In Vitro Regeneration of *Gloriosa supe*rba L.-An Overexploited Medicinal Plant in Bangladesh

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Abstract

Culture conditions were developed for *in vitro* regeneration of *Gloriosa superba* L. using shoot tip and nodal segment explants. In case of both explants, the best shoot induction was observed on MS+1.5 mg/l BAP+0.5 mg/l NAA. Nodal explants showed more effective for shoot proliferation than shoot tip explants. Among two types of explants, nodal segment explants produced the highest number of shoots (8±0.12) when they were cultured on above mentioned medium. Shoot tip explants also produced multiple shoots, but their performance was not as good as nodal explants. Addition of 10% coconut water (CW) to the above mentioned medium enhanced the number of shoots per culture and incorporation of 100 mg/l urea to the medium increased the length of shoots. Best rooting was obtained from shoots cultured on half-strength of MS fortified with 1.0 mg/l IBA+ 0.5 mg/l NAA. Within four weeks of transfer to the rooting medium, 80% microcuttings produced 8-10 roots. The regenerated plantlets were successfully transferred to potted soil, where survival rate was 80%.

Keywords: *In vitro* regeneration, explants, microcuttings, *Gloriosa superba* L.

1. Introduction

Gloriosa superba L. is a striking tuberous climbing plant with brilliant wavy-edged yellow and red flowers. The name *Gloriosa* comes from the word 'gloriosus' which means handsome and *superba* from the word 'superb' means splendid or majestic kind. It is an important medicinal plant which contains alkaloids, mainly colchicines and colchicoside. The seeds of this taxon are highly priced in the world market as they are the main source of colchicines and colchicoside.

In order to provide enough plant material for commercial exploitation, mass propagation through *in vitro* culture is urgently needed not only to conserve this taxon but also to meet the demands for this medicinal plant as a source of colchicine. Different parts of the plant have a wide variety of uses especially within traditional medicine practiced in tropical Africa and Asia. The tuber is used traditionally for the treatment of bruises and sprains, colic, chronic ulcers, hemorrhoids, cancer, leprosy and also for inducing labour pains. Medicinally, the tuber is used as abortifacient, and in smaller doses it acts as a tonic, stomachic and anthelmintic. It is also used in the treatment of gout because it contains colchicines. Paste of the tuber is externally applied for parasitic skin diseases [1]. The plant was under threatened category due to its imprudent harvesting from wild as it is extensively used by medicinal industries for its colchicines content. It also faces a low seed set problem, but due to its industrial demand it is now under cultivation [2]. Now-a-days, this herb is becoming rare in our country. It is commonly grown from seeds and tubers. Since in Bangladesh the seeds and tubers are harvested extensively for their therapeutic uses, the plant stands the chances of becoming a threatened species in Bangladesh. In order to protect such endangered species from possible extinction, the exploitation of medicinal plants must be accompanied by conservation measures [3]. *In vitro* micropropagation system of *G. superba* was reported by many researchers [4-7]. But their reports were not satisfactory for large scale propagation of plantlets as well as successful field transfer. Thus considering the above facts, the present study was undertaken to develop a suitable protocol for *in vitro* regeneration of *G. superba* L.

2. Materials and Method

Shoot tips and nodal segments of *Glorioa superba* L. were collected from Atomic Energy Research Establishment campus. The explant materials were kept in water, brought to the laboratory and washed thoroughly under running tap water for one hour in order to remove dirt on the stem surface. These were then cut into small pieces and surface sterilization was done with an aqueous solution of 0.1% $HgCl_2$ with two drops of tween 20 for six min under aseptic condition and rinsed five times with autoclaved distilled water to wash out any traces of $HgCl_2$. Shoot tips and nodal segments, approximately 2.0 cm in length were cut from surface sterilized stem for explants. Shoot tip and nodal segment explants were cultured on MS supplemented with different concentrations of cytokinins (BAP, Kin) singly or in combination with auxin (NAA) for shoot regeneration. For rooting, half-strength MS supplemented with auxin such as IBA, IAA and NAA were used. Coconut water (CW) (5-20%) and urea (50-200 mg/l) were added to the medium for shoot multiplication and elongation of individual shoots. The pH of the media were adjusted to 5.8 before adding agar. All media were gelled with 0.7% agar and autoclaved for 20 min at $121^{o}C$ under 1.1 kg/cm^2 pressure. The cultures were incubated at $25 \pm 1^{o}C$ under 16h photoperiod with a light intensity of 3000-4000 lux. The cultures were regularly subcultured at three weeks intervals on fresh medium. Observations were recorded every five days following implanting and subculturing. All experiments were repeated twice with at least 20 cultures

