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IN VITRO MICROPROPAGATION ON SHOOT TIP EXPLANTS OF *GLORIOSA SUPERBA* L. – AN ENDANGERED MEDICINAL PLANT

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The *in vitro* cultivation of mature plant shoot tip explants was used to construct an *in vitro* micropropagation strategy for the annual medicinally *Gloriosa superba* L. Shoot tips from 30-35-day-old field-grown plants were employed for *in vitro* micropropagation shoot tip explants culture. Surface sterilizing of shoot tips was found to be most effective after three minutes in 0.1% $HgCl_2$ solution. For the main establishment of isolated shoot tips on MS medium supplemented with (BAP/Kn/TDZ) (0.5-3.0mg/L), IAA (0.5 mg/L) + (BAP /Kn/ TDZ) (0.5-3.0 mg/L), and (BAP/Kn/TDZ) (0.5-3.0 mg/L) for multiple shoot induction. Within four weeks of culture, multiple shoot bud proliferation was seen at IAA (0.5 mg/L) + (2.0 mg/L) TDZ from shoot explants. The number of shoots per explant varied between 2 and 6. Several shoots were under aseptic conditions removed and subcultures in the same medium to extend the shoot. For root induction, the possibly resulting were transplanted to IAA/IBA (0.0mg/L-2.0mg/L). Within two weeks of culture, rooting was seen. Rooted plantlets were successfully toughened under culture conditions before being planted in the field. The recorded plant survival percentage was 86%. The plants appeared to be in good condition, with no visible phenotypic differences. With an 87% survival rate, the plantlets (12-16 weeks old) were successfully acclimatized to soil.

Key Words: *In Vitro* Micropropagation, Gloriosa superba L**,** shoot tip, multiple shoot buds, sub captured, and acclimatization

Introduction:

Gloriosa superba L. is a semi-woody herbaceous climbing plant that may grow up to 5 meters in height. One fleshy cylinder tuber with a V-shaped base can give rise to anywhere from one to four new plants. Originally from Africa's tropics, *G. superba* is now widely distributed in India, Sri Lanka, Malaysia, and Myanmar. Also, the southern United States is a major outdoor growing region for this plant. In colder temperate areas, this plant is best grown in a greenhouse or conservatory. The Mahadevapur reserved Forest of Karimnagar region of Telangana State is a natural habitat for this plant. Particularly in the traditional medicine of tropical Africa and Asia, various parts of the plant have several uses. The tuber has

a long medical history, having been treated for everything from sprains and bruises to colic and chronic ulcers to hemorrhoids, leukemia, leprosy, and even inducing labor. In certain cases, the plant is used instead of aconite as an adulterant because of its similar pharmacological effects (Aconitum sp.). Both the seeds and the root tubers are rich in colchicine, a useful alkaloid. On occasion, colchicine is used in cytogenetic and plant breeding research. The tuber, when taken in small doses, is effective as an abortifacient, tonic, stomachic, and anthelmintic. Due to the presence of colchicine, it is also effective against gout. Treatment of cutaneous parasites using tuber pastes (Ghani 1998). Because of its herbaceous nature, *G. superba* is rarely given any special care in the realms of propagation and nurturing. The seeds or tubers are the common starting point for cultivation. The widespread harvesting of the plant's seeds and tubers in India for medical uses has contributed to the species' threatened status.

To keep such vulnerable species from extinction, medicinal plant exploitation must be complemented by conservation efforts (Hamann 1991). The current work was carried out with the goals of

(A) Developing a system for mass multiplication of this valuable medicinal plant via *in vitro* culture and

(B) Assuring the generation of genetically similar plantlets for future field culture and conservation. This research aimed to develop a protocol for the successful micropropagation of *Gloriosa superba* L. seedling shoot tips explants.

