In vitro Shoot initiation from Apical shoot buds & Meristems of *Gloriosa superba* L. – An endangered medicinal herb of high commercial value

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Abstract: This research work has given a method for *in vitro* propagation by shoot induction in *Gloriosa superba* L., which is a medicinal plant of high commercial value. For shoot formation both apical shoot buds & meristems were used. This was achieved on MS medium containing BAP in combination with NAA & Kinetin alone or in combination with BAP was tested. Best shoot initiation response was achieved on MS Medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA on both apical shoot bud & meristem explants. The maximum percentage of explants (meristems & apical shoot bud) forming shoots are 90 ± 7.0 and 88 ± 6.2 respectively. Increase or decrease in concentration of BAP 2.0 mg/l + NAA 0.5 mg/l, increases, no. of days required for shoot initiation. Meristems showed more pronounced effect of shoot formation than apical shoot bud explants. These induced shoots were increase in their number & in size when were given subsequent incubation period. Addition of BAP in combination of Kn or Kn alone failed to show good shoot initiation response. The shoot induction protocol developed in this study provides a basis for germplasm conservation and for further investigation of medicinally active constituents of the elite medicinal plant. Further work for standardization of efficient *in vitro* protocol for best shoot multiplication & *in vitro* rooting is under progress in our laboratory.

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1. Introduction

Glory lily (*Gloriosa superba* L.) is a medicinal plant belonging to the family Liliaceae is a semi-woody herbaceous branched climber reaching approximately 5 meters height, with brilliant wavy-edged yellow and red flowers (Rajak & Rai, 1990). One to four stems arise from a single V-shaped fleshy cylindrical tuber. *Gloriosa superba* is one of the endangered species among the medicinal plants (Badola, 2002) commonly known as Kalihari in Hindi, Kal-lavi in Marathi, Manthori khizangu in Malayalam and Kazhappai kizhangu in Tamil. It is extensively scattered in the tropical and sub-tropical parts of the India. It is adapted to different soil texture and climatic variation. The plant grows in sandy-loam soil in the mixed deciduous forest in sunny positions. *Gloriosa superba* is an inhabitant of tropical Africa and now found growing naturally in many countries of topical Asia including India, Bangladesh, Malaysia and Myanmar. In India, it occurs commonly in tropical forests of Bengal and Karanataka (Sivakumar and Krishnamurthy, 2002). Studies reveal that all parts of the plant especially the tubers & seeds contain alkaloids such as colchicines and Gloriosine (Trease and Evans, 1983). Tubers and seeds of *Gloriosa superba* are an expensive export commodity. In the Indian systems of medicine, the tubers are used as tonic, antiperiodic, antihelmenthic, and also against snake bites (Gupta et al., 2005). Colchicine & Gloriosine are two commonly used phytochemicals for treatment of gout & rheumatism. Different parts of the plant have 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, haemorrhoids, cancer, impotence, nocturnal seminal emission, and leprosy and also for including labour pains and abortions (Kala et al., 2004). *Gloriosa superba* also used in wounds, skin related problems, Fever, Inflammation, piles, blood disorders, Uterine contractions, General body toner, Poisoning (Haroon et al., 2008). *Gloriosa superba* has gained the importance in medicine in recent years & is indicated promising drug for the production of colchicine on commercial scale (Kokate et al., 2004).

Application of tissue culture to plant conservation in India has been largely restricted to economically important species. However, the approach could usefully be extended to conserve all threatened plants so that vital biodiversity and the ecological network is sustains can be preserved (Jiten Chandra et al., 2011). *Gloriosa superba* is categorized as a rare and endangered species and is on the IUCN Red list of Endangered species (2001). The natural stocks of this plant are fast disappearing

& facing local extinction (Dhushara, 2004). Mortality on account of leaf blight disease is yet another major threat that this species experiences (Maiti et al., 2007). The tubers are only the propagating material, but there germination is not possible in different climatic conditions. In nature less seed germination with poor viability is responsible for its diminishing population size. The poor propagation coupled with over exploitation for its diverse medicinal applications *G. superba* has been endangered; therefore, there is urgent need to conserve the plant by biotechnological approach like tissue culture (Rajgopalan and Khader, 1994).

The use of *in vitro* techniques in germplasm conservation is increasing and has been successfully applied to the conservation of several rare & endangered species, both for propagation and for long-term storage (Chandra et al., 2006; Sarasan et al., 2006). In the present investigation, we report the successful *in vitro* shoot initiation of *G. superba* by means of apical shoot buds & meristems.

2. Material & methods

Healthy and profusely growing *Gloriosa superba* with rhizome were collected during the month of July, 2011 from Medicinal plant Conservative Area of Pachmarhi Biosphere reserve, Hoshangabad (Madhya Pradesh), India. The plant was identified by Dr. Amarjeet Bajaj (Head & Professor), PG and Research Department of Botany, Govt. Motilal Vigyan Mahavidyalaya, Bhopal, Madhya Pradesh, India. A voucher specimen (012/July/2011) was submitted in the Herbarium of Botany, Govt. Motilal Vigyan Mahavidyalaya, Bhopal. Young actively growing plants of *Gloriosa superba* were wash thoroughly in running tap water for 15 minutes to remove all the dirt and soil particles adhering to them. Apical shoot buds & meristems were used as explants for this experiment. Explants were cut and reduced to length of 2 cm using surgical blade, retaining the apical dome (1 cm). Then, they were kept immersed in water with a small amount of fungicide (Bavistin) for an hour followed by 3 times rinsing in distilled water. Thereafter, again explants were kept immersed in distilled water with few drops of wetting agent, labolene for ten minutes. It was immediately followed by five time rinses in distilled water to remove traces of labolene. Further sterilization procedures were carried out inside laminar air flow chamber, where explants were surface sterilization through single dip in 70% (v/v) for half minute followed by three times rinses in sterile distilled water. Thereafter mercuric chloride (0.10%) treatment were given to explants for 3 minutes followed by four times rinsed in sterile distilled water. Thereafter explants were carefully transferred to sterile blotting paper placed over sterile petri plate to remove excess water & were then inoculated into the culture establishment medium (MS Medium; Murashige & Skoog 1962) using sterile forceps under aseptic conditions. The explants placed vertically on the culture medium. The new shoots induced from the *in vitro* cultures were used as an explant for further experiment.

