RESEARCH ARTICLE

In Vitro shoots multiplication through callus culture of *Gloriosa superba* L., a threatened medicinal plant of Melghat Tiger Reserve, Maharashtra, India

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ABSTRACT

Melghat Tiger Reserve is situated in the mountainous region of Satpuras from Dharni and Chikhaldara Tahsils of Amravati District of Maharashtra State. *Gloriosa superba* is belonging to family liliaceae commonly known as Kal-lavi (Glory lily) useful in the treatment of parasitical affection of skin and scorpion bite. The callus is initiated and regenerated from nodal region on Murashige and Skoog medium supplemented with BAP (4 mg/l) + NAA (2 mg/l). Highest number of multiplied shoots obtained from callus cultured on MS medium supplemented with BAP (2 mg/l) without in combination with another growth regulator. The protocol established here is very much useful in the conservation of this threatened medicinal plant.

Keywords: Melghat, *In vitro*, multiplication, threatened, *Gloriosa superba*.

INTRODUCTION

Melghat Tiger Reserve is situated in the mountainous region of Satpuras from Dharni and Chikhaldara Tahsils of Amravati District of Maharashtra State. It is dry deciduous type of forest and consist of total 715 species belonging to 424 gnera are reported from Melghat, out of which 169 species are medicinal.

Gloriosa superba is belonging to family Liliaceae commonly known as 'Glory lily' (Fig a). It is widely distributed in the tropical and subtropical parts of India, Ceylon, Tropical Africa, Malacca (Cook, 1958; Hooker, 1894) up to an altitude of 2000 m. The plant grows in sandy-loam soil in the mixed deciduous forest all over India. It is branched herbaceous climber with showy, large flowers and borne solitary or in corymbose inflorescence. The fruit is loculicidal capsule with 3 lobes consisting of near about 30 – 150 rounded spongy seeds (Narain, 1977). The seeds are a rich source of colchicine and gloriosine (Farooqi *et al*., 1993). Colchicine is used in the treatment of cancer, gout and plant breeding work for inducing polyploidy. Various parts such as leaf, rhizome and seed of the

plants are claimed as useful parts by tribes of India. It is being used in 29 diseases like leprosy, lice, wound along with ulcers and sores, rheumatism, snakebite, scorpion bite, gout, abdominal pain; and useful as abortifacient, anthelmintic, etc. (Bhide and Acharya, 2012).

Colchicine is main alkaloid isolated from this plant and its practical use is more as in industries. This has led to exploitation of this plant all over the world. Once a very common plant on the bordering low hills of Satpura, Melghat forest of Amravati District facing the plains and in Pohra hills. However, plant population of this plant has decreased considerably in the last 10 years due to clearing of supporting vegetation (Dhore, 1986). If the present rate of demand for rhizome continues, the pressure of shortage from the interior forest areas will increase as soon as the availability from the easily accessible area diminishes. Poor seed germination, susceptibility towards many pests, and excessive collection in habitats for medicinal purposes have pushed this taxon to endanger.

Tissue culture studies can play an important role in the *in vitro* conservation of this threatened plant. A major objective of current plant cell and tissue culture work is more efficient exploitation of specific properties of plant genotypes (Johnson, 2002). Different workers all over the world have studied tissue culture studies of *G. superba* viz., Finnie and Staden, (1989); Somani *et al*., (1989); Samarajeewa *et al*., (1993); Hassan and Roy, (2005); Sivakumar and Krishnamurthy, (2000); Ade and Rai, (2011); Khandel *et al*., (2011); Venkatachalam *et al*., (2012); Yadav *et al*., (2012).

The objective of the present work is to frame tissue culture experiments, to induction, proliferation of callus and multiplication of shoots.

MATERIALS AND METHODS

Tubers were collected from Melghat Tiger Reserve core and buffer area forest of Amravati District. They were established and maintained in Botanical garden. Sprouted tuber buds and young shoots were collected, defoliated and washed in running tap water for about 15 min continuously. Tuber, shoot tips and nodal explants were soaked common soap solution for 10 min. After decanting soap solution it was washed with sterilized distilled water. Explants were surface sterilized with 70% alcohol for 30 seconds followed by 0.1% mercuric chloride for 8 min. In the intermediate of this sterilization process rinses of distilled water were given to the plant materials. Then they were cut into proper size and inoculated on Murashige and Skoog (1962) growth medium supplemented with different concentrations of growth regulators viz, (BAP) 6-Benzylamnopurine, (NAA) α-Naphthalene acetic acid, (IAA) Indol-3-acetic acid, (2,4-D) 2,4-Dichlorophenoxy acetic acid, (Kn) Kinetin (Himedia, Bombay); also consisting of sucrose and Agar. The pH was adjusted to 5.8 before autoclaving. All the culture vessels and media were sterilized at 121°C for 20 to 30 min. All the cultures were incubated at 16±1 Hr light and 8±1 Hr dark photoperiod and temperature of 20±2°. The cultures which show good results were sub-cultured into fresh medium. The data was analyzed statistically by using standard formula of standard deviation (SD) and standard error (SE).

RESULTS AND DISCUSSION

Mass propagation of plant species *in vitro* culture is one of the best and most successful examples of commercial application of plant tissue culture (Hassan, 2005). The callus started initiating from nodal explants after 6th week of culture on MS medium supplemented with BAP (4 mg/l) + NAA (2 mg/l). It was first white in colour (Fig b) but later on tuberous in texture and yellowish white in colour and hard. This callus was used further for regeneration of shoots. Related results were obtained by Roy and Pal (1995) in *Costus speciosus* where brown hard callus was proliferated on Schenk and Hildebrandt medium augmented with BA (0.5 m/l) in combination with NAA (0.5 mg/l); again Ahmad *et al*., (2007) in *Podophyllum hexandrum* obtained yellowish callus on MS medium with uniform concentration of BAP and NAA (1.5 mg/l). It was again confirmed that MS medium and B5 medium were supplemented with various concentrations and a combination of Auxin, Cytokinin, and organic acids was used, 98% of callus induction occurred in non-dormant corm bud explants of *G. superba* (Rishi, 2011).