Methodology: -

Young *G. superba* plants were obtained from the outside and nurtured in the aromatic and medicinal plants' experimental garden under partial shade conditions. Department of Botany Women's Degree College, Karimnagar Healthy plant shoot tips, including the apical dome and 2 or 3 leaf buds, were cut from one-year-old plants. The shoot tip explants were rinsed in a moderate nonphytotoxic liquid detergent (2% Labolene) for about 3 minutes before being washed in tap water. It was then immersed for 2 minutes in a 0.1% (w/v) Mercuric chloride (HgCl2) solution. Finally, before inoculating the axillary bud explants onto a sterilized nutritional agar medium produced in culture tubes, the explants were carefully rinsed with sterile water. All of the above procedures were carried out under aseptic circumstances in a laminar airflow cabinet.

Culture media and culture conditions: -

The explants were cultured in MS media with 3.0% sucrose and treated with cytokinins BAP, Kn, and TDZ (0.5-3.0mg/L) alone or in conjunction with IAA (0.5mg/L). To start, the pH of the culture media was adjusted to 5.8 and then agar-agar was added at a concentration of 0.8% (w/v). After being autoclaved at 121^0 C for 15 minutes, the medium was distributed into culture tubes (25 x 150 mm) containing 15 ml of the culture medium and equipped with non-absorbent cotton. One explant from a growing tip was placed in each test tube. The cultures were maintained at 25^0 C with white fluorescent tubes providing 16 hours of light (40 mol m-2s-1).

Four-week-old seedlings used to have their shoot apices trimmed to a length of 1.0 to 1.5 centimetres before being inoculated on a shoot bud induction medium that consisted of an MS basal medium supplemented with various concentrations of cytokines. These cytokines included 6-Benzyl Amino Purine (BAP), Kinetin (KIN), or Thioduzuron (TDZ) alone or in combinations of (1.0-3.0 mg/L (IAA). After a period of six weeks, a count was taken of both the total number of shoot buds and the proportion of explants that had become shoot buds. For the purpose of rooting shoot buds, the elongated shoots that were obtained from shoot-tip explants were excised and cultured in 250 ml flasks that contained 70 ml of rooting media. The rooting media consisted of an MS medium that had been supplemented with varying concentrations of auxins, ranging from (0.5 to 3.0 mg/L) of IAA or indole-3-butyric acid (IBA). (Table -1) (Fig-1). The number of rhizomes present (including the main roots and their branches). On rooted plantlets that were four weeks old, axillary branches were stimulated to grow. By cutting the ends off of these plantlets using a sterile blade, we were able to decapitate them so that axillary branch growth could occur. These plantlets had between 5 and 9 leaves. Axillary shoots that developed in the axils of the leaves of the severed plantlets were used for further shoot proliferation bud induction. This was accomplished by cultivating the axillary shoots on a medium supplemented (0.5-3.0 mg/L) BAP/Kn/TDZ alone or with (0.5mg/L) IAA. After six weeks, the number of shoot buds was counted.

The shoot buds were removed and cultivated on a rooting medium that consisted of MS media that had been treated with various concentrations of IAA or IBA (ranging from 0.5 to 2.0 mg/L). The shoot buds had multiplied from axillary shoot-tip explants. After carefully removing the rooted plantlets from the flasks, the roots were rinsed under running water to eliminate any remaining traces of agar. After that, the plantlets were transplanted into perforated paper cups that contained a mixture of soil and sand with a ratio of 1:1. The cups were then wrapped with clear polyethylene bags that had a few holes in them for the first ten days. The plantings were housed in a net house with a 50% shadow factor and were given daily irrigation with tap water in order to keep the relative humidity at a high level. After ten days, the moisture was gradually reduced by reducing the size of the openings in the polythene bags, and the bags were ultimately removed. This process was repeated until the humidity reached the desired level. After hardening off for four weeks, the plants were either moved into larger earthen pots or directly onto the field.

All culture was maintained in a growth chamber with a temperature of 25° ±2 degrees C and a photoperiod of 16 hours given by white fluorescent lamps emitting 30 micromoles per square meter per second. All of the studies were carried out three times, and each treatment for inducing shoot buds from shoot-tip explants and rooted shoot buds consists of ten separate trials each.

