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STUDY ON QUANTITATIVE ANALYSIS OF TWO IMPORTANT MEDICINALPLANTS GLORIOSA SUPERBA L.

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ABSTRACT

Application indigenous natural products has been alternative way to replacesynthetic medicineGloriosa superbais a well known ethnomedicinal plantwhich is used in Ayurveda. Photochemical studies ofG. superbashowspresence of colchicin, b-siltosterol, long chain fatty acids, b and g-lumiccolchicines, 2-hydroxy-6-methoxy benzoic, luterlin, N-formyl-deacetylcolchicines and new colchicine glycoside, 3-O-demethylcolchicine-3-O-alpha-D- glucopyranoside. FDA-approved use of Colchicine is to treat gout(it is one of the active ingredients of anti-gout tablets marketed by Merck &Co.). It is also used as an anticancer, antimicrobial, antifungal, anticoagulant,antilipoxygenase agent and antidote in snake bite. However, ingestion of allparts of the plant is extremely poisonous and can be fatal. The commonestclinical presentation of poisoning is severe gastroenteritis with nausea,vomiting, diarrhoea with b leading to dehydration, hypovolaemic shock andacute renal failure.Gloriosa superbausually multiply by corm and seeds butdue to low germination capability it restricts for the regeneration. Therefore,in order to safeguard and preserve this important plant biotechnologicalapproachs would be very useful. Micropropagation ofGloriosa superbameets ever increasing demands for colchicine. The availability from bothwild and cultivated sources make the plant ofGloriosa superbaa potentialsource of Colchicine in India.

Keywords:*Gloriosa superbaLinn. ,antitumor*

INTRODUCTION

According to one of the ancient proverbs in India," there is no plant on earthwhich has no medicinal property." A large number of plants have been usedby man from ancient times as medicine for curing various ailments. Inrecent times there is an upsurge of interest and focus on the importance ofmedicinal plants and traditional health systems in solving the health careproblems of the world .Although the modern medicine has developed somuch improves to useful in treating many horrible human diseases, but not inreasonable cost. Herbal renaissance is happing all over the globe as herbalproducts are symbol of safty as compare to synthetic medicine. Tradinalsystem of medicine is found to have utilities as many accounts. Due topopulation rise adequate supply of drug and high cost of treatment, sideeffects along with drug resistance has been encountered in synthetic drugs,which has leads to elevated emphasis for use of plants to treat humandiseases

The World Health Organization (WHO) has previouslyrecognized to re-establish the tradinal knowledge ofmedicine among our conventional theaters. Tradinalknowledge since 200 B.C. in Ayurveda is very wellrecognized especially in India among tribal people. In India,the population of tribal people is around 53

million alongwith 555 tribal groups or communities, which are reside inforest and surroundings. These people have enormousindigenous knowledge which is possible tool to explore fornovel cost-effective plants for medicine. Several medicinalplants were originally identified developed through theindigenous knowledge thus ethinomedicines have playedkey role in development of drugs in modern system ofmedicine.

OBJECTIVE OF THE STUDY

1. Molecular profiling will be used to investigate the genetic stability of in vitro produced plants.

2. The purpose of this research is to investigate the biochemical and phytochemical profiles of in vitro grown plants as well as mother plants.

RESEARCH METHODOLOGY

Celastruspaniculatus Wild, also known as the "Tree of Life" in Ayurvedic medicine, is a member of the Celastraceae plant family and has been used for as long as anyone can remember to cure brain-related diseases and to improve learning and memory. It is common knowledge that the Jyotishmati oil, which is derived from the seeds of C. paniculatus, has an influence on the Central Nervous System. The Indian subcontinent is its place of origin; nonetheless, it is known to grow wild in many locations across the world, including Australia, China, Taiwan, Cambodia, Indonesia, Laos, Malaysia, Burma, Nepal, Thailand, Vietnam, and a number of Pacific islands (Singh et al., 1996).

A huge shrub that is evergreen, climbs without thorns, and may reach a height of 10 metres. It has long, elongating branches that are reddish brown, and its stem can reach a diameter of 23 centimetres. The surface of the stem is covered with elongate lenticles. The leaves are simple and alternating, measuring 6-10 by 3-6 centimetres. oblong or obovate in shape, with a short acumen, crenate-serrate in the upper half, generally entire towards the base, crenulate, coriaceous, glabrous, base rounded or acute, with petioles that are between 6 and 12 millimetres in length. Inflorescence is paniculate. Flowers may be yellowish or greenish white in colour, and they are arranged in terminal pyramidal panicles that range in length from 5 to 15 centimetres. Pedicels are pubescent, and bracts are tiny and lanceolate.

