NUSANTARA BIOSCIENCE
Vol. 3, No. 2, pp. 68-72
July 2011

ISSN: 2087-3948
E-ISSN: 2087-3956
DOI: 10.13057/nusbiosci/n030203

Multiple shoot formation in *Gloriosa superba*: A rare and endangered Indian medicinal plant

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Manuscript received: 6 April 2011. Revision accepted: 24 July 2011.

Abstract. *Ade R, Rai M. 2011. Multiple shoot formation in Gloriosa superba: A rare and endangered Indian medicinal plant. Nusantara Bioscience 3: 68-72.* We report in vitro growth of callus and multiple shoots and standardized the culture conditions for *Gloriosa superba*. The main aim of the present study was to evaluate the effect of different growth media with different hormonal concentrations on callus induction and multiple shoot formation in *Gloriosa superba*. The results indicated that MS medium supplemented with 2,4-D 4.52 μM and BAP 13.30 μM promoted the formation of the maximum number of shoots compared to IAA, IBA and with Gamborg B_5 medium supplemented with kinetin, IBA and BAP were found to be superior.

Keywords: callus induction, *Gloriosa superba*, multiple shoots, kinetin.

Abstrak. *Ade R, Rai M. 2011. Pembentukan pucuk ganda pada* Gloriosa superba*: Sebuah tanaman obat dari India yang langka dan hampir punah. Nusantara Bioscience 3: 68-72*. Kami melaporkan pertumbuhan in vitro kalus dan pucuk ganda, serta standarisasi kondisi budibudaya untuk *Gloriosa superba*. Tujuan utama penelitian ini adalah untuk menguji pengaruh mediapertumbuhan yang berbeda dengan konsentrasi hormon yang berbeda terhadap induksi kalus dan pembentukan pucuk ganda pada *Gloriosa superba*. Hasilnya menunjukkan bahwa medium MS yang ditambah dengan 2,4-D 4,52 μM dan BAP 13,30 μM lebih unggul untuk meningkatkan pembentukan jumlah pucuk terbanyak dibandingkan dengan IAA, IBA dan media Gamborg B5 yang ditambah dengan kinetin, IBA dan BAP.

Kata kunci: induksi kalus, *Gloriosa superba*, pucuk ganda,kinetin.

INTRODUCTION

Gloriosa superba L. (Glory lily), a perennial tuberous climbing herb is widely distributed in tropical and sub-tropical parts of India including foothills of Himalayas (Kapoor 2001). It is known by different names in India, such as *Kalihari, Agnishikha, Languliata,* and *Nangulika*. The plant thrives from arid Bundelkhand to the humid Assam valley, India. It is one of the most important medicinal plants of Asia and Africa (Sivakumar and Krishnamurthy 2000, Jana and Shekhawat 2011). Almost all parts of it find diverse medicinal usage (Kapoor 2001). It has been a well-known plant in Indian Ayurveda and pharmacological industries as well (Asolkar et al. 1992). It is used in ayurvedic medicine as abortifacient, anti-gout, antileprotic, antipyretic, thermogenic and also anticancerous agent. It also provides relief to the swollen joints and in gout (Narain et al. 1981). The tubers have long been used as alternative source of colchicine and gloriosin (Sivakumar et al. 2003a; 2003b, Jana and Shekhawat 2011).

It contains alkaloids like colchicine ($C_{22}H_{25}O_6N$) and its derivative like gloriosin and colchicocide ($C_{27}H_{33}O_{11}N$) along with Benzoic acid, Salicylic acid, sterols and resinous substances and therefore, the demand of this plant is increasing day by day. The present cost of colchicine is about US $ 318/5g (www. sigmaaldrich.com). Food and drug administration of US approves colchicine for acute gout, Mediterranean fever in July 2009. FDA approved Colcrys Pvt. Ltd., USA to treat acute flairs in patient with gout, recurrent and painful form of arthritis and patient with familial Mediterranean fever, an inherited inflammatory disorder. These medications contain colchicine as active ingredients (FDA 2009). Its tubers are thermogenic, antipyretic, antihelmintic, purgative, anti-inflammatory and antileprotic. The tubers and seeds contain the maximum colchicine (0.7-0.9%) (Finnie and Van Staden 1989; Rajagopal and Kandhasamy 2009).

In conventional propagation method seeds and rhizome or tubers can propagate *Gloriosa.* The major problem in the regeneration of *Gloriosa superba* is dormancy of tuber. The germination capacity of seeds is very low (about 0.01%). Another basic problem is presence of hard seed-coat. The fungal contamination is associated with the seeds and tubers, such as *Curvularia lunata*, and tuber-rot caused by *Sclerotium* species (Prota 2010).