Results: -

Data on multiple shoot induction from shoot tip explants cultivated on MS medium supplemented with different concentrations of BAP/Kn/TDZ alone are presented in (Table 1), and BAP/Kn/TDZ in combination with (0.5mg/L) IAA are presented in (Table 2). Data on an ongoing strategy that helps from recreated shoot tip explants cultivated on MS medium supplemented with different concentrations of IAA and IBA are presented in (Table 3) and shown in (Fig-1).

Effect of BAP: -

There were no indications of callus development while cultivating shoot-tip explants, which led to the observed multiple-shoot proliferation. Multiple new shoot buds emerged following 6 weeks of explant culture from a single shoot tip (Fig-1). The highest number of shoots produced by any of the random drug testing on MS media BAP at (2.0 mg/L) was (6.0±0.32). However, shoot induction was observed to be significantly decreased at high BAP concentrations (3.0 mg/L). The number of numerous shoot buds rise up to a BAP concentration of 0.5 mg/L but decreased from a BAP concentration of 2.0 mg/L to 3.0 mg/L. (Table-1)

Effect of KIN: -

There was no confirmation of callus development while cultivating shoot-tip explants, which led to the observed multiple-shoot proliferation. Multiple new shoots emerged following 6 weeks of explant culture from a single shoot tip (Fig-1). The highest number of shoots produced by any of the random drug testing on MS media KIN at (2.0 mg/L) was (7.0 ± 0.35). However, shoot induction was observed to be significantly decreased at high BAP concentrations (3.0 mg/L). The number of numerous shoots rose up to a BAP concentration of 0.5 mg/L but decreased from a BAP concentration of 2.0 mg/L to 3.0 mg/L. (Table-1)

Table-I Direct multiple shoot buds proliferation from Shoot tip explants of *G. superba* L. on MS medium supplemented with various concentrations of BAP, KIN, and TDZ

Growth hormone concentration (mg / L)	% Of cultures responding	Average number of shot buds/explant (S.E) E.)*
BAP		
0.5	60	4.0 ± 0.32
1.0	70	5.0 ± 0.35
1.5	80	5.6 ± 0.32
2.0	90	6.0 ± 0.32
2.5	85	5.8 ± 0.36
3.0	60	5.5 ± 0.23
KIN		
0.5	62	4.3 ± 0.25
1.0	73	5.4 ± 0.32
1.5	85	5.8 ± 0.32
2.0	92	7.0 ± 0.35
2.5	88	6.6 ± 0.32
3.0	65	6.2 ± 0.23
TDZ		
0.5	65	5.0 ± 0.32
1.0	75	5.6 ± 0.32
1.5	86	6.0 ± 0.32
2.0	95	7.8 ± 0.42
2.5	80	7.0 ± 0.32
3.0	68	6.8 ± 0.32

***SE Standard Error**

Effect of TDZ:-

The impacts of TDZ (0.5-3.0 mg/L) on the shoot tip/axillary bud of *G. superba* were seen in the MS medium with TDZ supplementation. At (2.0 mg/L) TDZ, a significantly higher number of responsive cultures than at any other concentration tested were seen (95%). However, 2.0 mg/L TDZ resulted in the highest rate of shoot regeneration from shoot tip explants (7.8±0.42 shoots/explant), followed by 2.5 mg/L TDZ. (5.0±0.32), (5.6 ±0.32), and (6.0±0.32) new growths per plant were seen at 0.5, 1.0, and 1.5 mg/L TDZ, respectively. There were recorded responses from 65, 75, and 86 percent of cultures, respectively. (Table- 1) (Fig-1).

