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Callus induction from corm of *Gloriosa superba* Linn: An endangered medicinal plant

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ABSTRACT

In present study, an efficient protocol was developed for induction of callus derived from corms of *Gloriosa superba* Linn. MS medium with various concentrations and combinations of growth regulators or other supplements was used. The best growth of callus was obtained in MS medium containing 2.5 mg/l BAP + 1mg/l IAA + .5 mg/l KN+ 15% CW.

KEYWORDS

Gloriosa superba Linn.Callus;
MS Medium;
Micropropagation;
Medicinal plant.

INTRODUCTION

India is one of the twelve megadiversity countries of the world with a rich diversity of biotic resources. Out of 34 hotspots recognized, India has two major hotspots - the Eastern Himalayas and the Western Ghats. India harbours about 47000 species of plants of which 17000 are angiosperms. A total of 560 plant species of India have been included in the International Union for Conservation of Nature and Natural Resources (IUCN) Red List of Threatened species, out of which 247 species are in the threatened category[1]. From time immemorial, man has been dependent on nature for survival. This dependency led the aboriginal people living in harmony with nature to evolve a unique system of knowledge about plant wealth by trial and error methods[2]. Medicinal plants have been an integral part of life in various regional communities for food and drug both. India has more than 3,000 years of medicinal heritage based on medicinal plants[3].

Gloriosa superba Linn., is one of the endangered species among the medicinal plants which is a striking tuberous climbing plant with brilliant wavy edged yellow and red flowers that appears from November to March every year. It is one of the seven Upanishads in the Indian medicine, which cure many ailments but may prove fatal on misuse[4]. It is also used to treat intestinal worms, bruises, infertility and skin problem. So the art of use of plants medicine is herbalism. Man has been using this miraculous medicine for thousands of years but in couple of decades the practice of herbalism is seen very rare. Although the modern medicine has developed so much improves to be useful in treating many horrible human diseases, but not in reasonable cost[5]. *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. superb* L. contributing small part in Aravali flora. *G. superba* Linn. is a potential commercial source of colchicine and colchicoside[6]. *Gloriosa superba*

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Linn. usually multiply by corm and seeds but due to low germination capability it restricts for the regeneration. Therefore, in order to safeguard and preserve this important plant biotechnological approachs would be very useful[7]. In vitro culture based micropropagation technique has been successfully used for rapid and mass propagation of many medicinal plants. This technique has also been used in ex situ conservation of many plants species which are at threatened or rare state[8]. Plant tissue culture through micropropagation makes the rapid multiplication of selected genotypes possible, allowing the useful metabolites to be collected in greater quantities[9], as well as providing an alternative means of propagation, given that in the field, the presence of seed tegument dormancy hinders the swift production of uniform plants[10]. Plant tissue culture techniques have become powerful tools for studying and solving the basic and applied problems in plant propagation and genetic manipulation[11].

The present study was focus on the objective to develop a more efficient and reliable protocol for callus culture and rapid multiplication of endangered plant species and their ex situ conservation.

MATERIAL AND METHODS

Laboratory preparation: Maintenance of highly aseptic condition is vital factor for successful tissue culture. Thus the room of such lab should be washed with different disinfectants like 2% (v/v) sodium hypo chloride and 70% (v/v) ethyl alcohol. The final sterilization should be done by UV Radiation. The glass wares and all equipment washed and medium were prepared properly by adding all supplements or autoclaved at 120° c and 15 psi pressure for 30-40 minutes.

Explants preparation for culture: The plant used for callus culture should be healthy and free from any diseases and pest problems. The corms of *Gloriosa superba* L. were used as explants for callus culture. The explants were washed in running tap water properly to remove soil particles and soaked in detergent containing water for 30 minutes. After soaking the explants were washed with distilled water for 3 times. The explants were then surface sterilized in laminar air flow with 70% (v/v) ethyl alcohol for 1 minutes or 0.1% (w/v) Mercuric chloride for 3 min. followed by two to three rinses of sterile distilled water.

The sterilized explants were cut in small fractions than inoculated on the medium in aseptic condition.

Preparation of nutrient medium: The basal medium contained MS (Murashige and Skoog, 1962) salts, vitamins, sucrose 30gm/L and .7% agar. The basal medium was supplemented with various concentrations and combinations of growth regulators such as BAP (6-benzylamino purine), Kinetin and IAA (Indole acetic acid). The other supplement like 15 % coconut water was also added in medium. The medium was adjusted to pH 5.6-5.8 with 1N NaOH/HCl and dispensed in culture tubes and conical flasks of 100 ml capacity. The media was sterilized by autoclaving at 121°C and 15 psi pressure for 30 minutes.

Maintenance of culture condition: All the cultures were incubated at 24±2°C temperature and at photoperiod of 14 hours provided by cool-white fluorescent light with the intensity of 1000-2000 Lux and 50-60% relative humidity. Callus induction was obtained in various concentrations of growth regulators from corms. Various concentrations and combinations of BAP, IAA and KN in MS medium produced profuse, white, friable callus within three week. 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.

RESULT AND DISCUSSION

Callus formation from corms was initiated within three weeks after culturing in basal medium containing different supplements and various concentrations of growth regulators. The best results obtained at the concentration of 1mg/L IAA+ 2.5 mg/L BAP+ .5 mg/L KN. The carbon source was at 30g/L and 15% coconut water also used. The explants sterilized for 1minutes with 70% ethyl alcohol and 3 minutes with .1% mercuric chloride show best results. The highest growth was observed in corm derived callus.

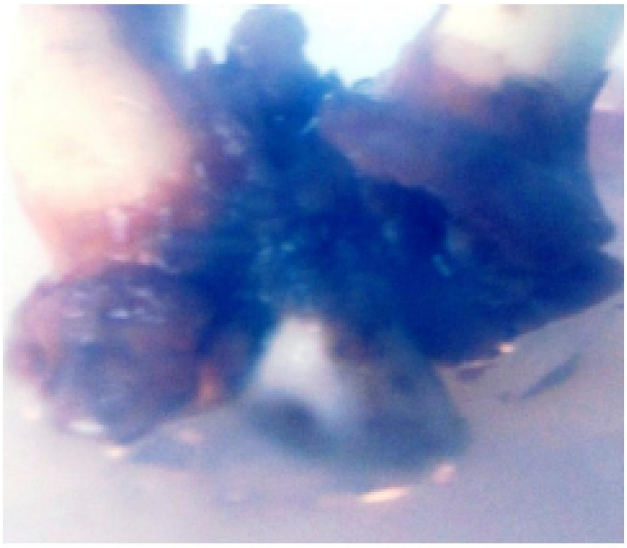

Figure1 Initiation of callus of *Gloriosa superba* L.

Figure2 Callus of *Gloriosa superb* L.

CONCLUSION

Herbs are being used since ancient time to maintain health, to treat disease and regain the healthy state of mind and body. They have been used in traditional forms of Indian medicine and have provided solutions to even those health problems that have defined modern science.

The conclusion of this study is that the explants were immersed in aqueous solutions of Savlon (Johnson & Johnson) for 5 minutes before surface sterilization. The explants were washed with sterile water and surface sterilized with 70% (v/v) ethyl alcohol for 1 minute or 0.1% Mercuric chloride (Hi Media) aqueous solution treatment for 3 minutes. The in vitro raised explants were inoculated in the media having different concentrations of BAP, IAA and KN. Callus formation started after three weeks at the ends of the explant. Globular and white callus was formed in the medium (MS + 2.5 mg/l BAP + 1mg/l IAA + .5 mg/l KN+ 15% CW).

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