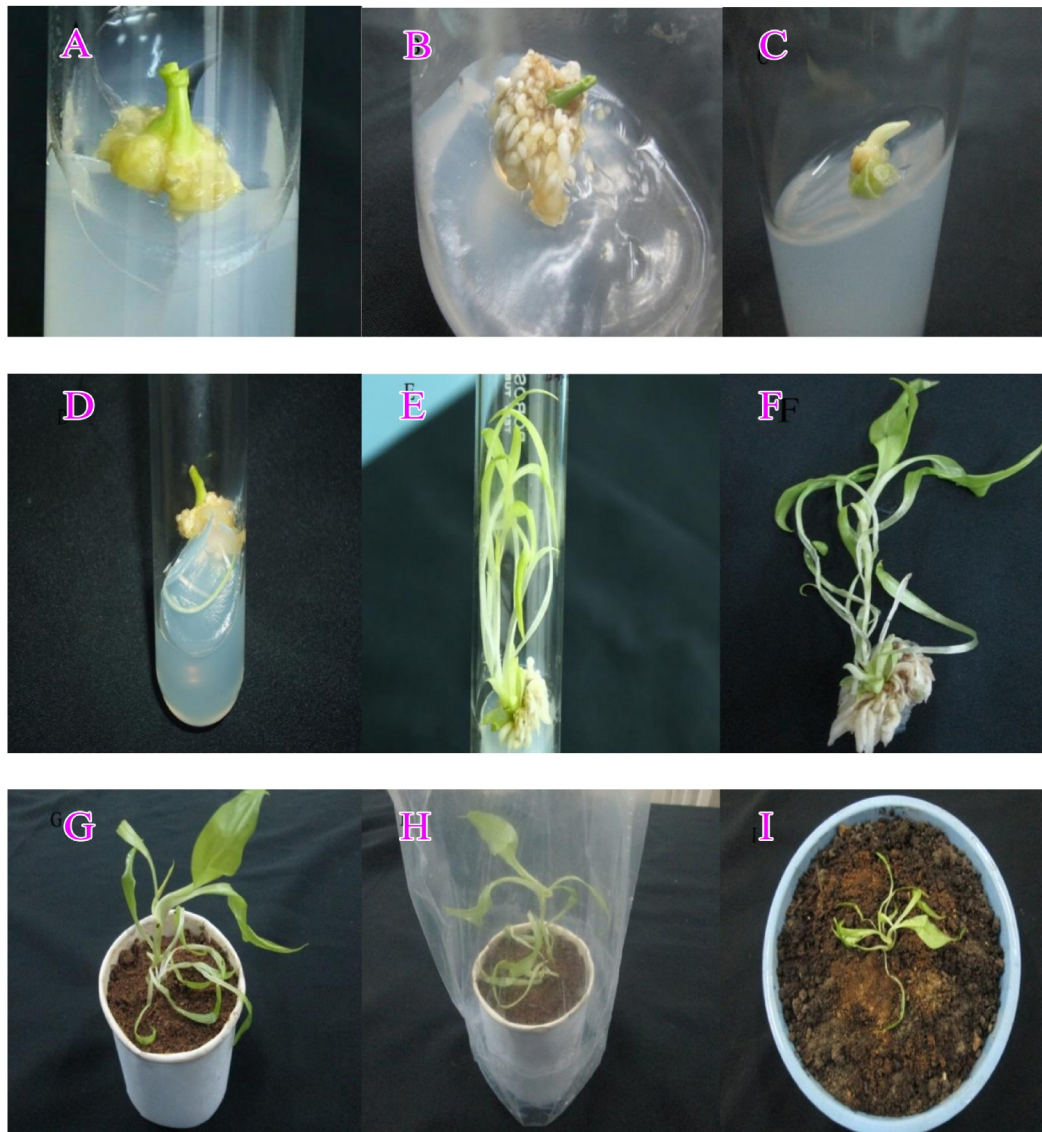
**Table 4.** Effect of PGRs on rooting response from internode (after 27 days)

No.	PGRs	PGRconc.	FRI (%)	MRN	MSL (cm)
1	NAA+BAP	0.2+0.15	84	19	2.1
		0.25+0.15	100	26	2
		0.25+0.2	72	14	5
2	IAA+BAP	0.2+0.5	-	-	-
		0.25+0.15	19	7	1.4
		0.25+0.2	24	1	2.5
3	IBA+BAP	0.2+0.5	32	4	1
		0.25+0.15	-	-	-
		0.25+0.2	-	-	-

FRI - Frequency of Root Initiation, MRN- Mean Root Number, MRL - Mean Root Length



**Root induction:** In the present study among the different hormone combinations tried, maximum frequency of root induction (100%) was observed in MS media supplemented with hormone combination of NAA (0.25 mg/l)+ BAP( 0.15 mg/l) after 27 days of inoculation from internode (Table 4 and Fig. 4). Indirect organogenesis with root induction of *Gloriosa superba* L. was done by Sayeed et al. (2005). The results showed that the maximum rooting induced on the medium supplemented with 1.0 mg/l IBA and 0.5 mg/l IAA.



**Plate 1**

A. Callus induction in internod; B Somatic embryogenesis in internode; C Shoot induction in internode; D Root; E-F Multiple shooting; G-I Acclimatization

**Acclimatization:** The plantlet is taken out from the rooting medium and washed in running tap water to remove the remnants of agar. If agar remains there it would invite microbes to grow and also destroy the young plantlets. Then the plantlet is put into Low Minimal Salt Medium (LMSM) and then transferred to a cup that containing sterilized sand and soil in the ratio 1:2. Polyethylene covers were placed over the plantlets for the first 2 weeks for acclimatization and then transferred to green house and finally acclimatized in the field (Plate 1).

Due to overexploitation and its unscientific collection *Gloriosa superba* has been endangered, therefore, there is urgent need to conserve the plant by biotechnological approaches like tissue culture. In the present study different hormonal combinations in MS medium suitable for callusing, high frequency somatic embryogenesis and organogenesis of *Gloriosa superba* was identified which will be helpful for conservation of the plant.

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