Effect of IAA + BAP:-

The results of cultivated shoot tip explants on MS medium supplemented with 0.5 mg/L IAA and varying concentrations of BAP ranging from 0.5 to 3.0 mg/L are provided in (Table-2) and displayed in (Fig-1). After a period of six weeks following the injection, direct multiple shoot proliferation was detected in the shoot tip culture. As the level of BAP grew, there was a proportional decrease in the number of shoots buds. On MS medium that had been supplemented with (0.5 mg/L) IAA and (0.5-3.0 mg/L) BAP, the various treatments were examined and assessed. At IAA (0.5 mg/L) and BAP (2.0 mg/L), the highest number of shoots was seen to be 6.5±0.23, and 85% of the cultures reacted at (0.5, 1.0, and 1.5 mg/L) BAP within the combination of (0.5 mg/L) IAA. IAA revealed an induction of (5.0± 0.35), (5.5± 0.34) and (6.2±0.33) shoots/explants. When the concentration of BAP was expanded gradually to 2.0 mg/L, the number of numerous shoots dropped. However, when the concentration of BAP was increased from 2.0 mg/L to 3.0 mg/L, the number of shoots and the percentage of responsive plants declined. (Table- 2).

Table –2

Direct multiple shoot buds Proliferation from Shoot tip explants of *G. superba* L. on MS medium supplemented with various concentrations of BAP, KIN, and TDZ.

Growth hormone concentration (mg / L)	% Of cultures responding	Average number of shot buds/explant (S.E) E.) *
IAA+BAP		
0.5+0.5	63	5.0 ± 0.35
0.5+1.0	65	5.5 ± 0.34
0.5+1.5	70	6.2 ± 0.33
0.5+2.0	85	6.5 ± 0.23
0.5+2.5	70	6.0 ± 0.24
0.5+3.0	50	5.3 ± 0.45
IAA+KIN		
0.5+0.5	65	5.3 ± 0.43
0.5+1.0	75	5.8 ± 0.32
0.5+1.5	86	6.8 ± 0.23
0.5+2.0	90	7.3 ± 0.23
0.5+2.5	75	6.8 ± 0.32
0.5+3.0	56	6.0 ± 0.45
IAA+TDZ		
0.5+0.5	65	5.8 ± 0.23
0.5+1.0	78	6.0 ± 0.35
0.5+1.5	88	7.0 ± 0.28

0.5+2.0	93	8.0 ± 0.32
0.5+2.5	78	7.2 ± 0.36
0.5+3.0	60	5.6 ± 0.32

***SE Standard Error**

Effect of IAA + KIN:-

As the concentration of BAP was increased, considering the number of shoots induction was found to be reduced, the morphological reaction of stem cuttings culture on different concentrations of cytokinin such as KIN in combined effect with (0.5 mg/L) IAA is presented in (Table-2) on MS + (0.5 mg/L) IAA + (2.0 mg/L) KIN actually results in a maximum number of shoots (7.3± 0.23) shoots/ explants (Fig-1). The smallest number of shoots (5.3± 0.43 shoots/ explant) was generated when KIN was added at a concentration of (0.5 mg/L) and IAA was at the same concentration.