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per treatment. For hardening, the test tubes containing rooted shoots were kept at normal room temperature and light for 10 days. Thereafter, the rooted shoots were taken out from the test tubes and gently washed to remove remnants of agar from roots. Then they were transplanted to earthen pots containing a mixture of soil, sand and compost (2:1:1) and covered with transparent polyethylene bag for 9 days to ensure high humidity while irrigating regularly. After two months the plantlets were transferred to open field.

3. Results and Discussion

After surface sterilization, shoot tip and nodal segment explants were cultured onto MS supplemented with different concentrations of BAP, Kin and NAA alone or in different combinations for multiple shoot regeneration. Induction of shoots were observed in media containing BAP or Kin alone and in combination with NAA but the number of shoots induced from the explants varied according to the concentration of cytokinin or auxin used. Among the different concentrations and combinations used, the combination MS+1.5 mg/l BAP+0.5 mg/l NAA was found to be the best. In the case of nodal segments, 90% explants developed shoots within six weeks with 8±0.12 shoots per culture on this medium (Table 1, Fig. 2). In the same medium, 80% shoot tip explants produced shoots and the number of shoots per culture was 6±0.22 (Table 1, Fig. 1).

For further development of the medium and enhanced shoot proliferation, coconut water (CW) (5-20%) was added to the medium. Addition of 10% CW to the medium, increased the number of shoots (15 and 10 in case of nodal segment and shoot tip explants, respectively) per culture (Figs. 3, 4). Positive effect of urea on shoot elongation was also observed. Addition of 100 mg/l urea to the medium increased the length of shoots (Fig. 5). Thus the medium as determined for multiplication of large number of shoots with proper length was MS+1.5 mg/l BAP+0.5 mg/l NAA +10 %CW+ 100 mg/l urea. Well developed and elongated shoots were taken out carefully from the culture vessels and individual shoots were separated and implanted in the rooting media containing half-strength MS with different concentrations and combinations of IBA, IAA and NAA. The best result was obtained in half-strength MS medium supplemented with 1.0mg/l IBA+0.5 mg/l NAA (Table 2). In this combination, it was observed that 80 % shoots rooted within four weeks of culture and each microcutting produced 8-10 roots (Fig. 6). The rooting response varied with the type and concentration of auxins used in the media (Table 2).

For hardening and establishment of plantlets under the natural conditions, the well-rooted plantlets were transferred to earthen pots containing soil, sand and compost. During hardening 80% plantlets survived and those were subsequently transferred to experimental field.

Several researchers reported that MS medium supplemented with Kin and IBA at a concentration of 1.0 mg/l each was optimum to regenerate multiple shoot in *G. superba* from shoot tip explant [4]. Another group of scientists developed a medium for *in vitro* regeneration of *G. superba* from apical and axiallary buds explants, where they used MS + 1.5 mg/l BAP +0.5 mg/l NAA [5]. In the present experiment, it was observed that MS + 1.5 mg/l BAP +0.5 mg/l NAA was optimum for maximum number of shoot proliferation from the cultured explants of *G. superba*. This result is similar with the previous report. Addition of 10% CW to the medium increased the number of shoots per culture. We got some research articles where researchers reported more or less similar effect of CW on *in vitro* shoot development [8]. In the case of shoot elongation, 100mg/l urea was found to be optimal. Beneficial effects of the addition of complex organic substances on the growth of cultures have been reported [9-11]. Addition of urea in culture medium was also fruitful in another important medicinal plant- *Andrographis paniculata* Nees [12].