Apical shoot bud & meristems were culture on MS basal medium supplemented with 3 % (w/v) sucrose (Sd-fine Chemicals, India) for culture initiation which also served as explants sources for subsequent experiments. The pH of the medium (supplemented with respective growth regulators) was adjusted to 5.7 with 1N NaOH or 1N HCl before gelling with 0.8 % (w/v) agar. In all the experiments, the chemicals used were of analytical grade (Merck and Sd-fine Chemicals, India). The medium was dispensed into culture vessels (Borosil, Mumbai, India) and autoclaved at 105 kPa at 121°C for 15 minute. The surface sterilized explants were placed vertically on the culture medium. All the cultures were incubated at 25 ± 2°C under 16 h light /8 h dark photoperiod with irradiance of 45 – 50 µ mol/ m² / s photo synthetically active radiation (PAR) provided by cool white fluorescent tubes (Philip, India) and with 60 – 65 % relative humidity. All subsequent subcultures were done at four weeks intervals.

Culture media consisted of MS (Murashige and Skoog 1962) supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar (Himedia, India) was evaluated for their effects on *in vitro* growth and development of *G. superba*. For induction of shoots, explants were cultured on MS medium supplemented with different concentration of cytokinins, including BAP (0.5-5.0 mg/ l), NAA (0.1-0.1.0 mg/ l) and Kinetin (0.2-2.0 mg/ l) either individually or in combination.

During shoot initiation as well as further growth of shoots observation on percentage of apical shoot buds and meristems sprouting, number of shoots, shoot length and days required for shoot initiation were recorded after six weeks. Each treatment consist of three replication (Twelve cultures each replication). The effects of different treatments were quantified and the data were subjected to statistical analysis using standard error of the mean. Medium lacking growth regulators served as control.

3. Results & Discussion

In order to establish an efficient *in vitro* micropropagation system for *Gloriosa superba* from apical shoot buds & meristems explants were incubated on MS solid medium supplemented with varying levels of BAP (0.5-0.5 mg/l), NAA (0.1-1.0

mg/l and Kinetin (0.2-2.0 mg/l) either individually or in combination. MS basal medium without growth regulator did not show any response. However, MS basal medium supplemented with various concentration of cytokinine alone or in combination with auxin swelled in their size after 1-2 weeks of culture and initiated axillary shoots in another two weeks (Table 1 & table 2).

The results showed that BAP alone or in combination with NAA was more effective for shoot formation then kinetin alone or in combination with BAP. A higher degree of shoot initiation was observed when explants were cultured in MS with 2.0 mg/l BAP + 0.5 mg/l NAA. Further, increase or decrease in this concentration, increases the shoot initiation time (Table 1 & 2; Graph 1, 2 & 3). The association of BAP and NAA positively affected the shoot formation/ shoot initiation rate of *G. superba* compared with BAP alone. Increasing the concentration of Plant growth regulators (BAP + NAA) in both explants (Apical shoot buds & meristems) enhanced the response of shoot initiation & best shoot initiation achieved at 2.0 mg/l BAP + 0.5 mg/l NAA. Further increase in concentration decrease the shoot initiation. Kinetin alone or in combination with BAP is not effective in shoot formation.

Table 1. Effects of type and concentration of plant growth regulators on *in vitro* shoot initiation from apical shoot buds of *Gloriosa superba* L. after six weeks of culture