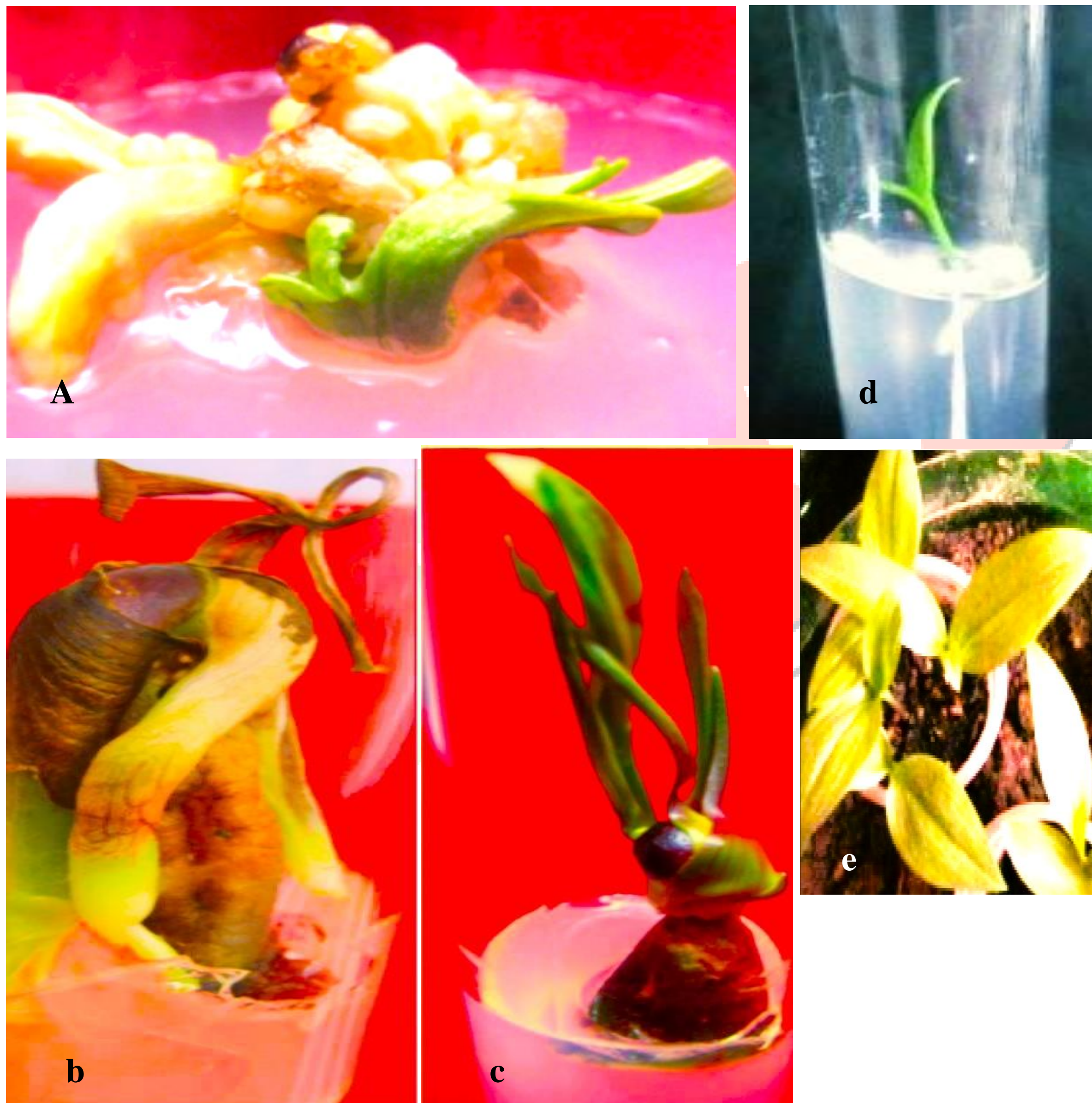

Figure-1: *In Vitro* Micropropagation of Plantlet regeneration from shoot tip explants in ***G. superba***. a) In Vitro Plantlets on MS+ (2.0/L) BAP b) Multiple shoots on MS+ (2.0 mg/L) KIN C) *In Vitro* shoots formation on MS+TDZ (2.0mg/L) after six weeks d) *In Vitro* rooting from Micro shoots on MS+ 3.0 mg/L IBA after six-week e) Hardening of plantlets

Effect of IAA+TDZ:-

IAA + TDZ (2.0mg/L) resulted in (8.0±0.32 Shoots/ explant), whereas on MS medium (0.5 mg/L), the greatest number of shoots that could be generated by IAA + TDZ was developed when they were at a concentration of just 0.5 mg/L. At concentrations of (0.5 mg. /L) IAA and (2.0 mg/L) TDZ, the highest percentage of reaction was seen (Plate -I). Up to a concentration of 2.0 mg/L TDZ, the percentage of reaction was raised; however, as it reached higher concentrations, it gradually dropped. At a concentration of 2.0 mg./L of TDZ, a high frequency of shoot buds (7.3± 0.23) was produced per explant, and the capacity to stimulate shoot formation declined as the concentration of TDZ increased. At concentrations of 0.5, 1.0, and 1.5 mg/L of TDZ, the plants reacted with 65, 78, and 88% cultural response respectively (6.0± 0.23, 6.6± 0.35, and 7.0± 0.28 shoots / explants). Following reaching 2.0 mg/L of TDZ, the proportion of responsive cultures was then dropped, after which it had been steadily increased. At a concentration of 3.0 mg/L TDZ and 0.5 mg/L IAA, a low number of shoots were reported per explant.