For the induction of roots in *in vitro* raised shoots of *G. superba*, maximum researchers reported that IBA and IAA combination with half-strength MS is more effective for root induction [4, 6-7]. In the present investigation it was observed that 1.0 mg/l IBA and 0.5 mg/l NAA in half-strength MS medium was optimal for root formation. The findings will be useful for propagation and conservation of this medicinal plant-*Gloriosa superba* L.

Table 1. Effect of different concentrations and combinations of growth regulators in MS mediun on shoot proliferation from shoot tip and nodal explants of *Gloriosa superba* L. Data were recorded after six weeks of culture.

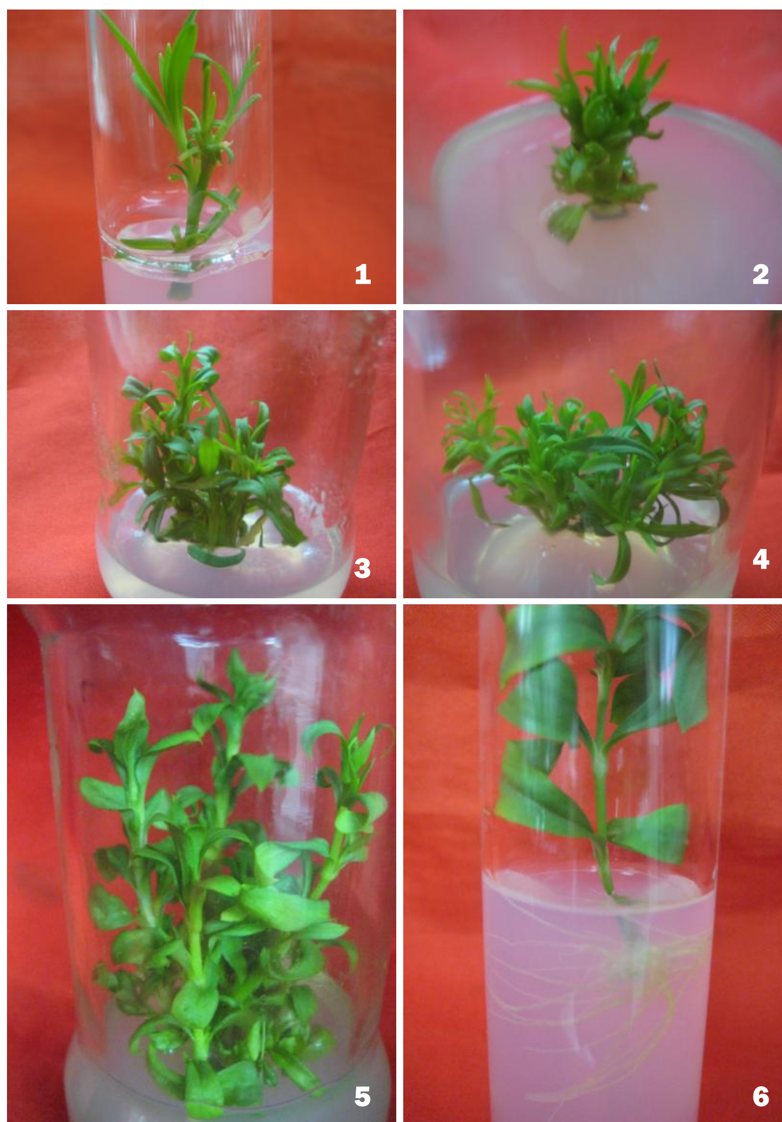
Growth regulators (mg/l)	% of explants produced shoots		Average number of shoots/explant	
	Shoot tip	Nodal segment	Shoot tip	Nodal segment
BAP				
0.5	-	-	-	-
1.0	-	-	-	-
1.5	30	40	3±0.12	3±0.22
2.0	50	60	3±0.22	4±0.24
2.5	20	20	2±0.22	3±0.22
3.0	-	-	-	-
Kin				
0.5	-	-	-	-
1.0	-	-	-	-