Plant Growth Regulators Composition (mg/l)			**Media**	**% of Apical shoot buds Initiated ± S.E.[a]**	**Mean number of shoots/ explants ± S.E.[a]**	**Mean shoot length (cm) ± S.E.[a]**	**Days required for shoot induction ± S.E.[a]**
BAP	NAA	Kinetin					
0.0[b]	0.0[b]	0.0[b]	MS1	0.0	0.0	0.0	00.00
0.5	0.0	0.0	MS2	34 ± 1.3	1.2 ± 0.2	1.5 ± 0.1	25.6 ± 0.66
1.5	0.0	0.0	MS3	35 ± 1.6	1.4 ± 0.3	1.8 ± 0.1	19.2 ± 0.58
2.0	0.0	0.0	MS4	38 ± 1.8	2.7 ± 0.5	2.0 ± 0.1	15.4 ± 0.48
2.5	0.0	0.0	MS5	40 ± 2.1	1.5 ± 0.6	2.8 ± 0.1	14.5 ± 0.34
0.5	0.0	0.0	MS6	41 ± 3.0	1.2 ± 0.7	3.0 ± 0.1	12.1 ± 0.22
1.0	0.1	0.0	MS7	42 ± 3.1	2.5 ± 0.9	3.1 ± 0.1	10.6 ± 0.33
1.5	0.2	0.0	MS8	60 ± 4.1	3.1 ± 0.1	4.2 ± 0.1	9.4 ± 0.35
1.5	0.4	0.0	MS9	69 ± 6.7	3.8 ± 0.4	5.5 ± 0.1	7.7 ± 0.52
2.0	**0.5**	**0.0**	**MS10**	**88 ± 6.2**	**4.4 ± 0.3**	**6.7 ± 0.2**	**7.2 ± 0.43**
2.0	1.0	0.0	MS11	70 ± 4.2	3.7 ± 0.4	5.8 ±0.1	8.3 ± 0.23
2.5	0.5	0.0	MS12	63 ± 4.7	3.5 ± 0.5	4.7 ± 0.1	9.1 ± 0.42
2.5	1.0	0.0	MS13	58 ± 4.1	3.3 ± 0.3	4.5 ± 0.1	11.2 ± 0.50
3.0	0.5	0.0	MS14	52 ± 4.0	3.6 ± 0.4	2.8 ± 0.1	16.8 ± 0.48
3.0	1.0	0.0	MS15	47 ± 3.9	3.2 ± 0.3	2.5 ± 0.1	18.1±0.62
3.5	0.5	0.0	MS16	40 ± 3.7	2.8 ± 0.3	1.8 ± 0.1	21.5 ± 0.64
3.5	1.0	0.0	MS17	38 ± 3.2	2.6 ± 0.2	1.7 ± 0.1	25.6 ± 0.71
4.0	0.5	0.0	MS18	32 ±3.1	2.4 ± 0.2	1.5 ± 0.1	28.1 ± 0.88
4.0	1.0	0.0	MS19	27 ± 2.9	2.2 ± 0.1	1.3 ± 0.1	30.1 ± 85
4.5	0.5	0.0	MS20	25 ± 2.6	2.1 ± 0.1	1.1 ± 0.1	32.7 ± 88
4.5	1.0	0.0	MS21	22 ± 2.1	2.0 ± 0.1	1.0 ± 0.1	34.2 ± 0.89
5.0		0.2	MS22	25 ± 3.1	2.6 ± 0.3	2.7 ± 0.1	28.9 ± 0.53
5.0		0.5	MS23	22 ± 2.9	2.0 ± 0.2	2.0 ± 0.1	32.4 ± 0.62
		1.0	MS24	20 ± 2.6	1.8 ± 0.1	1.5 ± 0.1	40.8 ± 0.77
		1.5	MS25	15 ± 1.5	1.5 ± 0.1	1.1 ± 0.1	43.2 ± 0.78
		2.0	MS26	12 ± 1.2	1.2 ± 0.1	1.0 ± 0.1	45.3 ± 80

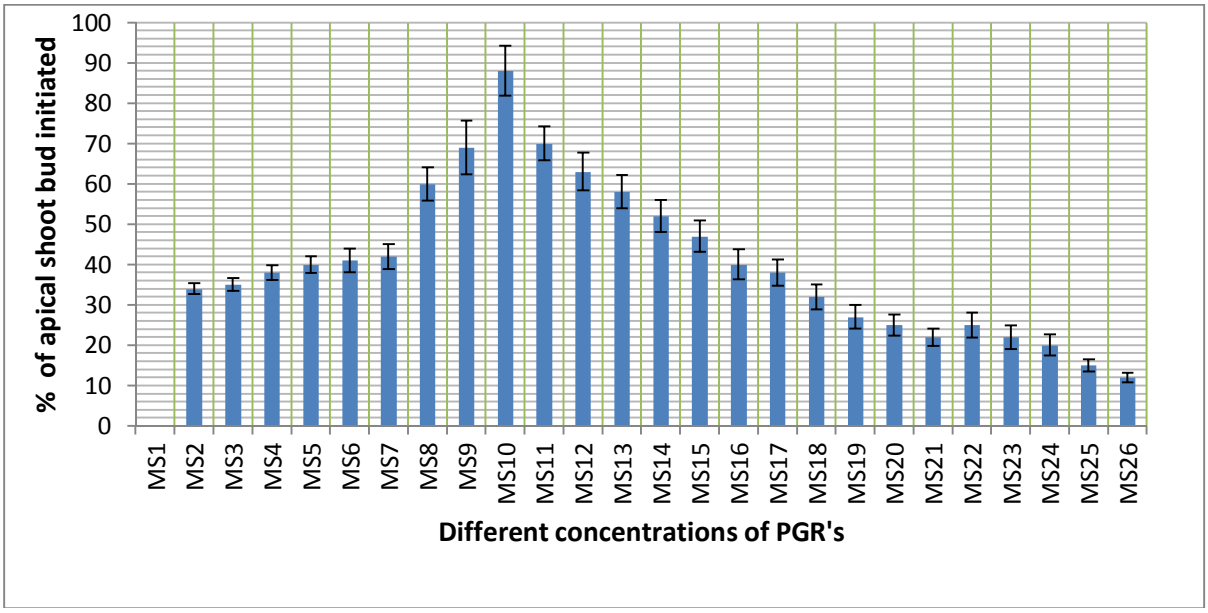
[a]) = Mean ± S. E. for three replications (12 cultures for each replication); data scored after six weeks

