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Micropropagation of Gloriosa superb L. An Endangered Medicinal Plant

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Abstract

The present study deals with the micropropagation of Gloriosa superb L (Liliaceae) a medicinal plant of high commercial value .It is a rare and endangered Indian medicinal plant hence got place in "IUCN red data book". The present study on micropropagation of Gloriosa superba through nodal explants showed better result in BAP (2 mg/l) along with NAA (0.5 mg/l) combination. 0.2% Bvistine (w/v) for 30 min followed by 0.1% $Hgcl_2$ for 3-5 min. treatment produced 75-80% contamination free culture.

Keywords: : Gloriosa Superba, Micropropagation.

Introduction

Gloriosa superba L., a medicinal plants belonging to family Liliaceae is semi-woody climbing herb. It is commonly known as Kalahari in hindi. The seeds and tubers of this plant have been used in traditional Indian medicine for the treatment of gout rheumatic arthritis, in disease of skin, level and several other purposes [Shankar and Mishra., 2011., Khandel et al, 2011]. It was reported that antimicrobial potential of G.superba extracts in which excellent antifungal activity was confirmed against Candida albicans [Khan et al., 2008].

In the present era of knowledge some basic and applied sciences are fast emerging as a tool for human welfare in which tissue culture has gained much attention. The primary advantage of micropropagation is the rapid production of quality, disease free and uniform plant material. The plant can be multiplied throughout the year without dependence on season.

Micropropagation insures a good regular supply of medicinal plants, using minimum space of time [Prakash and Staden, 2007]. Although Gloriosa superba wild is multiplied through vegetative propagation in nature [Ravindra Ade, 2011]. There is always problem of heterogeneity in obtaining uniform seed stock. Using seeds for propagation indicated that plants developed through seeds are poor in vigour. They have been reported to have a low viability and they have a slow rate of growth [Selvarasu and Kandhasamy., 2012].

Aim of the Study

The present study was undertaken with a view to developing protocol for mass propagation of this important medicinal plant through in vitro culture. This is important because the plant cannot be propagated by seed and vegetative reproduction is also very difficult.

Material and Methods

Apical and nodal shoot segments of Gloriosa superba were obtained from selected donor plant collected from field of institute of forest and productivity extention of lalgutwa at mander.

Nodal explants of 1-2 cm size were excised and used as explants source for all the experiments. Young actively growing plants of Gloriosa superba were washed thoroughly in running tap water for 15 min to remove all the dirt and soil particles adhering to them. Large ex-plants were cut into 1-2 cm pieces with fine scalpel and were washed under running tap water 3-4 times. Then they were kept immersed in water with a fungicide (Bavestein) for up to 30 min followed by 3 times rinsing in distilled water. Further sterilization procedures were carried out inside laminar air flow chamber where $Hgcl_2$ (.1%w/v) treatment were given to explants for 3-5 min followed by four times rinsed in sterile distilled water.

Ex-plants were carefully transferred to sterile blotting paper placed over sterile petri plate to remove excess water and were then inoculated into the culture establishment medium using sterile forceps under aseptic conditions. Inoculated explants were then kept in a dust free culture room. The temperature of the culture room was maintained at

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25±2°c and related humidity 60-70%. A twelve hour photoperiod with a light intensity of 2000 lux was provided using cool white fluorescent tubes. Responding cultures were periodically sub cultured. Explants were designed for the selection of the most desirable plant parts and optimum concentration of growth regulator and medium. MS medium enriched with different concentration of BAP (0.5-3 mg/l) alone and BAP (1.5-3 mg/l) in combination with NAA (0.2-0.5 mg/l) were used for culturing nodal segments.

Result and Discussion

In the present study several factors limited the successful establishment of the explants in culture. The selection of explants is an important step for the success of tissue culture. Here best results were achieved when the explants were harvested during the active phase of growth [Torres, 1989]. The sterilization procedure reduced the degree of contamination of explants to 80% when it was surface sterilized with $Hgcl_2$ (.1%) for 5 min along with Bavestein (Table 1).

$Hgcl_2$ is a widely accepted surface sterilizer [Zryd, 1988][Megala and Elango., 2013].

[Table 1: Effect of duration of exposure of sterilants on contamination of explants.

Bavestein Treatment (min)	$Hgcl_2$ Treatment (%)	Exposure Time (min)	Contamination (%)
30	0.1	3	60
30	0.1	4	45
30	0.1	5	20

Initially ten different composition of BAP (0.5-5 mg/l) were used. The results obtained are compiled in Table 2. The best growths of the explants were obtained in the medium consisting of MS and BAP (3 mg/l) alone. As the concentration of BAP increased the growth rate of explants were decreased.

Table 2: Effect of plant growth regulator BAP with MS medium on in vitro shoot initiation from apical shoot buds of Gloriosa superba.

Plant Growth Regulators BAP	Media MS	Growth %	Days required
0.5	MS1	0	30
1.0	MS2	0	30
1.5	MS3	10	28
2.0	MS4	30	25
2.5	MS5	45	28
3.0	MS6	60	20
3.5	MS7	45	27
4.0	MS8	30	29
4.5	MS9	25	29

Finally different combinations of BAP (1-2.5 mg/l) and NAA (0.1-0.5 mg/l) were used for final result. The results are shown in the Table 3. Comparatively with two phase of experiment the best result were obtained in the medium consisting of BAP (2mg/l) along with NAA (0.5 mg/l) from the nodal explants after 4 week.

Table 3: Effect of various growth regulators with MS medium on shoot formation.tai

Plant growth regulators (mg/l).		Media	No. Of growth	Days required
BAP	**NAA**			
1.0	0.1	MS1	36±2.9	30
1.5	0.2	MS2	58±4.1	28
2.0	0.2	MS3	61±4.0	27
2.0	0.5	MS4	89±6.0	20
2.5	0.2	MS5	62±4.2	29
2.5	0.5	MS6	60±4.2	29

The number of multiple shoot formation per explants were recorded at regular interval of time.

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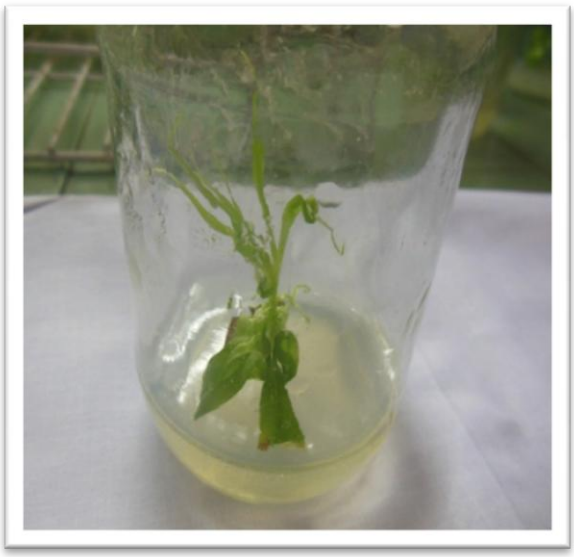

Figure: A. Mature plant of Gloriosa superba. B. Fresh inoculation of apical shoot. C. Transfer of explants. D. E. Initiation of shoots. F. Multiple shoots formation.

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