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Full Length Research Paper

In vitro tuberization of glory lily (*Gloriosa superba* L.)

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***Gloriosa superba* L., a climber belonging to the family, Colchicaceae is a high value medicinal plant cultivated in Tamil Nadu. Colchicine is one major tropalone alkaloids of medicinal importance which cures gout, cancer, rheumatism. Vegetative propagation through V or L shaped tubers is a commonly followed practice but is considered slow with poor multiplication ratio of 1: 1 every year. The cost involved towards planting material (800 kg of tubers/acre) alone accounts to 2.0 lakhs at Rs. 250 per kg of tuber prevailing for the last three years. In this context, a suitable protocol for *in vitro* tuberisation using non-dormant tubers of *Gloriosa superba* L. on Murashige and Skoog medium supplemented with various concentrations growth regulators have been reported in the present study. Sprouted tuber node explants were sterilized with 70% ethanol for 30 s followed by 60 s in $HgCl_2$ which reduced the contamination percentage (8.00%). MS medium supplemented with 4.0 mgL^{-1} BAP and 1.0 mgL^{-1} NAA recorded the highest response for primary tuber (100%) and secondary tuber (100%) formation. This also recorded the maximum number of tuber (1.77) from single explants. GA_3 (1.0 mgL^{-1}) was observed to be vital for the elongation of shoot whereas, IAA (1.0 mgL^{-1}) in combination with IBA (0.5 mgL^{-1}) was effective for induction of roots on MS medium.**

Key words: Glory lily, ms medium, tuber node, shooting, rooting, primary tubers.

INTRODUCTION

Gloriosa superba L., commonly known as Kalahari (Hindi), glory lily, superb lily and tiger lily is an export oriented medicinal plant and a member of the Colchicaceae family. *G. superba* is propagated by 'V' or 'L' shaped tubers which sprouts during the rainy season. Vegetative propagation through tubers is a commonly followed practice but is considered slow with poor multiplication ratio of 1: 1 every year (Krause, 1986). Through micro propagation technique, elite plants are produced with high multiplication rates (1: 8) which also produce disease-free planting materials (Seemanti et al., 2007). Suprio (2011) reported that *in vitro* regeneration by multiple auxiliary shoot induction from sprouted tuber and seedlings derived from shoot tip explants was successful, particularly with the use of TDZ at 0.1 mg/L concentration which induced up to 14.1 auxiliary shoots per explants. Rapid multiplication through micro propagation is an attempt towards aiming for higher multiplication ratio and for development of quality planting materials in the present study.

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MATERIALS AND METHODS

Mature tubers of *G. superba* were collected from healthy plants from farmer's field, Mulanoor of Tirupur district, Tamil Nadu. The sprouted tuber node was the explants, that is, 1 cm long portion of tuber containing 3 to 5 mm long whitish apical buds. The explants were excised and washed thoroughly with detergent solution (Teepol 0.1%) followed by 3 to 4 washings with sterile distilled water. After that, explants were rinsed with 70% ethanol for 30 s, and was then washed with sterile distilled water for about 3 times followed by washing with 0.1% $HgCl_2$ solution at different duration (35, 40, 45, 50, 55 and 60 s) and washed 3 to 5 times in sterile water. The explants excised in appropriate size were implanted in

Table 1. Effect of duration of exposure of sterilants on tuber nodal explants of *Gloriosa superb.*

Treatment		Tuber node		
$HgCl_2$ concentration (%)	Duration of exposure (s)	Contamination (%)	Survival (%)	Mortality (%)
0.1	35	98.00(83.08)	2.00(8.13)	0.00(0.74)
0.1	40	90.00(71.58)	10.00(18.44)	0.00(0.74)
0.1	45	86.00(68.08)	10.00(18.44)	4.00(11.54)
0.1	50	35.00(36.27)	62.00(51.95)	3.00(9.97)
0.1	55	9.00(17.46)	91.00(72.67)	0.00(0.74)
0.1	60	8.00(16.43)	80.00(63.46)	12.00(20.27)
	Mean	54.33(48.82)	42.00(38.85)	3.17(7.33)
	SE (d)	1.953	1.2502	0.1472
	CD (0.05)	4.2567	2.7240	0.3207

*Values in parenthesis are arcsine transformed.

the MS medium. The different growth regulators like BAP (3.0, 4.0, 5.0 mgL^{-1}), GA_3 (0.5 and 1.0 mgL^{-1}) and NAA (0.5, 1.0 mgL^{-1}) were used in the medium both separately and in combinations.

RESULTS AND DISCUSSION

In the present study on *G. superba*, the sterilization procedure reduced the degree of contamination of explant (tuber node) to 8.00% when it was surface sterilized with 70% ethanol for 30 s followed by 60 s in $HgCl_2$. When the herbaceous part of explants was treated with lower concentration of sterilants with lower period of exposure, the soft tissues were not affected (Table. 1). This also supports the results of Custers and Bergervoet (1994), Sivakumar and Krishnamoorthy (2000) and Sivakumar et al. (2003) in *G. superba,* that surface sterilization using 0.1% mercuric chloride for 5 and 10 min respectively produced contamination free cultures. Similar reports were also given by Hassan and Roy (2005).

