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RESEARCH ARTICLE

IN VITRO PROPAGATION OF *GLORIOSA SUPERBA* L. IN TERAI REGIONOF UTTAR PRADESH

Pallavi Sharma[1*] and Tulika Mishra[2]

[1]Research Scholar, Department of Botany, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur-273009, Uttar Pradesh, India; [2]Assistant Professor, Department of Botany, Deen Dayal Upadhyaya Gorakhpur University, Gorakhpur-273009, Uttar Pradesh, India

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***Corresponding author:**
Francisco Palemón-Alberto

ABSTRACT

Gloriosa superba ***L.*** is associated with a plethora of medicinal benefits and ethnomedical applications since time immemorial. The current rare state of this plant is the result of over-exploitation for its medical uses. To address *Gloriosa superba's* low seed set issue, tissue culture and somatic embryogenesis techniques needed to be standardized. In the present study, we tried to develop a standard protocol for *in-vitro* propagation of **G.** *superba* in the Terai region of Uttar Pradesh. **G.** *superba* was cultured under circumstances designed for *in vitro* regeneration utilizing nodal segment explants. The combination of MS+1.5 mg/l BAP+0.5 mg/l NAA produced the most number of shoots (7±0.18), demonstrating the best shoot induction. The number of shoots per culture increased by adding 10% coconut water (CW) to the previously mentioned medium, and the length of the shoots increased by adding 100 mg/l of urea to the media. Shoots cultivated on half-strength MS supplemented with 1.0 mg/l IBA + 0.5 mg/l NAA showed the best rooting. The medicinal and conservation components of *G. superba* will benefit from this established approach, enabling it to satisfy future economic demands.

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INTRODUCTION

Man's life has always been closely entwined with the natural world around him, and plants have an impact on many facets of human existence. Humans have utilized plants for a wide range of needs, including food, fibre, medicine, defence, and decoration. Since ancient times, people have used substances obtained from plants to heal illnesses. In addition to giving the impoverished access to inexpensive medicine, medicinal plants have enormous potential for alternative therapies and the creation of jobs, revenue, and foreign exchange for developing nations. Typically, plants generate a large number of secondary metabolites, which are a major source of insecticides, microbicides, and numerous other significant medicinal products. Medicinal plants and their secondary metabolites have been used for a long time, either directly or indirectly to treat illnesses. Because of their many uses, these plants have recently attracted much attention. It is well recognized that the richest bioresource of pharmaceutical intermediates, modern and traditional medicines, food supplements, neutraceuticals, and chemical entities or synthetic medications is found in medicinal plants (Tiwari et. al., 2011).

A significant member of the Colchicaceae family, *Gloriosa superba* L. is also referred to as Agnishikha, Tiger claw, Kalihari, Creeping lily, Flame lily, and Glory lily. Because of the presence of alkaloids, including colchicine and its derivatives, gloriosine and colchicocide, as well as the presence of benzoic acid, salicylic acid, and resinous compounds, all portions of this plant are utilized medicinally in the Siddha, Ayurvedic, and Yunani systems of medicine. The Flame Lily has a wide range of uses, including in the treatment of constipation, respiratory disorders, skin illnesses, larvicidal tests, antibacterial energy, antidepressant energy, enzyme resistance, and anti-inflammatory efficacy. It is discovered to be effective in treating sprains and bruises, resentment, long-term wounds, haemorrhoids, malignancies, labour discomfort, and abortion. A decade ago, *Gloriosa* was the only known to exist in the wild. However, due to economic needs, it was domesticated, and today, Indian medicine uses all parts of the plant. This plant species has a wealth of physiologically active chemicals, which makes it a promising candidate for pharmaceuticals that could supplement conventional treatments (Sharma, 2021). Glory lily demand is high this time around. The reason for its high incidence of wild harvesting is the low production compared to the high demand.