[b]) = Medium lacking growth regulators served as control

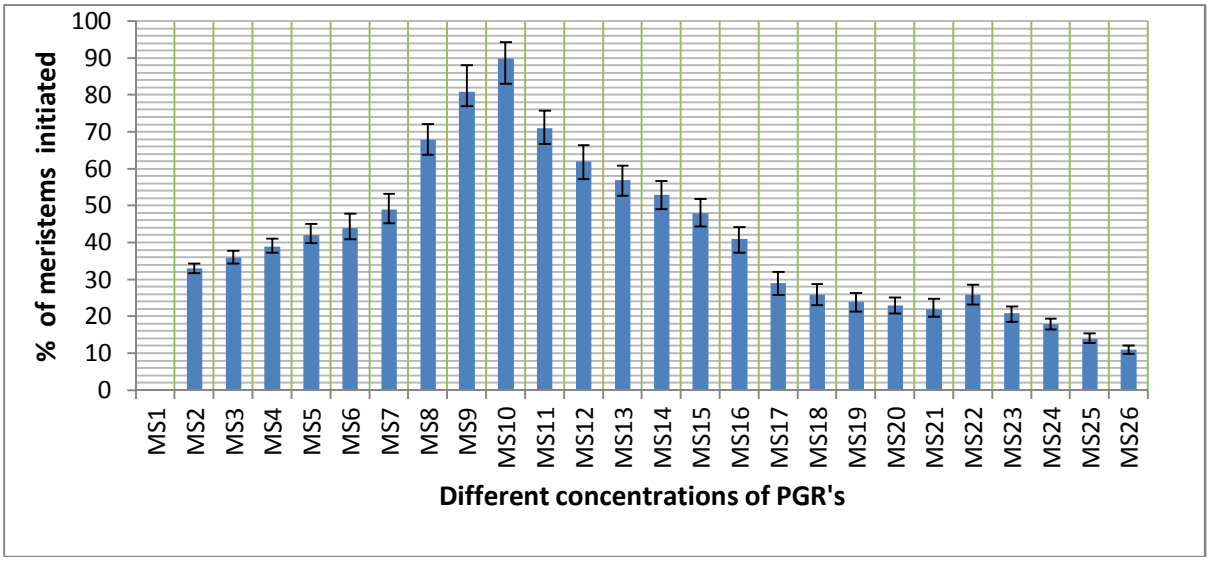
Table 2. Effects of type and concentration of plant growth regulators on *in vitro* shoot initiation from meristems of *Gloriosa superba* L. after six weeks of culture

Plant Growth Regulators Composition (mg/l)			**Media**	**% of meristems Initiated ± S.E.[a]**	**Mean number of shoots/ explants ± S.E.[a]**	**Mean shoot length (cm) ± S.E.[a]**	**Days required for shoot induction ± S.E.[a]**
BAP	NAA	Kinetin					
0.0[b]	0.0[b]	0.0[b]	MS1	0.0	0.0	0.0	00.00
0.5	0.0	0.0	MS2	33 ± 1.3	1.3 ± 0.3	1.4 ± 0.1	26.6 ± 0.66
1.5	0.0	0.0	MS3	36 ± 1.7	1.4 ± 0.4	1.9 ± 0.1	18.2 ± 0.48
2.0	0.0	0.0	MS4	39 ± 1.8	2.6 ± 0.4	2.0 ± 0.1	17.4 ± 0.38
2.5	0.0	0.0	MS5	42 ± 2.1	1.5 ± 0.7	2.7 ± 0.1	16.5 ± 0.34
0.5	0.0	0.0	MS6	44 ± 3.1	1.3 ± 0.6	3.1 ± 0.1	13.1 ± 0.22
1.0	0.1	0.0	MS7	49 ± 3.8	2.7 ± 0.8	3.2 ± 0.1	11.6 ± 0.33
1.5	0.2	0.0	MS8	68 ± 4.2	3.2 ± 0.1	4.1 ± 0.1	10.4 ± 0.55
1.5	0.4	0.0	MS9	81 ± 41	3.9 ± 0.3	3.9 ± 0.1	7.9 ± 0.50
2.0	**0.5**	**0.0**	**MS10**	**90 ± 7.0**	**4.8 ± 0.3**	**6.5 ± 0.2**	**7.5 ± 0.45**
2.0	1.0	0.0	MS11	71 ± 4.3	3.9 ± 0.4	5.9 ± 0.1	7.3 ± 0.24
2.5	0.5	0.0	MS12	62 ± 4.8	3.6 ± 0.4	4.6 ± 0.1	10.1 ± 0.42
2.5	1.0	0.0	MS13	57 ± 4.3	3.4 ± 0.5	44 ± 0.1	12.2 ± 0.55
3.0	0.5	0.0	MS14	53 ± 3.9	3.4 ± 0.3	2.9 ± 0.1	17.8 ± 0.47
3.0	1.0	0.0	MS15	48 ± 3.6	3.5 ± 0.4	2.6 ± 0.1	16.1±0.63
3.5	0.5	0.0	MS16	41 ± 3.8	3.3 ± 0.2	1.7 ± 0.1	20.5 ± 0.64
3.5	1.0	0.0	MS17	29 ± 3.2	2.9 ± 0.3	1.8 ± 0.1	24.6 ± 0.73
4.0	0.5	0.0	MS18	26 ± 3.0	2.5 ± 0.2	1.6 ± 0.1	29.1 ± 0.78
4.0	1.0	0.0	MS19	24 ± 2.8	2.3 ± 0.3	1.3 ± 0.1	33.1 ± 86
4.5	0.5	0.0	MS20	23 ± 2.3	2.1 ± 0.1	1.2 ± 0.1	32.7 ± 88
4.5	1.0	0.0	MS21	22 ± 2.1	2.0 ± 0.1	1.1 ± 0.1	36.2 ± 0.87
5.0		0.2	MS22	26 ± 2.8	2.7 ± 0.3	2.6 ± 0.1	29.9 ± 0.54
5.0		0.5	MS23	21 ± 2.5	2.1 ± 0.2	2.1 ± 0.1	35.4 ± 0.66
		1.0	MS24	18 ± 1.6	1.7 ± 0.1	1.4 ± 0.1	40.5 ± 0.79
		1.5	MS25	14 ± 1.3	1.4 ± 0.1	1.2 ± 0.1	47.3 ± 0.78
		2.0	MS26	11 ± 1.1	1.1 ± 0.1	1.1 ± 0.1	49.3 ± 0.80