The exterior of the calyx is pubescent; the lobes are semi-orbicular and ciliate; the petals are three millimetres long, oblong, and rounded at the apex. Female flowers have ovary globose, narrowed into a short stout style, glabrous stigma large 3-lobed; stamens inserted on edge of disc which is larger than disc in male flowers; anthers small without pollen, ovate, acute or subtriangular, less than 1mm long. Male flowers have stamens inserted on margin of disc, filament short; anthers oblong about 2 mm long; rudiment 9–12 millimetres in diameter, subglobose, bright yellow, transversely wrinkled, three-valved, with the valves spreading after dehiscing and staying joined at the base, revealing seeds. Capsules are subglobose in shape. Seeds 1-6, which are frequently seen alone, are entirely encased in a crimson, meaty aril. According to the findings of our review of the relevant literature, the plant is collected for its seeds without regard to whether or not the plant was grown in cultivation or in the wild due to the widespread use of its medicinal qualities.

This is done mostly in order to extract the seed oil. Because of this, the species is considered to be extremely precarious (Rajashekharan et al., 2002), and it is a plant that is critically endangered in both the Western and Eastern Ghats (Pattanaik et al., 2009).At the Plant Physiology and Plant Tissue Culture Laboratory, Department of Life Sciences, Faculty of Botany, Hemchandracharya North Gujarat University, Patan, Gujarat,

India, various experiments on Gloriosa superba L. and Cilantros pandiculates Wild. were conducted using standard techniques. This research was carried out in India.Experiments relating to the present investigation were carried out in both plants using the following procedures:Tissue culture studies.Evaluation of the plants' genetic integrity after being grown in vitro.Variations in biochemical processes that take place during in vitro regeneration.Research on the phytochemicals.

DATA / ANALYSICS

THE RESULTS OF STUDIES CONCERNING TISSUE CULTURE

Gloriosa superba Linn., often known as glory lily, is a plant that is used in traditional medicine and is a member of the Liliaceae family. The name Gloriosa superba comes from two words: the first, Gloriosa, comes from the term "Glorious," which means lovely, and the second, superba, comes from the word "superb," which means magnificent or outstanding in sort. From ancient times, people have used this plant as a source of medicine. It has a long history of use. Brilliant yellow and scarlet blooms with wavy edges adorn this semi-woody herbaceous-branched climber that may reach a height of roughly 5 metres. One to four stems can emerge from a single tuber that has the form of a V and is fleshy and cylindrical. It is considered to be one of the semi-toxic medications used in traditional Indian medicine, and while it is effective in treating a wide range of conditions, its abuse can be deadly. The tropical regions of Asia and Africa are where Gloriosa superba was first discovered. The North-West Himalayas, Assam, and the Deccan peninsula are only few of the places in tropical India where you may find its plants growing3,4. Phytochemicals are a broad category of the bioactive components that may be found in many plant species. In general, phytochemicals have been organised into six primary groups, which are comprised of carbohydrates, lipids, phenolics, alkaloids, and terpenoids respectively. Compounds found in plants called phytochemicals are the ones responsible for causing biological effects. 5-15. Investigations into the phytochemistry of the Gloriosa superba plant found evidence of the existence of carbohydrates, alkaloids, glycosides, flavonoids, steroids, terpenoids, and phenolic chemicals.

Results of Gloriosa superba L.

In tropical Africa, several components of the Gloriosa superba plant serve a broad range of purposes, particularly in the practise of traditional medicine. In Côte d'Ivoire, a cough and general soreness can be alleviated by using a leaf decoction as a liniment, and the juice of the leaves can be inhaled via the nose if someone is feeling faint. As a method of treating congestion, an enema containing the leaves is used medically in Côte d'Ivoire and Burkina Faso. In Congo, a treatment for asthma that involves applying crushed leaves to the chest is used.