Micro tuber formation

Finnie and Vanstaden (1989) reported that multiple productions of tubers were important rather than shoot *in vitro*. The highest response (100%) to *in vitro* tuber formation in glory lily in the present study was observed on MS medium supplemented with 5.0 mgL^{-1} BAP +1.0 mgL^{-1} NAA. Similar report was published by Sivakumar et al. (2003) who stated that auxin and cytokinin were the effective inducers of micro tuberization in different yam species cultured *in vitro*, although, certain types of these regulators are more effective than others for increasing micro tuber induction frequencies. For example, naphthalene acetic acid (NAA) and kinetin (KN) have been shown to promote micro tuber induction in *G. superba* (Suprio, 2011) and *Dioscorea bulbifera* (Ammirato, 1982) and *Dioscorea alata* (Mantell and Hugo, 1989).

In the present study, response to micro tuber formation was higher only in the medium supplemented with NAA at 1.0 mgL^{-1}. Similar results were given by Alizadeh et al. (1998), in which NAA at 1.0 and 2.0 mgL^{-1} respectively, showed promotive effect but the effect was short termed and less than one tuber was obtained from single explants in the course of 12 weeks on MS medium supplemented with 2.0 mgl^{-1} NAA.

In the present study, medium supplemented with BAP and NAA resulted in maximum number (1.77) of micro tuber formation. The medium supplemented with BAP and NAA gave maximum (100%) response to primary and secondary tuber formation (Table 2). This result is in accordance with Islam et al. (2004) who stated that BAP and NAA yielded a better response of *in vitro* micro rhizome induction in *Curcuma longa*. Nayak (2000) and Sharma and Singh (1995) also reported that 5.0 mgL^{-1} BAP in *Curcuma aromatica* and 8.0 mgL^{-1} BAP in ginger, respectively enhanced micro rhizome production. Iglesias et al. (1999) reported, that the combination of BA and NAA 0.5 µM gave better response of bulblet formation *in vitro* in *Hyacinthoides paivae.*

Micro rooting

In the present study, maximum value for response to rooting (49.41%), number of roots (3.06), root length (1.90 cm) was observed in the MS medium supplemented with 1.0 mgL^{-1} IAA + 0.5 mgL^{-1} IBA, while the medium with either IAA or IBA alone exhibited a poor response for the aforementioned characters (Table 3). This results are in accordance with Sayeed and Shyamal (2005) who stated that rooting in regenerated shoots of *G. superba* was achieved at 90% when the excised shoots were cultured individually on root induction medium, consisting of half strength MS salts with 1.0 mgL^{-1} IAA + 0.5 mgL^{-1} IBA. Similarly Suprio (2011) also reported that NAA (0.5 mg/L) and IBA(0.1 mg/L) was the best *in vitro* rooting medium for *G. superba*.

Table 2. Effect of growth regulators on response to primary and secondary tuber formation, days for tuber formation and number of tubers formed per explants.

Treatment no.	Growth regulators (mgL^{-1})			Response to tuber formation (%)				Days for tuber formation (days)		Number of tubers per explants
	GA_3	BAP	NAA	Primary tuber		Secondary tuber		Primary tuber	Secondary tuber	
T_1	-	-	-	61.66	(51.80)	0	(0.55)	33.11	0.00	0.77
T_2	0.5	-	-	35.00	(36.27)	0	(0.55)	31.11	0.00	0.44
T_3	1.0	-	-	56.66	(48.86)	0	(0.55)	31.55	0.00	0.66
T_4	-	5.0	-	43.33	(41.15)	0	(0.55)	21.77	0.00	0.55
T_5	1.0	5.0	-	68.33	(55.97)	78.33	(43.73)	21.00	54.55	1.22
T_6	-	10.0	-	41.33	(41.16)	0	(0.55)	21.55	0.00	0.55
T_7	1.0	10.0	-	75.00	(60.31)	60	(50.95)	22.55	55.66	1.33
T_8	-	4.0	1.0	95.00	(79.36)	86.66	(69.24)	21.55	50.33	1.44
T_9	-	5.0	1.0	100.00	(89.45)	100	(89.45)	19.88	45.11	1.77
			Mean	64.25	(56.04)	33.51	(28.46)	24.89	22.86	0.97
			SE (d)	4.1070		10.5942		1.4399	8.5751	0.2100
			CD (0.05)	8.6286		22.2578		3.0253	18.0159	0.4412

*Values in parenthesis are arcsine transformed.

Table 3. Effect of growth regulators on response to rooting (%), days for rooting (days), no. of roots and length of root (cm).

Treatment no.	Growth regulators (mgl^{-1})		Response to rooting (%)		Days for rooting (days)	Number of roots per plantlet	Root length (cm)
	IAA	IBA					
T_1	0.5	-	41.33	(40.00)	27.94	1.46	0.76
T_2	0.5	0.5	43.18	(41.07)	22.65	1.80	1.20
T_3	0.5	1.0	46.00	(42.70)	18.99	1.96	1.76
T_4	1.0	-	46.26	(42.85)	15.83	1.86	1.26
T_5	1.0	0.5	49.41	(44.65)	13.60	3.06	1.90
T_6	1.0	1.0	46.78	(43.15)	17.14	2.63	1.83
		Mean	45.49	(42.40)	19.36	2.133	1.45
		SE(d)	0.7887		2.9799	0.3226	0.3388
		CD(0.05)	1.7185		6.4928	0.7029	0.7383

*Values in parenthesis are arcsine transformed.

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