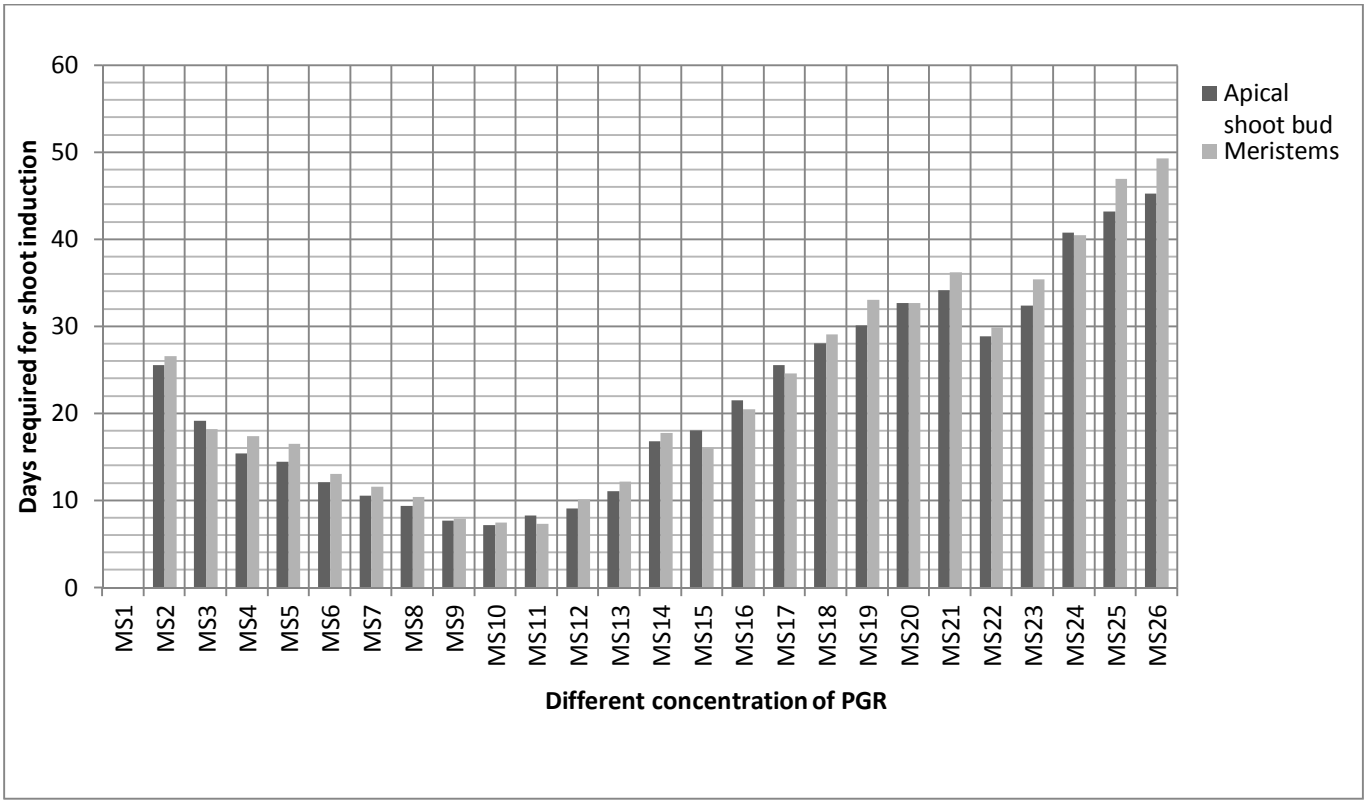
[a]) = Values are mean ± S. E. for three replications (12 cultures for each replication); data scored after six weeks
[b]) = Medium lacking growth regulators served as control



Graph 1. Effects of type and concentration of plant growth regulators on *in vitro* shoot initiation from apical shoot buds of *Gloriosa superba* L. after six weeks of culture



Graph 2. Effects of type and concentration of plant growth regulators on *in vitro* shoot initiation from meristems of *Gloriosa superba* L. after six weeks of culture



Graph 3. Showing days required for shoot initiation on different media composition

Data shown in Table 1 & 2 indicate that different concentration of BAP, NAA and Kinetin alone or in combination with basal media having variable effects on shoot induction on apical shoot buds & meristems explants of *Gloriosa superba*. BAP in combination with NAA was found to be more effective than kinetin alone or in combination with BAP from apical shoot buds and meristems explants. For shoot initiation MS medium was supplemented with different concentrations of plant growth regulators ranging from BAP (0.5-5.0 mg/l) + NAA (0.1-1.0). Best sprouting & shoot formation response was obtained in MS medium containing 2.0 mg/l BAP + 0.5 mg/l NAA. At this concentration produced an average of 4.4 ± 0.3 shoots per explants with 88 % ± 6.2 % of shoots initiated with 6.7 ± 0.2 cm shoot length from apical shoot buds after 7.2 + 0.43 days of inoculation. Where as in meristems explant at this concentration an average of 4.8 ± 0.3 shoots per explants with 90 % ± 7.0 of shoots initiated with 6.5 ± 0.2 cm shoot length after 7.5 ± 0.45 days of inoculation (Table 1 & 2; Graph 1, 2 & 3). By increase or decrease in the concentration of BAP+NAA not only sprouting frequency of explants, decreased but also time taken for shoot induction was also increased.

When, combination of 5.0 mg/l BAP with different concentrations of kinetin was used. It was observed that by the addition of Kinetin the rate of shoot formation was decreased. When 0.2 mg/l Kinetin was used with 5.0 mg/l BAP 25 ± 3.1 shoots sprouted after 28.9 ± 0.53 days of inoculation from apical shoot buds and 26 ± 2.8% shoots after 29.9 ± 0.54 days of inoculation from meristem explants. All the concentration of Kinetin alone or in combination with same concentration of BAP failed to give satisfactory results for shoot initiation & formation in both kinds of explants.