Growth regulators (mg/l)	% of explants produced shoots		Average number of shoots/explant	
	Shoot tip	Nodal segment	Shoot tip	Nodal segment
1.5	30	30	3±0.22	3±0.22
2.0	-	-	-	-
2.5	-	-	-	-
BAP+NAA				
1.0 + 0.25	-	-	-	-
1.5 + 0.25	-	-	-	-
2.0 + 0.25	-	-	-	-
2.5 + 0.25	40	50	4±0.22	4±0.32
3.0 + 0.25	60	60	4±0.22	4±0.44
1.0 + 0.5	70	78	5±0.12	5±0.14
1.5 + 0.5	80	90	6±0.22	8±0.12
2.0 + 0.5	50	60	4±0.34	5±0.14
2.5 + 0.5	30	34	3±0.22	4±0.24
3.0 + 0.5	-	-	-	-
Kin+NAA				
0.5 + 0.25	-	-	-	-
1.0 + 0.25	-	-	-	-
1.5 + 0.25	-	-	-	-
2.0 + 0.25	-	-	-	-
2.5 + 0.25	-	-	-	-
1.0 + 0.5	30	40	2±0.24	3±0.24
1.5 + 0.5	20	30	2±0.12	3±0.12
2.0 + 0.5	-	-	-	-
2.5 + 0.5	-	-	-	-

Table 2. Influence of different auxins (IBA, IAA 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)	Percentage of rooting	Required time for rooting (days)	No. of roots per shoot	Average length of roots (cm)
IBA				
0.5	-	-	-	-
1.0	30	26-28	3-4	2.4
1.5	20	22-27	2-3	2.2
2.0	-	-	-	-
2.5	-	-	-	-
IAA				
0.5	-	-	-	-
1.0	-	-	-	-
1.5	20	20-25	2-3	2.5
2.0	-	-	-	-
2.5	-	-	-	-
IBA+IAA				
0.5+0.5	-	-	-	-
1.0+0.5	-	-	-	-
1.5+0.5	-	-	-	-
2.0+0.5	30	20-25	2-3	3.0
2.5+0.5	20	20-25	1-2	2.5
0.5+1.0	-	-	-	-
1.0+1.0	-	-	-	-
1.5+1.0	50	20-25	3-4	3.0
2.0+1.0	20	25-28	2-3	2.8

Auxins (mg/l)	Percentage of rooting	Required time for rooting (days)	No. of roots per shoot	Average length of roots (cm)
IBA+NAA				
1.0+0.25	70	22-26	6-8	3.0
1.0+0.5	80	20-22	8-10	3.2
1.5+0.5	50	22-24	4-6	2.5
2.0+0.5	20	24-26	3-5	2.4
1.0+1.0	-	-	-	-
1.5+1.0	-	-	-	-
2.0+1.0	-	-	-	-
IAA+NAA				
1.0+0.5	-	-	-	-
1.5+0.5	-	-	-	-
2.0+0.5	-	-	-	-



Figs. 1-6. *In vitro* regeneration of *Gloriosa superba* L., (1) Multiple shoot regeneration from shoot tip explant on MS+1.5 mg/l BAP+0.5 mg/l NAA, (2) Multiple shoot regeneration from nodal segment explant on the same medium, (3-4) Positive effect of 10% CW on shoot proliferation in both shoot tip explant (Fig. 3) and nodal segment explant (Fig. 4), (5) Elongated shoots on MS+1.5 mg/l BAP+0.5 mg/l NAA+10% CW +100 mg/l urea and (6) Root induction on half-strength MS+1.0 mg/l IBA+ 0.5 mg/l NAA.

4. Conclusion

Through this study we established an *in vitro* regeneration protocol of *Gloriosa superba* L. Two types of explants named- shoot tip and nodal segment were used in the experiment. Among two types of explants, nodal segment explants produced the highest number of shoots on shoot induction medium. Shoot tip explants also produced multiple shoots, but their performance was not as good as nodal explants. Shoots rooted well on half-strength of MS fortified with 1.0 mg/l IBA+ 0.5 mg/l NAA. The regenerated plantlets were successfully transferred to potted soil and thereafter the experimental field.

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