A leaf decoction is indicated for treating dropsy of the scrotum in Burundi, while the pulp of the leaf is used to cure rheumatism. The Ulanga people of Tanzania traditionally use the ash from the plant after it has been burned to treat wounds. In addition, the juice of the plant is used as a treatment for malaria. The tuber has a wide variety of applications in the medical field when taken in modest quantities. In traditional medicine, it is utilised for the treatment of a variety of conditions, including cancer, colic, chronic ulcers, haemorrhoids, and bruising. Moreover, it is used as a tonic and purgative. Poultices made of it are used to cure neuralgia, and it is applied topically to treat arthritic disorders, swellings of the joints, sprains, and dislocations. Poultices are also used to relieve neuralgia. It is said that the tuber possesses antidotal qualities that can treat snakebites.

In Sudan wild 1, blooming stem; 2, tubers; 3, fruit. The tuber sap of the PROSEA plant, which is used to make a drink that puts people to sleep, is the source. A tuber decoction is used by the Marakwet people of Kenya for the treatment of stomach ailments and to induce miscarriage. In addition, macerated tuber is used to treat conditions such as ringworm, eczema, itching, leprosy, and smallpox. In the Democratic Republic of the Congo, the tuber, after being rubbed and cleansed, is applied topically to cure stomach aches and venereal illnesses. It is common knowledge that the tuber, fruits, and leaves have anthelmintic characteristics.

These features are put to use in the treatment of diseases caused by Guinea worms, schistosomes (which can cause bilharzia), roundworms, tapeworms, liver fluke, and filaria. The Pygmy people have a treatment for female sterility that involves injecting the juice that is extracted from crushed leaves into the rectal cavity. In order to ease the process of labour and delivery, the tuber is ground up and used to make a paste. People in Tanzania who speak Ulanga use ear drops made from tuber juice to alleviate earaches, but those in Zimbabwe who speak Shona use tuber juice drops directly to hurting teeth. In Zambia, the tuber is both an abortifacient and part of a treatment for impotence. It is also used in the preparation. Women who suffer from sterility, delayed puberty, delayed childbearing, or menstruation issues are given a soup that is produced from the sap of either leaves or tubers. It is common practise to eliminate head lice with the use of leaf juice, unripe fruits combined with butter, and tuber macerate.

Callus formation

For the purpose of inducing callus, several explants such as shoot apexes, leaves, and nodes were harvested. After that, the explants were inoculated again on MS media that had been enriched with several quantities and combinations of 2,4-D, BAP, Kin., and NAA. Under the impact of a variety of hormones, the explants exhibited a positive reaction. The variation in induction frequency was dependent not only on the hormones that were employed, but also on the explants.

Formation of a callus from the shoot's apex

After two weeks of hormone supplementation, the callus initiation process was initiated at the basal cut end section of the plant. Table 1 and Plate 1 illustrate the findings of the effects of different hormones on the formation of calluses and the features of calluses, respectively. This particular sort of callus was rigid and compact in appearance, with a yellowish-white coloration. Under the impact of a number of different hormone concentrations, it was discovered that the results were substantial but unsatisfactory. The frequency of callus induction ranged from 16% all the way up to 86%, depending on the concentrations and combinations that were utilised. The highest level of callus induction was seen at a concentration of 2.0 mg/l out of all the different individual concentrations of 2,4-D that ranged from 1.0 to 3.0 mg/l. Nevertheless, the induction frequency was only 32%. Nevertheless, a decrease in the incidence of callus induction was seen at concentrations as low as 1.0 mg/l and as high as 3.0 mg/l. When MS medium is supplemented with individual BAP at concentrations ranging from 1.0 to 3.0 mg/l, substantial findings for callus induction are obtained from all of the doses.

The greatest outcomes were shown with 74.002.46% of the explants when the BAP concentration was 2.0 mg/l, followed by 1.0 mg/l, which had a callus induction frequency of 54.004.01%. At a greater dosage of 3.0 mg/l 2,4-D, only 40.003.17% demonstrated callus formation with poor development. In this particular example, induced callus turned necrotic after 15 days of callus induction.In order to achieve an even higher

level of callus induction, the impact of a variety of different combinations of 2,4-D and Kin. was investigated. Induction of callus was seen to occur with each of the several combinations tried, and the resulting callus was rigid, compact, nodular, white, and yellowish. Maximum callus induction and development was seen at a dosage of 1.5 mg/l 2,4-D+1.0 mg/l Kin. with the greatest (86.002.46%) induction frequency.