This plant species is included in the "Red Data Book" of International Union for Conservation of Nature (IUCN) and is threatened with local extinction in Asian countries where it has become endangered. Glory lily is naturally reproduce by seeds and tubers, however its tubers seldom regenerate and its seeds have poor germination rates that range from three weeks to months. Its application in commercial cultivation is limited by the hard seed coat that is impermeable to water, resulting in poor germination. Only after recurrent bouts of extreme heat and cold do seeds become water-permeable. After being treated with gibberellic acid, cold water, hot water, water soaking, and other acids, the seed can germinate more quickly. Numerous studies demonstrate that concentrated sulfuric acid increases the percentage of seeds that germinate by softening the stiff seed coverings. Pests, fungal diseases, soil-borne illnesses, and poor propagation are issues that arise when growing glory lily (Sharma and Mishra, 2022).

Gloriosa superba has long been associated with a plethora of medicinal benefits and ethnomedical applications. The current state of this plant is the result of over-exploitation for its medical uses. One of the worry for humanity nowadays is preventing the extinction of therapeutic plants (Mishra and Sharma, 2020). Further research on the the plant *G. superba* is necessary in the future. It is important to prioritize increasing Glory lily colchicine production to satisfy industrial demands. To address this plant's low seed set issue, tissue culture and somatic embryogenesis techniques need to be standardized. In the present study, we tried to develop a standard protocol for *in-vitro* propagation of *G. superba* in the Terai region of Uttar Pradesh.

MATERIALS AND METHODS

Healthy nodal explants of *Gloriosa supeba* were collected from the the Terai region of Uttar Pradesh (from Gorakhpur). The explant materials thoroughly cleaned under running tap water for an hour. After cutting them into small pieces, they were surface sterilized for six minutes under aseptic conditions using an aqueous solution of 0.1% $HgCl_2$ with two drops of tween 20. To remove any remaining $HgCl_2$, it was rinsed five times with autoclaved distilled water. For explants, nodal segments measuring around 2.0 cm in length were removed from surface-sterilized stems. Explants were cultivated on MS supplemented with varying doses of auxin (NAA) and cytokinins (BAP) to promote shoot regeneration. Half-strength MS supplemented with auxin (IBA and NAA) was employed for rooting. The medium was supplemented with urea (50-200 mg/l) and coconut water (CW) (5-20%) to promote individual shoot elongation and multiplication. Before adding agar, the media's pH was adjusted to 5.8. Every medium was gelled using 0.7% agar and subjected to a 20-minute autoclave at 121°C and 1.1 kg/cm^2 of pressure. The cultures were kept at 25±1°C with a 16-hour photoperiod and 3000–4000 lux of light intensity. Every three weeks, the cultures were routinely subcultured on fresh medium. Five-day intervals were used for recording observations after implanting and subculturing. Every experiment was conducted twice, using a minimum of 20 cultures for each treatment. The test tubes with the rooted shoots were placed at normal room temperature and light for ten days to allow them to harden. After that, any remaining agar was carefully rinsed from the roots of the rooted shoots by removing them from the test tubes. After that, they were moved into earthen pots filled with a 2:1:1 mixture of soil, sand, and compost. For nine days, they were covered with a clear polyethene bag to maintain high humidity and regular irrigation. The plantlets were moved to an open field after two months (Akter et. al., 2014).

RESULTS

Multiple shoot induction: Following surface sterilization, explants from the shoot tip and nodal segment were cultivated on MS supplemented with varying amounts of BAP and NAA either separately or in various combinations to promote repeated shoot regeneration. The number of shoots induced from the explants varied depending on the dose of cytokinin or auxin employed. The optimal combination among the various concentrations and combinations was determined to be MS+1.5 mg/l BAP+0.5 mg/l NAA which resulted in 7±0.08 shoots per culture on this medium emerged from 88% of explants in less than six weeks (Table 1).