Though it has been originated in our country, its germplasm is very rare and far to meet the present increasing demands from pharmacological industries all over the world. (http://database.prota.org/dbtw-wpd/exec/

dbtwpab). The demands of its seeds in pharma industries at home and abroad have been rapidly increasing (Sivakumar 2000). Due to its diverse usage in medicine and over-exploitation for colchicine in industries and excessive use of planting material, i.e. seeds and tubers for local purpose, susceptibility towards many pests, and excessive collection in habitats for medicinal purposes have pushed this taxon to endangered. Due to overexploitation and unscientific collection, *G. superba* 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). In order to provide enough plant material for commercial exploitation, the cultivation-using corm is not sufficient. Thus, mass clonal multiplication through tissue culture is urgently needed not only to conserve this taxon but also to meet the tremendous demands of this medicinal plant as a source of colchicine.

In the present study, we have developed an effective protocol for the rapid propagation and thus regeneration of this medicinal, ornamental and commercially useful plant.

MATERIALS AND METHODS

Germplasm

Germplasm of *Gloriosa superba* L. was collected from Warud, Melghat and Chikhaldara of district Amravati, Central India and planted in Garden of Department of Biotechnology, Sant Gadge Baba Amravati University, Amravati, India (Figure 1A).

Ex-plants

Leaves, non-dormant corm bud (Figure 1B), auxiliary bud, nodal portion and seeds

Surface sterilization of explants

Large ex-plants were cut into 1-2 cm pieces with fine scalpel and were washed under running tap water 3-4 times and rinsed in double distilled water 4 to 5 times in hood. The surface sterilization was carried out by using mercuric chloride 0.2% ($HgCl_2$) for delicate explants (leaf base, and axillary buds) for 3 minutes and 1% mercuric chloride ($HgCl_2$) for tubers and seeds for 3-4 minutes respectively. Later, rinsed in sterile double distilled water 4 to 5 times and then dried on the sterile petridish containing sterile blotting paper.

Growth media and culture conditions

Murashige and Skoog, Gamborg's B_5, Nitsch medium, White's medium, Chu's N_6 and Coconut water were used. The solidifying agent was Phytagel (0.25%) and 5.8 pH was maintained. High-quality chemicals of Hi-media, Mumbai were used. All the treatments were replicated thrice with 10 culture tubes in each set. The cultures were maintained at 25±2^{0}C with 16 hours illumination at 1500-2000 lux and a relative humidity of 60%. Initiation of callusing and multiple shoots was recorded at regular intervals. Results were analyzed statistically.

RESULTS AND DISCUSSION

In the present study, we have tried different growth media for the maximum induction of calli and multiple shoots formations. It includes Murashige and Skoog, Gamborg's B_5, Nitsch, White's and Chu's N_6 media having different hormonal combinations of auxins (2,4-D, IAA and IBA) and cytokinins, (BA, BAP, and BAP). Murashige and Skoog's medium was found to be suitable for the induction of calli and multiple shoot formation followed by Gamborg's medium, while all other media showed no response (Table 1).

Table 1. Effect of different media on the growth of callus and formation of multiple shoots of *Gloriosa superba*

Culture medium	Hormonal combination		Callus and multiple shoots observed (%)
	Auxin	Cytokinin	
Murashige and Skoog's medium	2,4-D	BAP	70 %
Gamborg's B_5 medium	IBA	BAP	50%
Nitsch,s medium	2,4-D, IAA, IBA	BA, BAP	No response
White's medium	2,4-D, IAA, IBA	BAP	No response
Chu N_6 medium	2,4-D, IAA, IBA	BAP	No response

Number of calli and multiple shoots formed per explant were recorded at regular interval. The MS medium when fortified with auxins and cytokinin at different concentrations showed variation in number of calli and shoots formation (Table 2). Initiation of callus and multiple shoot formation was noted after 30-35 days of inoculations (Figure 1C, D, E, F, G, H, I, J, K).