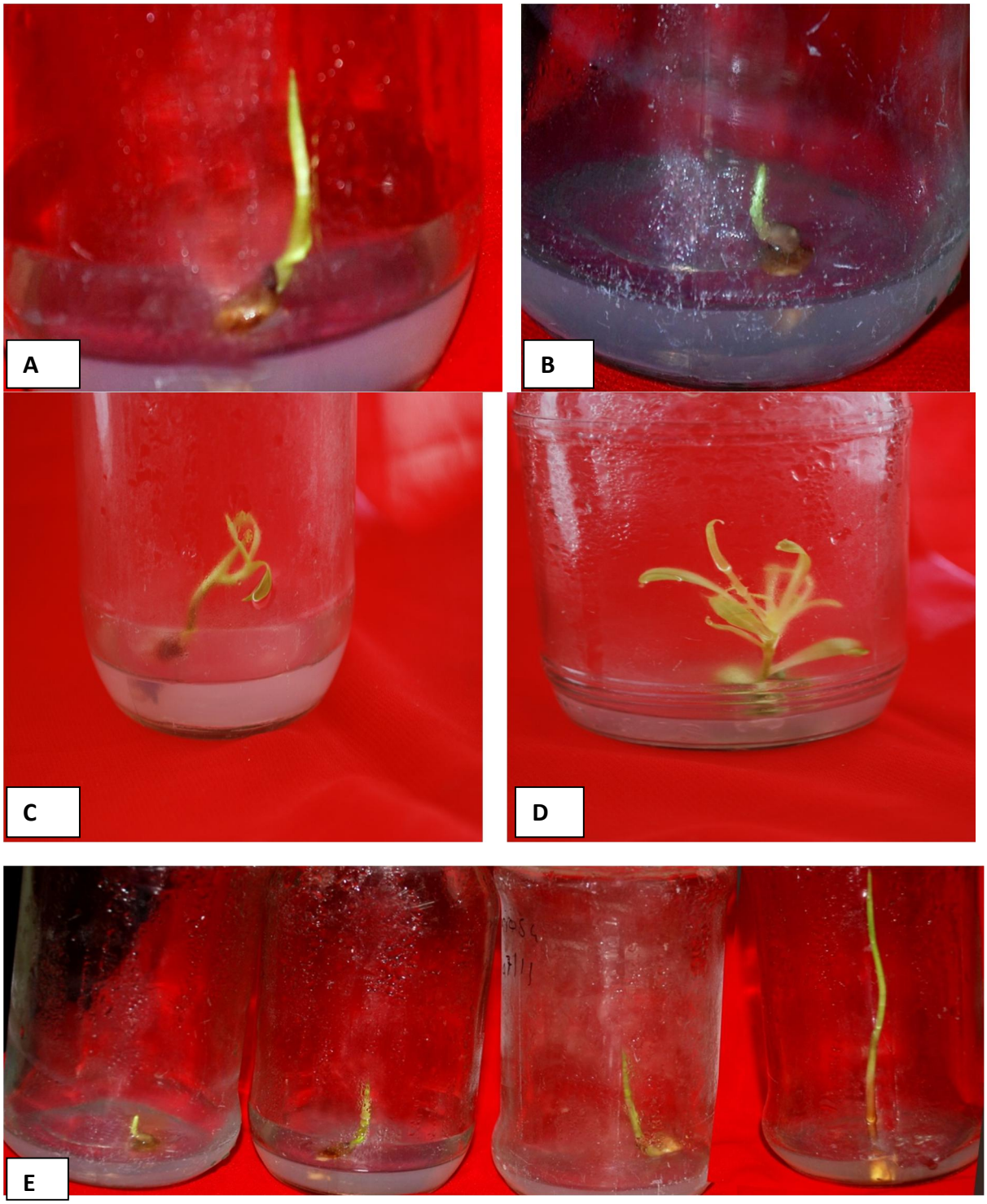


Figure. 1. *In vitro* shoot induction of G. superba. (A) *In vitro* initiation of apical bud on MS medium containing 2.0 mg/l BAP + 0.5 NAA after 7 days of inoculation; (B) *In vitro* initiation of meristems on MS medium containing 2.0 mg/l BAP + 0.5 NAA after 7 days of inoculation; (C) *In vitro* grown shoots of *G. superba* from meristems on MS medium containing 2.0 mg/l BAP + 0.5 NAA after 25 days of inoculation; (D) *In vitro* grown shoots of *G. superba* from meristems on MS medium containing 2.0 mg/l BAP + 0.5 NAA after 35 days of Inoculation; (E) Different stages of apical bud initiation for shoot induction of *G. superba.*

In vitro techniques offer new possibilities in commercial clonal propagation of plants as well as in high valued secondary products (Short, 1991). Development of efficient and reproducible regeneration protocol from cells/tissues holds tremendous potential for the production of high quality plant-based medicines (Murch et al., 2000). The present study was also undertaken to propagate important cultivar of *Gloriosa superba*. For shoot induction both apical shoot buds and meristems were used. Propagation from existing meristems & apical shoot buds yields plants that are genetically identical with donor plants (Tripathi & Tripathi 2003). The choice of explants is of cardinal importance and makes an absolute difference between success & failure in inducing regeneration *in vitro* (Mustasim Mohamed khalafalla et al., 2007). *In vitro* micropropagation from meristems & apical shoot buds has yielded encouraging results in medicinal plants like *Rauwolfia micrantha* (C.G Sudhha & S. Seeni 1996), *Isoplexis canariensis* (Perez et al., 2002), *Dianthus caryophyllus* (Aamir Ali et al., 2008), *Portulaca grandiflora*. Hook (Ashok K Jain & Mudasir Bashir, 2010).