This level was determined using all of the hormonal concentrations that were used Of all of the concentrations that were utilised, either on their own or in combination, this combination proved to be the most effective one. Just 50.003.17% of the explants in this example produced callus when treated with 0.5 mg/l 2,4-D and 0.2 mg/l Kin. Explants exhibited callus formation at a concentration of 1.0 mg/l 2,4-D with 0.5 mg/l Kin. After analysing the data, the researchers found that there was a discernible rise in the rate of growth as the concentration rose. Jadhav and Hegde, 2001 found a response pattern in Gloriosa that was analogous to what they saw in other species, such as Withaniasomnifera (L.) Dunal (Chakraborty, 2013). According to Enric et alresearch .'s from 2000, the 2, 4-D plays a significant part in the induction of calluses in bamboo. Nevertheless, the researchers found that a high concentration of the chemical reduced the capacity of calluses (Huang et al., 1989). According to Lin et al. (2003), the optimum concentration of 2, 4-D for bamboo varies depending on the species).

Table 1 The effect of plant growth regulators on the production of callus from the apex of the shoot of Gloriosa

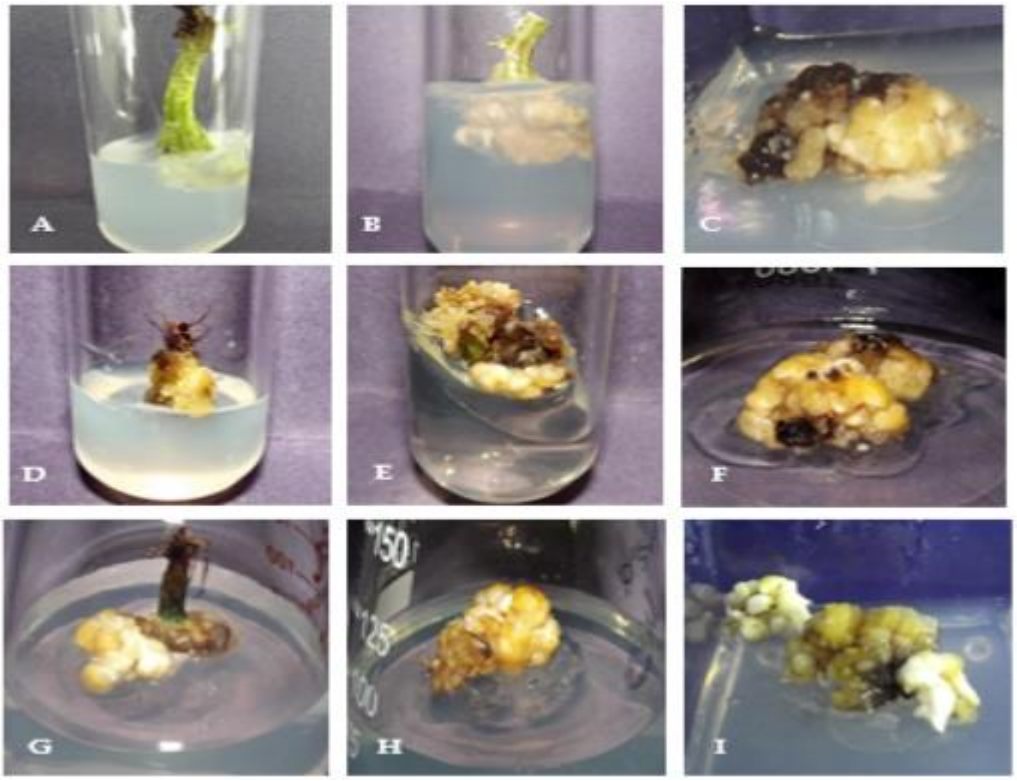
PGRs type	Conc.(mg/l)	Days to initiatecallus	% Growthfrequency	Calluscharacteristics
BAP	1.0	10	54.00±4.01b	Creamish compact
	2.0	8	74.00±2.46a	Creamish compact
	3.0	15	40.00±3.17bc	Creamish compact
2,4-D	1.0	17	22.00±3.75de	Creamish compact
	2.0	15	34.00±2.46cd	Creamish compact
	3.0	18	16.00±4.01e	Creamish compact
2,4-D+Kin.	0.5+0.2	12	46.00±5.11bc	Yellowish compact
	1.0+0.5	11	66.00±4.01b	Yellowish compact
	1.5+1.0	8	86.00±2.46a	Yellowish compact