Table 2. Effect of 2,4-D, BAP with MS medium on callus and multiple shoot formation in *Gloriosa superba*

Treatment (µM/L)	Number of multiple shoots*
MS+BAP (4.40)+2,4-D (4.52)	11.00 ± 1.04
MS+BAP (8.90)+2,4-D (4.52)	15.00 ± 0.30
MS+BAP (13.30)+2,4-D (4.52)	21.00 ± 1.05
MS+BAP (17.80)+2,4-D (4.52)	03.86 ± 0.30
MS+BAP (22.20)+2,4-D (4.52)	06.00 ± 0.91
MS+BAP (4.40)+2,4-D (4.52)+ADS (5.28)	11.00 ± 1.57
MS+BAP (8.90)+2,4-D (4.52)+ADS (10.57)	09.00 ± 0.26
MS+BAP (13.30)+2,4-D (4.52)+ADS (13.86)	05.00 ± 1.05
MS+BAP (17.80)+2,4-D (4.52) +ADS (21.14)	04.00 ± 0.80
MS+BAP (22.20)+2,4-D (4.52)+ADS (26.43)	04.00 ± 0.70

Note: *Each value is the average± SD

Figure: 1. A. Two-month mature plant of *G. Superba*, B. "V" shape macro tubers of *G. superba*, C. Initiation of callus, D, E. Callus, F, G. Initiation of multiple shoot, H, I, J, K. Multiple shoots formation in *Gloriosa superba*

Shivkumar and Krishnamurthy (2000) reported that MS medium with combination of BAP (7.77µM) and ADS (5.44 µM) showed the maximum formation of shoots. Finnie and Van Staden (1989) observed that MS basal medium with only 2,4-D showed the callus formation. Jadhav and Hegde (2001) also reported that the callus formation occurs at 2,4-D (18.08µM) + Kn 23.20µM + CH (10 mg/l)+ CW (20%). On the contrary, in the present study, we have found that the MS medium with 2,4-D (4.52µM) and BAP (13.30µM) showed the maximum response in case of calli and shoot formation (Figure 2), while MS medium with 2,4-D (4.52µM) and BAP (17.80µM) showed the minimum response (average no. of calli and shoot formation=3.86). On the other hand, MS medium having hormonal combination of BAP, 2,4-D, and ADS did not show any significant response as compared to BAP and 2,4 D combination used above (Figure 3).

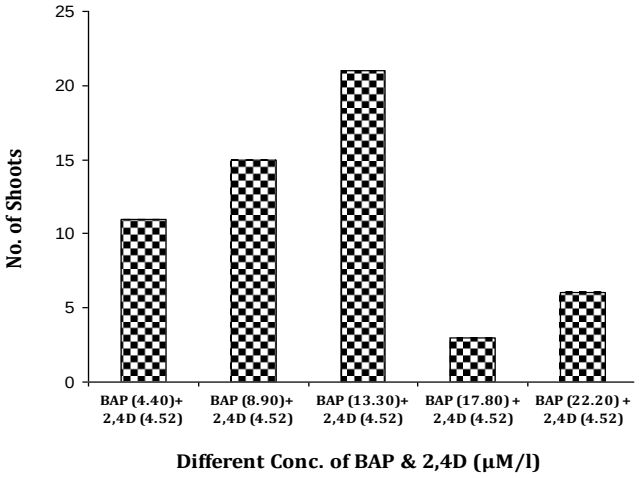

Figure 2. Effect of different conc. of BAP and 2.4-D with MS medium on multiple shoot formation

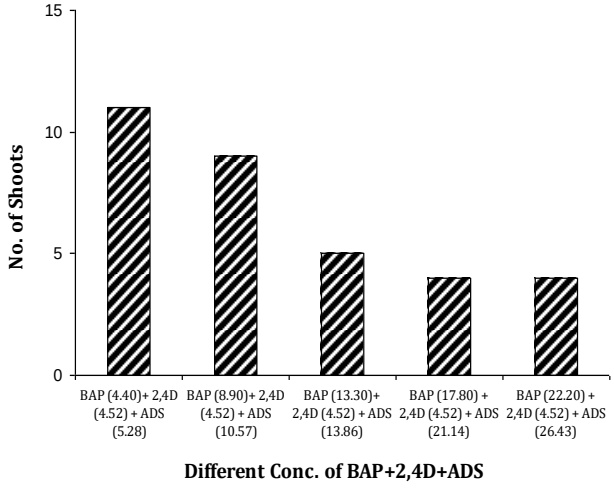

Figure 3. Effect of combination of BAP and 2.4-D and ADS with MS medium on multiple shoot formation

Samarajeewa et al. (1993) reported the callus and multiple shoots on Gamborg's B_5 medium with kinetin 0.46µM along with BAP and IAA 0.04-0.28µM/L the explants were non-dormant corm bud. In the present study, we have tried Gamborg's B_5 along with BAP and IBA (Table 3). Initially, there was no response at lower concentration but on increase of concentration better response was observed at BAP (13.30 µM/L) +IBA (9.80 µM/L) (Figure 4). To assess the effect of IBA and BAP with Gamborg B_5 medium in combination with NAA after inoculation with all necessary culture conditions such as photoperiod 16 hour at 25 ± 2^0C, the maximum response with respect to initiation of callus was observed at the concentration of 4.90µM/L and 17.80µM/L respectively, while the other combinations of BAP, IBA and NAA (Figure 5) were not suitable for growth of callus (Table 3).