In vitro micropropagation of *Gloriosa* was described by many scientists by using different explants (Samarjeeva et al., 1993 from apical bud & node segments of shoot tip; Custers & Bergervoet, 1994 from shoot cuttings & nodes, internodes; Sivakumar and Krishnamurthy (2002, 2004) studied induction of embryoids from leaf tissue *of G. superba*. (Hassan & Roy, 2005) from apical & axillary buds. In the present study shoot initiation/ shoot formation medium was standardized. The results of present study reveals that best shoot induction was obtained in MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA for both apical shoot bud & meristems explants (Table 1 & 2; Fig. 1 (A-E) ; Graph; 1 2). By increase or decrease in concentration of BAP + NAA shoot initiation/ formation response was decreased. These results are similar in accordance to, the earlier findings by (S. Prakash et al., 2004) in *Curcuma amada*, (Hiregoudar et al., 2005) in *Feronia limonia* & in *in vitro* cultures of *Mentha pipertia* by (P. Sujana & C.V. Naidu 2011).

Samarjeeva et al., (1993) achieved shoot initiation of *G.superba* on Gamborg's B5 medium containing 0.5 to 1 mg/l BA and 0.01-0.5 mg/l IAA, IBA, NAA. However, Bergervoet (1994) reported addition of low level of BA (1 mg/l) improved shoot growth & high level of BA (10 mg/l) caused proliferation of multiple shoots, while Hassan & Roy (2005) used MS medium supplemented with 1.5 mg/l BAP + 0.2 mg NAA for shoot induction from shoot tips & nodal segments, where 92 & 93 percentage of explants forming shoots respectively. But in present study it was observed that BAP in combination with Kinetin or Kinetin alone failed to stimulate satisfactory shoot induction response. On the other hand there was a decline in shoot induction & increase in shoot induction timings. The similar phenomenon was also observed in *Gloriosa superba* by (Hassan & Roy, 2005) and in *Dianthus caryophyllus* by (Aamir et al., 2008).

In the present study it was also observed that when MS medium supplemented with 2.0 mg/l BAP + 0.5 mg/l NAA, meristems explants initiated is higher than the apical shoot bud explants. This potential effect of explants is also discussed by Bressan et al., 1982 and Aamir et al., 2008.

In present investigation a synergistic influence was evident when different concentrations of BAP, NAA and Kinetin were tried either individually or in combination (Table 1 & 2). When MS medium supplemented with BAP in combination with NAA is more effective for efficient shoot induction in *Gloriosa superba,* which is in similarity with earlier reports of *Gloriosa superba* (Hassan & Roy, 2005). Continued exposure of explants to concentrations higher than 2.0 mg/l BAP during shoot induction caused high accumulation of cytokinin which inhibited further shoots from developing. (Bhau and Wakhlu, 2003) observed that high concentration of BAP resulted in decrease in shoot multiplication rate of mulberry. High concentrations of cytokinin also have been reported for the reduction of shoot-bud induction frequency in *Bacopa monniera* nodal explants (Tiwari et al., 2001). MS medium supplemented with BAP in combination with Kinetin. Additions of lower concentration (0.5 mg/l) of Auxin (NAA) to the MS medium containing 2.0 mg/l BAP enhanced shoot elongations as well as sprouting frequency of explants. A low concentration of auxins can promote growth of axillary shoot by counteracting the inhibitory effects of high cytokinin concentrations on shoot elongation (Nehra & Kartha, 1994). The times taken for explants initiation were also better on BAP + NAA as compared to BAP + Kn. The superiority of BAP over Kinetin has been well demonstrated in tissue culture techniques for the adventitious shoot induction (Speer 1993; List at al., 1996). Further, *In vitro* induced shoot of *Gloriosa superba* will be tried for best shoot multiplication and *in vitro* rooting.

4. Conclusions

In conclusion, this study describes a procedure for *in vitro* micropropagation through shoot induction and successful growth of induced

shoots of *Gloriosa superba* in *in vitro* conditions. The results of this study shown that tissue culture techniques can play an important role in clonal propagation of elite genotypes of *G. superba* which has diverse medicinal applications and eventually due to over exploitation this plant is facing local extinction. It has been affirmed as endangered plant by IUCN and hence there is pressing need to conserve this medicinal herb of high commercial value. *Gloriosa superba* 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 approach would be very useful (Sivakumar & Krishnamurthy 2002). It seems likely that this protocol for shoot induction, possibly with modification, can be used for *in vitro* shoot induction of other species of the genus *Gloriosa* using apical shoot bud & meristem segments.