***In vitro* rooting: -**

Fully elongated and healthy shoots were moved into ½ MS root medium supplemented (RIM) (Murashige and Skoog 1962). This medium was reinforced with varying doses of IAA (0.5–2.0 mg/L) and IBA (0.5–2.0 mg/L). On MS media containing 1.5 mg/L IBA, abundant rhizogenesis was found at 1.5 mg/L IAA, in contrast to (0.5 -2.0 mg/L) IAA/ IBA on MS medium at the same concentration, although 96% of plants developed roots at a rate of 14.3 0.27 roots per explant. (Table -3) (Fig-1).

Acclimatization: -

After the rooted plantlets had been taken from the culture media, the agar from the roots was removed by washing them under running water from the faucet. The plantings were then moved into poly pots that had been filled with vermiculite that had been soaked beforehand, and they were kept alive in a growth chamber that had a temperature of 28 degrees Celsius and relative humidity of 70–80 percent. After a time of three weeks, they were transferred to poly bags that contained a mixture of soil, sand, and manure in a ratio of 1: 1: 1. These bags were then placed in a shade house for a further duration of three weeks. During the course of three weeks, the plantings in their pots were given irrigation of Hogland's solution once every three days. (Fig-1).

Table -3:

Rooting ability of regenerated shoots from Leaf explants/cotyledonary explants /Shoot tip explants culture of *G. superba* L. cultured on MS medium supplemented with IAA and IBA.

Growth Hormones (mg/L)		Percentage of response	Average no of roots (S.E)*
IAA	IBA		
00	00	23	1.0 ± 0.12
0.5	-	60	2.3 ± 0.37
1.0	-	70	3.2 ± 0.38
2.0	-	73	5.6 ± 0.38
-	0.5	54	4.3 ± 0.36
-	1.0	73	8.3 ± 0.87
-	2.0	70	6.3 ± 0.36

*** Mean ± Standard Error**

Discussion: -

One of the most recognized and successful examples of the commercial operation of plant tissue culture technology is the process of widespread (*In Vitro*) multiplication of plant species through the use of *in vitro* culture. Recent years have seen significant development in this technique, which has allowed for significant advancements in clonal enhancement and the preservation of genetic resources (Barz *et al.* 1977; Datta and Datta 1985; Kukreja *et al.* 1989; Jusekutty *et al.* 1993; Maskay 1996; Wawrosch and Kopp 1999). It has been demonstrated that a diverse variety of species are capable of undergoing rapid shoot regeneration, with first explants being harvested from the typical aerial shoots of field-grown herbaceous medicinal plant species (Jaiswal *et al.* 1989; Mathur *et al.* 1993; Maskay 1996; Rai 2002; Hall and Camper 2002). Pierik (1987) showed that the addition of AC can frequently have a stimulating influence on the process of organogenesis and development in several plant species. In addition, the medicinal properties of activated charcoal were shown to be present in *Muscari armeniacum* (Pierik 1987). Explants of *G. superba* shoot tip were used in the present experiments, which resulted in the regeneration of shoot buds directly from these sources. Continuous generation of healthy shoot buds and shoots was accomplished by performing repeated subcultures of explants on fresh shoot proliferation media. This was accomplished at least through five to ten subculture cycles. To elaborate more, When the excised shoots were cultured individually on a root induction medium consisting of half-strength MS salts with 1.0 mg/l IBA and 0.5 mg/l IAA, rooting in regenerated shoots of *G. superba* was achieved at a rate of 90 percent. This was achieved when rooting was induced in regenerated shoots of *G. superba* (Table-3). By the third week of culture, roots have begun to form, and by the fifth week, a tuberous structure has appeared at the root's base (Fig-I). Several publications have documented the use of auxin(s) for rooting, either singularly or in combination with another auxin (s) (Sahoo and Chand 1998; Ajith and Seeni 1998; Rai 2002).