Influence of Coconut water (CW) and urea on the number and growth of shoots: Coconut water (CW) (5–20%) was added to the medium to promote shoot proliferation and subsequent development. The number of shoots (15 per culture) increased with the addition of 10% CW to the medium. Additionally, urea was shown to have a positive influence on shoot elongation. The length of the shoots increased when 100 mg/l urea was added to the media. To multiply a large number of shoots of the appropriate length, the medium was found to be MS+1.5 mg/l BAP+0.5 mg/l NAA + 10% CW + 100 mg/l urea.

Rooting of elongated shoots: Carefully removed from the culture vessels, well-developed and elongated shoots were divided into individual shoots and inserted into the rooting media containing half-strength MS with various ratios and mixes of IBA, IAA, and NAA. Half-strength MS medium supplemented with 1.0 mg/l IBA+0.5 mg/l NAA produced the greatest results. Eighty percent of the shoots in this combination were rooted after three weeks of culture, and each microcutting yielded 7-9 roots. The kind and amount of auxins utilized in the media had an impact on the rooting response (Table 2).

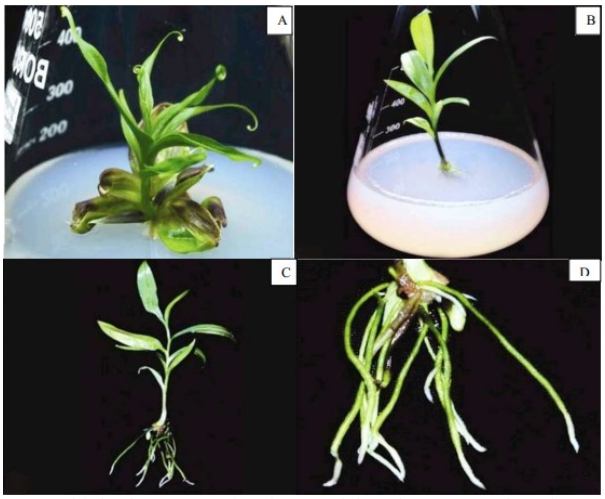
Plantlet hardening: After removing the in vitro-grown plantlets from the culture tubes, the adhering media was removed from the roots by washing them with tap water.

Table 1: Impact of various growth regulator combinations and concentrations in MS medium on the proliferation of shoots from *Gloriosa superba* L. nodal explants. Data were collected following a six-week culture period

Growth regulators (mg/l)		% of explants produced shoots	Average number of shoots/explant
BAP	NAA		
1.0	0.5	75	4±0.16
1.5	0.5	88	7±0.18
2.0	0.5	56	4±0.16
2.5	0.5	36	3±0.29
3.0	0.5	-	-

Table 2. Influence of different auxins (IBA and NAA) in half-strength MS medium on adventitious root formation from in vitro raised shoots of *Gloriosa superba* L. after six weeks of culture

Auxins (mg/l)		% of rooting	Days required for rooting	No. of roots per shoots	Average length of roots (cm)
IBA	NAA				
1.0	-	28	28-30	4-5	2.5
1.5	0.25	68	23-27	5-7	3.0
1.0	0.5	80	21 -23	7-9	3.1
1.5	0.5	52	22-24	3-5	2.6
2.0	0.5	18	24 -26	2-3	2.4



(A) Multiple shoot regeration from nodal explant, (B) A shoot inoculated in rooting medium, (C) and (D) Well developed rooting

Figure 1. *In vitro* regeneration of *Gloriosa superba* L.

In a paper cup filled with red soil, sand, and vermicomposite (2:1:1), the plantlets were allowed to harden. For ten days, the platelets were kept at 25±2 °C in a growth chamber with polyethene bags covering them. 80% of the plantlets made it through the hardening process, and those were then moved to the experimental field. The plantlets were transported to a greenhouse to become acclimated to the environment after ten days, at which point the polythene bags were removed.