Formation of a Callus from the Leaf and Node

In the first step of the experiment, leaf explants were tested with varying concentrations of 2,4-D (ranging from 1.0 to 3.0 mg/l). After trying combinations of 2,4-D and Kin, BAP and NAA, and BAP and 2,4-D together with little success, researchers decided to try other approaches. Nodal segments were cultured on MS

media that had been augmented with several concentrations of 2,4-D (ranging from 1.0 to 3.0 mg/l) as well as combinations of 2,4-D and Kin, BAP and NAA, and BAP and 2,4-D. No findings were found regarding the induction of callus, regardless of the concentration that was tested. The colour of all the cultivated explants changed to brown, and not long after that they began to decompose (Data was not represented). In the result portion, therefore, only the data pertaining to callus generation from the shoot apex were displayed.

Plate 1 The influence that plant growth regulators have on the production of callus from Gloriosa shoot apex explants



A-C: Callus formation in MS+ 2.0 mg/l BAP after 3,6 and 9 weeks

D-F: Callus formation in MS+ 2.0 mg/l 2,4-D after 3,6 and 9 weeks

G-I: Callus formation in MS+0.5 mg/l 2,4-D+ 1.0 mg/l Kin. after 3, 6 and 9 weeks

Formation of tubers in a roundabout way

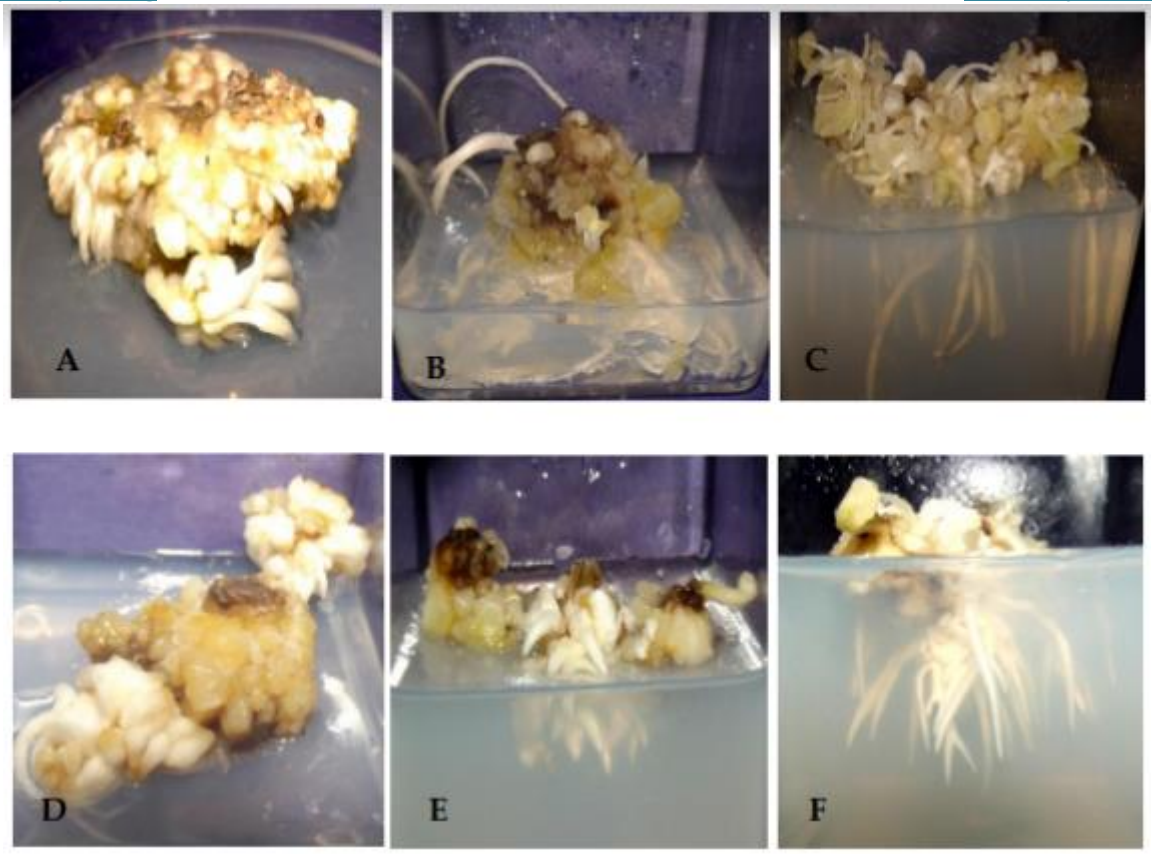
After obtaining a callus from shoot apex explants, further attempts were made to induce organogenesis in the form of shoot production and root formation using the callus. The research was carried out in collaboration with BAP and Kin., as well as NAA and IAA. Even after being cultured for a total of sixty days on shoot induction media, the callus exhibited no signs of change. According to the findings of the study, shoot organogenesis was unable to take place in the callus that was formed from shoot apex explants, and it finally became a brownish colour.In addition, attempts were made to generate tubers and roots from the acquired calluses by using a variety of hormone combinations in conjunction with MS media. The findings are reported in Table 2 and Plate 2 respectively. The medium with the highest growth frequency (92.002.00%) and the highest number of in vitro formed tubers (22.40.75) was MS medium supplemented with 3.0 mg/l BAP. This