Following a period of between four and six weeks invested in the rooting media, the rooted shoots were moved into pots. If the plants in the rooting culture tubes were maintained at a normal room temperature for seven days prior to organ transplants in pots and reared for three weeks, then none of the plantlets survived when they were directly transplanted. However, the transplanted plants of *G. superba* did

survive when they were reared. The plantings were nurtured in a space that had a temperature range of 30 to 20 degrees Celsius, light levels of 2,000 lux, and relative humidity of 80 percent. The plant was moved from the rooting media to the pot in natural circumstances during this acclimation phase. During this time, the shoots were longer, the leaves became larger, and they became a dark green color. (Fig. I) The plant appeared to be in better health. It's estimated that somewhere between 85 and 90 percent of clonally grown plants will have similar phytochemical profiles (Roja and Heble 1993)

The findings of the current experiment indicate that the shoot tip explants taken from mature *G. superba* plants may be encouraged to grow numerous shoots *in vitro* using a specific induction protocol. On MS media that was enriched with varying doses of BAP, KIN, and TDZ, the production of the greatest number of shoots was stimulated. These findings are also in accordance with those obtained from *Tectona grandis* (Gupta *et. al.,* 1980) Multiple shoot inductions similar to those shown in Abizzia lebbeck (Gharyl and Maheshwari 1982) were also seen in *Ziziphus manritiana* (Sudharshan *et. al.,* 2000) and *Vanilla plantifolia* (Geetha *et. al.,* 2000) shoot tips cultivated on MS + cytokinin alone, just as they were in the current investigations.

After three weeks, the plants were moved to a more open area where they were progressively acclimated to the circumstances of the outside environment. Ninety percent of the plants survived. The method that is described here looks to be easily applicable for large-scale clonal replication and planting for the purpose of relevant quantities in the industry. In addition, it is feasible to obtain a tenfold rise in the number of goods produced per unit cultivation area by standardizing the techniques for the clonal multiplication of selected top plants. The capability of *G. superba* to differentiate shoot buds and to proliferate shoots from shoot tip explants was dependent on hormonal variation. Only when cytokinin was present was their response in terms of good shoot bud formation and proliferation. The control media had no effect on the plants. Results that are comparable have been seen and thoroughly documented in a number of medicinal herbs, including *Withania somnifera* (Pattnaik and Chand, 1996), *Bixa ovellana* L. (Sharon and Marie, 2000), *Emblica officinale* (Verma and Kant, 1996), and others (Deka *et.al.,* 1999). According to the results of our research, a concentration of 2.0 mg/L BAP, KN, or TDZ was much more effective than other concentrations at inducing shoot organogenesis. When well-developed shoot tips were moved to rooting media containing (1.5 mg/L) IBA/IAA, a greater frequency of roots was produced than was previously seen. It was shown that IBA had a comparable impact on *Ocimum americanum*, *O.canum,* and *O.sanctum* (Pattnaik and Chand, 1996), in addition to Heracleum candicans (Wakhlu and Sharma, 1999). However, compared to other auxins, IBA at a concentration of 1.5 mg/L was determined to be the most effective rooting hormone. Similar Auxin support *in vitro* proliferation findings was seen in Sunflower (Patil *et al.,* 1993), Mulberry (Naik and Lata, 1996), and Coriander (Patil *et al.,* 1997). (Stephan and Jayabalan, 1998). Based on the results of our experiments, it is clear that BAP and KN are the most effective agents for producing numerous shoots, whereas IBA is the most effective agent for roots and in vitro blooming. This communication concludes by describing an effective quick propagation mechanism for *G. superba*.

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