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References

1. The Wealth of India, a dictionary of Indian raw material & Industrial products, 2002, vol-IV (F-G), 139
2. Sivakumar, G. and Krishnamurthy, K. V. (2002). *Gloriosa superba* L.- a very useful medicinal plant. In: Recent Progress In Medicinal plants, Vol. 7 – *Ethnomedicine and Pharmacognosy* Part II. Singh, V. K. Govil, J. N., Hashmi, S., Singh, G. (eds). Series Sci. Tech. Pub., Texas, USA, pp. 465-82
3. Trease, S. E. and Evans, D. (1983). Colchicum seed and corm. In: *Pharmacognosy*, 12th Balliere Tindall, London, pp. 593-597.
4. Gupta, L. M., Rana, R. C., Raina R., and Meenakshi Gupta (2005). Colchicine contents in *Gloriosa superba* L. *SKUAST-J*, 4: 238-241.
5. Kokate, C. K., Purohit, A. P., Gokhale, S.B. (2004). *Pharmacognosy*, Nirali Prakashan, Pune, pp. 506.
6. Badola, H. K. (2002). Endangered medicinal plant species in Himachal Pradesh. A report on the International Workshop on 'Endangered Medicinal plant species in Himachal Pradesh', organized by G. B. Pant Institute of Himalayan Environment and Development at Himachal Unit, Mohal-Kullu during 18-19 March 2002 *Curr. Sci*., 83: 797-798.
7. Jiten Chandra Dang, Suman Kumaria, Shrawan Kumar & Pramod Tandon (2011). Micropropagation of Ilex Khasiana, a critically endangered and endemic holly of Northeast India. *AoB PLANTS* plro012.
8. Kala, C., Farooquee N., Dhar U. (2004). Prioritization of medicinal plants on the basis of available knowledge, existing practices and use value status in Uttaranchal, India. *Biodiversity and Conservation*, 13(2): 453-469.
9. Dhushara (2004) http://www.dhushara.com/book/med/med.htm
10. Chandra B., Palni, LMS., Nandi, S. K. (2006). Propagation & conservation of *Picrorhiza kurrooa* Royle ex Benth.: an endangered Himalayan medicinal herb of high commercial value. *Biodiversity and conservation*, 15: 2325-2338
11. Maiti, C. K., Sen, S., Paul, A. K., and Acharya, K. (2007). First report of leaf blight disease of *Gloriosa superba* L. caused by *Alternaria alternata* (Fr.) Keissler *in India. J. Gen. Plant Pathol*., 73: 377-378.
12. Rajgopalan, A., and J.B.M. Md. Abdul Khader (1994). A comparision of different method of estimation of colchicine in *Gloriosa superba*. *Crop Research*, 8: 549-551
13. Prakash, S., Elangomathavan, R., Seshadri, S., Kathiravan and Ignacimuthu, S. (2004). Efficient regeneration of *Curcuma amada* Roxb. plantlets from rhizome and leaf sheath explants. *Plant Cell Tissue and Organ Culture*, 78: 159-165.
14. Hiregoudar,L .V., Kumar, H. G. A., and Murthy, H. N. (2005). *In vitro* culture of *Feronia limonia* (L). Swingle from hypocotyls and intermodal explants. *Biol. Plant*, 49: 41 – 45.
15. P. Sujana and C.V. Naidu (2005). High Frequency Rapid Plant Regeneration from Shoot Tip and Nodal Explants of *Mentha*

piperita (L.) – An Important Multipurpose Medicinal Plant. *Journal of Plant Physiology*, 3: 5-9-13.

16. Short, K. C. and A. V. Robert (1991). In vitro culture, micropropagation and the production of secondary products. *Biotechnology in Agriculture & Forestry*, Vol. 15. Medicinal & aromatic plants III (Ed.): Y. P.S. Bajaj. Springer-Verlag Berlin Heidelberg.
17. Haroon K., Murad. A.K., Iqbal, H. (2008). Enzyme inhibition activities of the extracts from rhizomes of *Gloriosa superba* Linn (Colchicaceae). *Journal of enzyme inhibition and medicinal chemistry*, 22 (6) 722-5.
18. Rajak, R.C. and M.K. Rai (1990) Herbal Medicines Biodiversity and Conservation Strategies. International Book Distributors, pp. 75-79.
19. Sarasan, V., Cripps, R., Ramsay, M.M., Atherton, C., McMichen, M., Prendergast, G., Rowntree, J. K. (2006).Conservation in vitro of threatened plants-progress in the past decade. *In vitro Cellular & Developmental Biology-Plant*, 42: 206-214.
20. Murch, S. J., Krishna, R. S., Saxena, P. K. (2000). Tryptophan as a precursor for melatonin and serotonin biosynthesis in in-vitro regenerated St. John's wort (*Hypericum perforatim*. Cv. Anthos lants. *Plant Cell Reports*, 19: 698-704.
21. Tripathi, L. & Tripathi, J. N. (2003). Role of biotechnology in medicinal plants. Tropical *Journal of Pharmaceutical Research*, 2 (2): 243-253.
22. Mutasim Mohamed Khalafalla, Eisa Ibraheem Elgaali & Magda Mohamed Ahmed (2007). In vitro multiple shoot regeneration from Nodal explants of *Vernonia amygdalina*- An important medicinal plant. *African Crop Science Proceedings*, 8: 747-752.
23. C. G. Sudha, S. Seeni (1996). In vitro propagation of *Rauwolfia micrantha*, a rare medicinal plant. *Plant Cell, Tissue & Organ Culture*, 44: 243- 248.
24. Perez-Bermudez, P. Seitz, H. U. & Gavidia, I. (2002). A protocol for rapid micropropagation of endangered Isoplexis. *In vitro Cell Dev Bio- Plant*, 38: 178-82
25. Aamir Ali, Humera Afrasiab, Shagufta Naz, Mamoona Rauf and Javed Iqbal (2008). An efficient protocol for *In vitro* propagation of Carnation (*Dianthus caryophyllus*). *Pak. J. Bot*., 40 (1): 111-121.
26. Ashok K Jain and Mudasir Bashir (2010). Efficient micropropagation protocol for Portulaca grandiflora. Hook. Using Shoot Tip Explants. *New York Science Journal*, 3 (10): 142-146.
27. Samarajeewa, P.K., M.D. Dassanayake, and S.D.G. Jayawardena (1993). Clonal propagation of *Gloriosa superba. Indian Journal of Experimental Biology*, 31: 719-720.
28. Custers, J.B.M. and J.H.W. Bergervoet (1994). Micropropagation of *Gloriosa*: towards a practical protocol. *Scientia Horticulturae.* 57: 323-334.
29. Sivakumar, G. and K.V. Krishnamurthy (2004). In vitro organogenetic responses *of Gloriosa superba*. *Russian Journal of Plant Physiology* 51: 790-798.
30. Hassan, S.A.K.M. and S.K. Roy (2005). Micropropagation of *Gloriosa superba L.* through high frequency shoot proliferation. *Plant Tissue Culture* 15 (1): 67-74.
31. Bhau, B. S. Wakhlu, A. K. (2003). Rapid micropropagation of five cultivars of mulberry. *Biol Planta*., 46 (3): 349-355.
32. Nehra, N. S. & Kartha, K. K. (1994). Meristem and shoot tip culture: requirements and applications. In: nasil IK, Thorpe T (eds)., *Plant Cell Tissue Culture*. Kluwer Academy, pp 37-70.

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