resulted in the highest number of in vitro created tubers. Combinational effects showed that BAP (2.0 mg/l) and NAA (1.0 mg/l) were the most effective treatments, producing the greatest growth frequency (90.003.17%).

Table 2 The influence of plant growth regulators on the formation of indirect tubers from shoot apex callus in Gloriosa

PGRs Type	Conc.(mg/l)	% Growthfrequency	Mean No. of invitro tubers	Mean Length(cm)
BAP	1.0	0.0±0.0e	0.0±0.0f	0.0±0.0f
	2.0	80.00±3.17b	18.0±1.22b	2.6±0.09c
	3.0	92.00±2.00a	22.4±0.75a	3.74±0.08a
BAP+NAA	0.5+0.2	46.00±4.01d	6.6±0.40e	1.7±0.07e
	1.0+0.2	48.00±3.75d	10.6±0.40d	2.1±0.12d
	2.0+0.2	64.00±4.01c	14.8±0.80c	3.36±0.06b
	2.0+1.0	90.00±3.17a	19.0±1.22b	1.52±0.09e

Plate 2 The influence of plant growth regulators on the in vitro tuberization of Gloriosa derived from shoot apex callus



A-C: In direct tuber formation in MS+ 2.0 mg/l BAP+0.2 NAA after 3, 6 and 9 weeks

D-F: In direct tuber formation in MS+ 3.0 mg/l BAP after 3, 6 and 9 weeks

Formation of direct shoots in conjunction with tuber development

Culture of the shoot tips allowed for the production of direct shoots and the extension of existing ones. Explants of shoot tips taken from mature plants were used to inoculate MS media that had been enriched with varying doses of BAP and Kn (ranging from 1.0 to 4.0 mg/l), either alone or in conjunction with NAA (ranging from 0.2 to 0.5 mg/l) (Table 9). There was no sign of reaction in the MS medium since it lacked growth regulators. BAP at a concentration of 4.0 mg/l was shown to be the most effective among cytokinins for shoot induction and elongation. The frequency of shoot induction was 98.002.00%, although only one shoot was induced. The most promising findings for shoot proliferation were seen in Kin. medium that had been reinforced with 4.0 mg/l of Kin. According to the findings, it was discovered that the percentage of shoot induction and the length of the shoot became longer with increasing concentrations of both types of cytokines that were tested (Table 9, Plate 3).Multiple shoot development was seen in MS media that had been treated with either Kin. or BAP and NAA. This was the combinational effect. The highest rate of bud break, 74.002.45%, was seen in MS medium containing 0.5mg/l Kin.+0.2mg/l NAA, with 1.40.33 number of shoots and 7.50.08cm shoot length. On the other hand, the lowest rate of bud break, 36.004.01%, was recorded in at higher concentrations of 3.0 mg/l Kin.+0.2 mg/l NAA. In addition, it was discovered that 1.0 mg/l BAP with 0.2 mg/l NAA produced the best results, with a maximum of 1.60.33 number of shoots. Microtuberization and shoot development were both observed concurrently when cytokinins and auxins were combined in the

growth medium. At a growth frequency of 64.002.45%, the maximum number of tubers, 11.60.51, was reported in a solution containing 2.0 mg/l Kin. and 0.2 mg/l NAA (Table 9, Plate 4).

