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Short Communication

In Vitro Callus Induction and Regeneration of Healthy Plants of *Gloriosa superba* Linn.

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ABSTRACT
Gloriosa superba Linn. a member of the Liliaceae family, is a very important medical plant due to the presence of alkaloids, mainly colchicine and colchicoside. Poor seed germination, susceptibility toward many pests, and excessive collection in habitats for medicinal purposes have pushed this taxon to endanger. An efficient protocol was developed for regeneration of healthy plant derived from different explants from both *in vivo* and *in vitro* raised plants, viz. roots, corm buds (dormant and non-dormant), young leaves, stems, pedicels, and shoot tips from aerial shoots. MS medium and B_5 medium 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. The best callus obtained on the concentration of NAA 2 ppm and Kinetin 0.5 ppm in B5 medium. The greatest numbers of multiple shoots (43) were observed in corm-derived callus.

Key Words: *Gloriosa supe*rba, Medicinal plant, multiple shoots, Corm

INTRODUCTION
Gloriosa (Liliaceae) is a small genus of mostly tropical African and Asiatic distribution. The plants are climbing herbs characterised by their leaf tips modified into tendrils. The rhizomes and seeds yield a variety of alkaloids. Only one species occurs indigenously in India namely *G. superba* contributing small part in Aravali flora. *G. superba* is a potential commercial source of colchicine and colchicoside (Sivakumar and Krishnamurthy, 2002). Due to Poor seed germination, susceptibility toward many pests and excessive collection in habitats for medicinal purposes this taxon has been pushed to endanger. *G. superba* has been tried in the past for multiplication through micropropagation (Somani *et al.,* 1989, Finnie and van Staden, 1989, Samarajeewa *et al.,* 1993). The present investigation pertains to the development of an efficient protocol for the callus induction and regeneration of *G. superba in vitro* tissue culture.

MATERIALS AND METHODS
Plant Material
Gloriosa superba L. plants were collected from Botanical garden of Dr. Y. S. Parmar University of Horticulture & Forestry, Nauni, Solan (Himachal Pradesh) and Jhalana Dungari hills Jaipur (Rajasthan) India. The voucher specimen was deposited in the Herbarium, Department of Botany, University of Rajasthan, Jaipur, India,

Culture Explants
Terminal shoot tips, stem nodes with single auxiliary bud, dormant and non dormant rhizomes were used as explants. The explants were washed with running tap water pre soaked in 0.1% liquid detergent for about 30 min. The explants were then surface sterilized with 0.1% (w/v) Mercuric chloride for 3 min. followed by two to three rinses of sterile distilled water.

Culture Medium
The basal medium contained MS (Murashige and Skoog, 1962) salts, B5 (Gamborg *et al.,* 1968) vitamins, 3% sucrose and 0.9% agar. The basal medium was supplemented with various concentrations and combinations of growth regulators such as 2,4-D (2,4-dichlorophenoxy acetic acid), BAP (6-benzylamino purine), NAA (Naphthalene acetic acid), Kinetin, IBA (Indole Butyric Acetic acid), and IAA (Indole acetic acid). The medium was adjusted to pH 5.8 with NaOH/HCl and dispensed in culture tubes and conical flasks of 100 ml capacity. The media was sterilized by autoclaving at 121 ^{0}C and 15.lbs pressure for 20 minutes.

Culture Conditions
All the cultures were incubated at 24±2^{0}C and at photoperiod of 16.h provided by cool-white fluorescent light with the of intensity 3mmol m^{-2} s^{-2}. Callus induction was obtained in various concentrations of growth regulators from corm, leaf explants. Various concentrations and combinations of 2, 4-D, NAA and Kn in B_5 and MS medium produced profuse, white, friable callus within two to three week .The callus initiated in the media supplemented with NAA and BA combination was compact and semi-hard. The explants with induced calluses were transferred to fresh media every two weeks depending on the rate of callus growth. Explants showing no visible callus growth or with slow growing callus were

transferred to fresh media every four weeks. The growth index was calculated after every two weeks time interval.

Multiple shoot proliferation from shoot tips, nodal explants and callus was obtained in various concentrations of IBA, NAA, BAP and Kn. Best combination for multiple shoot is BAP and NAA from shoot tips and nodal explants and from callus.

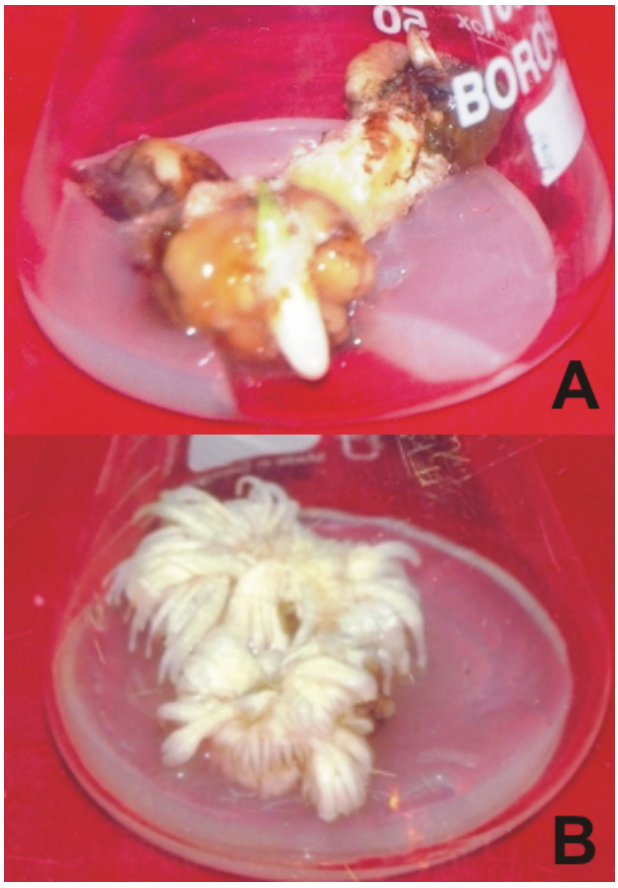

Fig.1. Micropropagation in *G. superba.* A: Explant showing shoot bud and tuber formation; B: *In vitro* multiple shoots and roots formation.

RESULTS AND DISCUSSION

Callus formation from six different explants from both *in vivo* and *in vitro* raised plants, viz. roots, corm buds (dormant and non dormant), young leaves, stems, pedicels, and shoot tips from aerial shoots was initatied within four to five weeks after culturing in basal medium supplemented with various concentration of auxin and cytokinins. The best results obtained at the concentration of NAA 2 ppm + 0.5 ppm kinetin in all the cultured explants. About 70 % of the non-dormant corm bud explants, 55% of the young leaf explants, 76% of the shoot bud explants, 45% of the root explants, 87% of the dormant corm explants, 66% of the stem explants and 57% of the pedicel explants successfully produced callus after 5 weeks of culture. The callus was maintained on NAA 1.0 ppm + 0.5 ppm Kn. Maximum growth index was shown in 4-6 weeks old callus. The highest number of multiple shoots was observed in corm-derived callus (43), nodal explants (36) and shoot tips (39). Vigorous root formation occurred in all cases when multiple shoots were derived (Fig. 1B). The optimum B5 medium for the microtuberization of *G. superba* supplemented with 3% of sucrose produced about 180-240 tubers within six months.

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