Highest number of multiplied shoots obtained from callus cultured on MS medium supplemented with BAP (2 mg/l) without in combination with another growth regulator (Table 1).

Table1: Effect of growth regulators on shoots response of callus in *G. superba*

Cytokinin (mg/l)		Auxin (mg/l)	% of shoot	Length of shoot (cm)	Multiplication of shoot
BAP	Kn	NAA	initiation	Mean ± SE	Mean ± SE
2	-	-	100	2.6 ±0.18	5.3 ±0.29
4	-	3	80	3.75 ±0.16	2.63 ±0.18
4	-	2.5	90	4.44 ±0.19	4.67 ±0.18
-	3	1.5	70	1.71 ±0.17	-

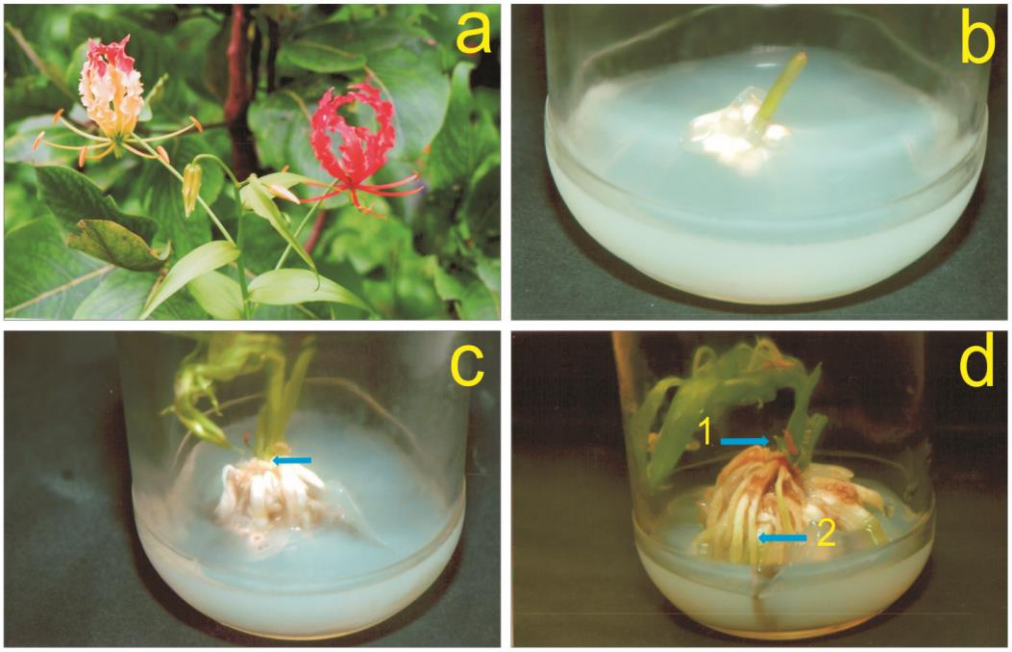

Figure- 1: *G. superba* (a) A flowering twig, (b) Initiation of callus, (c) shoot emergence, (d 1) multiplied shoots, (d 2) roots

MS medium containing BAP 4 mg/l + NAA (3 mg/l) regenerated 80 % shoots (Fig c and d1), showing average shoot number i.e., 2.63 ± 0.18, shoot length 3.75 ± 0.16. In another combination, MS medium supplemented with BAP (4 mg/l) + NAA (2.5 mg/l) showed 90% initiation with maximum shoot number recorded 4.67 ±0.18 having soot length 4.44 ± 0.19 cm. From the above two combinations, best results was obtained in second combination. Frequency of shoot number and shoot length declined markedly at higher concentration of NAA than the other growth regulator. Hence it was important to note that BAP was responsible for shoot initiation and growth. Previous results of Benmoussa *et al*., (1996) in *Asparagus densiflorus* are in accordance with present findings; 6BA promoted more shoots per callus than Kn with low concentration of NAA; the number of shoot buds decreased sharply in the presence of higher doses of cytokinin. There is another example where BAP along with low concentration NAA initiated shoot differentiated from callus; however, the process was retarded at higher concentration of BAP in the cultivars of *Cucurbita pepo* (*Pal et al*., 2007). Moreover, in another combination of Kn (3 mg/l) + NAA (1.5 mg/l) shows very low percentage of shoot initiation and growth 1.71 ± 0.17 cm without multiplying. Samarajeewa (1993) reported that Kinetin was less effective than BAP for shoot multiplication which supports the view of present author. The multiplied shoots showed extensive rooting without transfer in fresh medium (Fig d2).

In words of Singh *et al*., (2013), that *G. superba* will conserved by tissue culture technique; not only we cultivate the good quality of plant but also enhance the valuable component of plant and reduce the over harvesting of plant from its natural habitat.

CONCLUSION

G. superba is occurred as a threatened category medicinal plant in Melghat Tiger Reserve,

Maharashtra. Present report of shoot regeneration and multiplication through callus culture can provide wide prospect for further research and study for *in vitro* conservation of this threatened and medicinally useful herb.

Conflicts of interest: The authors stated that no conflicts of interest.

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