Table 3. Effect of Gamborg's B_5 growth medium supplemented with BAP, IBA, and NAA on calli and multiple shoot formation in *G. superba.*

Hormonal treatment (µM/L)	No. of callus induction and multiple shoot*
B_5+BAP (4.40)+IBA (9.80)	09.00 ± 0.36
B_5+BAP (8.90)+IBA (9.80)	11.00 ± 1.04
B_5+BAP (13.30)+IBA (9.80)	18.33 ± 0.92
B_5+BAP (17.80)+IBA (9.80)	15.02 ± 0.36
B_5+BAP (22.20)+IBA (9.80)	17.00 ± 0.96
B_5+BAP (4.40)+IBA (4.90)+NAA (5.37)	14.10 ± 0.45
B_5+BAP (8.90)+IBA (4.90)+NAA (10.74)	14.00 ± 0.87
B_5+BAP (13.30)+IBA (4.90)+NAA (16.11)	18.00 ± 0.60
B_5+BAP (17.80)+IBA (4.90)+NAA (21.48)	No response
B_5+BAP (22.20)+IBA (4.90)+NAA (26.85)	No response

Note: *Each value is the Average± SD

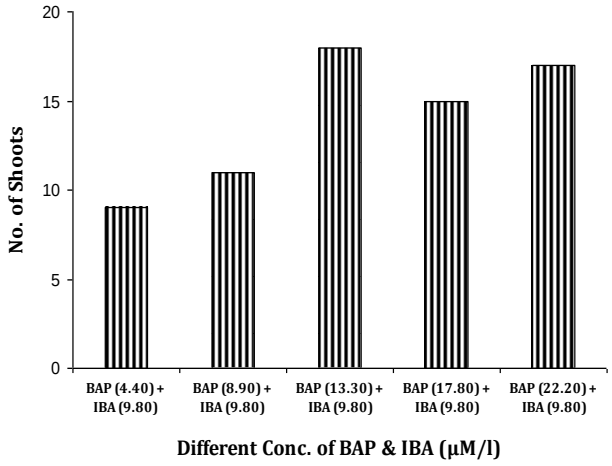

Figure 4. Effect of different conc. of BAP and IBA with Gomborg's B_5 medium on multiple shoot formation

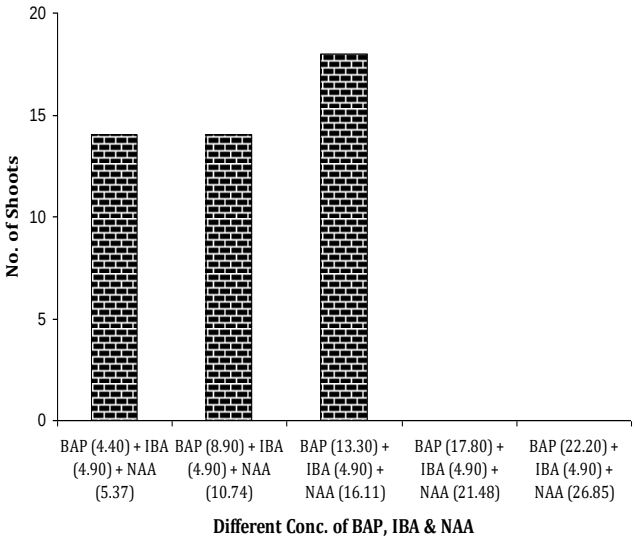

Figure 5. Effect of different conc. of BAP, IBA, and NAA with Gomborg's B_5 medium on multiple shoot formation

The calli were independently sub-cultured for maximum shooting in MS medium with varying concentrations of Kinetin and BA ranging from 2.32 - 17.80µM/L. But profuse shooting was noted in 2,4-D 9.05 and BAP 17.80µM/L after 20 days sub-culturing. Differential response of tissue to any growth hormone depends upon their endogenous level in the explant used for the initiation of culture. Thus, there is need for optimum concentration of the growth hormone at which the response of culture initiation and growth of tissue is maximum.

CONCLUSION

It can be concluded that Murashige and Skoog's (MS) and Gamborg's B_5 media were found to be ideal for the induction of callus and multiple shoot formation as compared to White's medium, N_6 and Nitsch media, which did not give any response. Required culture conditions such as photoperiod 16 hours at 1500-2000 lux, humidity 60% and temperatures 25±2^{0}C were maintained.

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