DISCUSSION

Although *G. superba* naturally exhibits low frequencies of regeneration and poor seed germination, the plant is normally propagated using corms and seeds. The traditional propagation method has several drawbacks, such as the need to set aside 50% of the yield to raise the next generation and the potential for soil-borne disease transmission between crops, between locations, and during the two to three-month storage period between harvest and the next crop's raising. It is imperative to create a proficient mechanism for **in vitro** mass growth of the plant to preserve this medicinally significant species. Higher rates of organogenic callus were obtained by Finn and Van Staden (1994) with 2,4-D (1.5 mg l^{-1}) and Kin (0.9 mg l^{-1}), and maximum callus proliferation was observed with 2, 4-D (1 mg l^{-1}) and Kin (1 mg l^{-1}). Sivakumar and Krishnamurthy (2000) achieved multiple shoot induction from non-dormant corm bud explants with BAP (7.77 µM) + ADS (5.44 µM) and shoot tip explant on 2iP (8.61 µM) + Kin (2.32 µM). Manju et al. (2010) detected shoot initiation on MS media supplemented with Kin (2.0 mg l^{-1}) and 2, 4-D (1.0 mg l^{-1}) and generated organogenic callus from internodal explants using a combination of 2, 4-D (3.0 mg l^{-1}) and BAP (0.5 mg l^{-1}). Gopinath and Arumugam (2012) obtained multiple shoot production with 93.40 mean number of shoots from rooted micro rhizomes on ½ MS medium supplemented with Kin (1.0 mg l^{-1}) + BAP (1.5 mg l^{-1}) + 20% CW. From rhizome explants, they obtained yellowish textured callus with 99.40% callus initiation efficiency. Gopinath et al. (2014) also noted the development of dark greenish shoots on ½ MS media containing BAP (3.0 mg l^{-1}), IBA (1.0 mg l^{-1}), and IPA (0.75 mg l^{-1}), as well as the generation of yellowish callus from root explants with 2,4-D (2.0 mg l^{-1}) + IAA (1.0 mg l^{-1}) + NAA 0.75 (mg l^{-1}). The findings of Ade and Rai (2009), who saw more shoots per callus on MS basal medium supplemented with 1.5 mg l^{-1} BA + 0.5 mg l^{-1} NAA, are consistent with our findings. Custers and Bergervoet (1994) found that adding BA (1 mg l^{-1}) at low concentrations enhanced plant growth, but high concentrations of BA (10 mg l^{-1}) caused multiple shoot proliferation, which is similar to the findings of Sivakumar et al., 2019. In our investigation, on average 7 shoots per explant were obtained using 1.5 mg l^{-1} of BA and 0.5 mg l^{-1} of NAA. The current study shows that contrary to the earlier studies, CW and urea can also be utilized to encourage the formation of shoots from elongated shoots. In medicinal plants produced *in vitro*, the addition of CW to the substrate improved shoot growth and development (Tefera and Wannakrairoj, 2004). According to Loc et al. (2005), *in vitro* tests have shown that CW can be utilized for shoot induction and multiplication in conjunction with synthetic auxins like IBA and NAA. The best results for shoot induction were obtained with half-strength MS medium supplemented with 1.0 mg/l IBA+0.5 mg/l NAA.

CONCLUSION

The current work devised an effective *in vitro* mass propagation protocol for *G. superba* employing corm bud explant. We developed an in vitro *Gloriosa superba* L. regeneration procedure through this work. In the experiment, the explants used were nodal segments. Well-rooted shoots on half-strength MS supplemented with 0.5 mg/l NAA and 1.0 mg/l IBA. The plantlets that had recovered were effectively moved to potted soil and then to the experimental field. Plant growth regulators improved the induction of numerous shoots, and our findings unmistakably showed that CW and urea promoted the roots of elongated shoots. The cultural conditions, growth and medium composition could all be responsible for this difference. The method developed here is incredibly effective for mass-propagating *G. superba* and offers a useful approach to the species' restoration. The technique devised in this work might be used for further investigations on *G. superba* species cultivated *in vitro* to examine and comprehend the metabolic composition and profiling of the plants with high therapeutic value grown in different parts of India.

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