The obtained results indicated that the individual BAP at higher concentration or in combination with NAA was more effective for shoot formation as compared to Kin./BAP alone as MS medium fortified with Kin./BAP alone gave only one shoot per one shoot apex explants. This was the case because MS medium fortified with Kin./BAP alone gave only one shoot per one shoot apex explants. Chandrawanshi et al., 2015 came to comparable conclusions when they employed the shoot tip of Gloriosa as explants in their study. Khandel et al., 2011 also showed that the improved shoot initiation response was obtained on MS media supplemented with 2.0 mg/L BAP and 0.5 mg/L NAA by utilising apical shoot bud and meristem explants of G. superba. This was done by using the explants.

Hassan and Roy (2005) in Gloriosa discovered a report that was comparable about the induction of shoots. They found two or three shoots in MS medium with BA alone within three to four weeks after inoculation. Furthermore, the number of shoots increased in MS medium with a combination of 1.5 mg/l BAP with 0.2 mg/l NAA with 15% coconut water and 2 g/l activated charcoal. Shoot induction was not observed in their study when they used MS medium; however, they did find two or three shoots in MS medium with BA alone. Sivakumar and Krishnamurthy (2000) found that the largest number of shoots could be obtained from apical shoot apex explants of G. superba when they were grown on MS media that was supplemented with 2ip and Kin. This species was also reported to have undergone organogenesis by Sivakumar and Krishnamurthy (2004b).

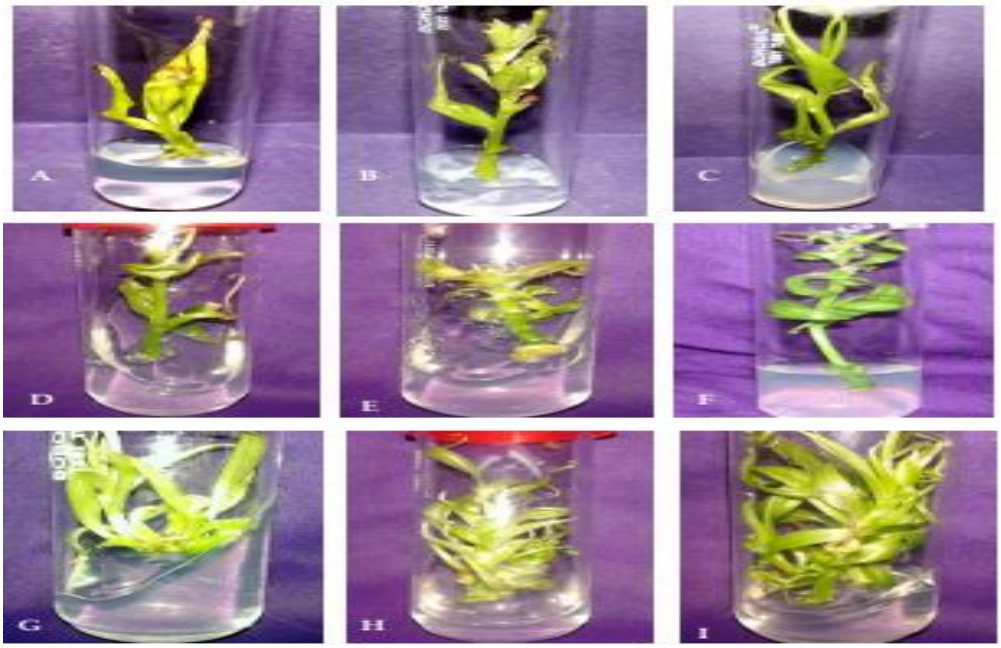
Throughout the course of this research, we saw both the development of tubers and the lengthening of the shoots. In MS medium that was supplemented with 2.0 mg/l Kin. in conjunction with 0.2 mg/l NAA, the shoot induction frequency was found to be at its greatest and the number of tubers produced were at their highest. Nonetheless, other combinations were equally effective at promoting strong tuber formation in vitro. But direct shoot formation from the shoot apex was very slow and laborious. It required perfect standardisation of the media and hormones for shooting, and once shoot induction occurred, periodic transfer of it on the same media with the same hormones promoted it multiplication and elongation along with basal in vitro tuberization. Direct shoot formation from the shoot apex was very difficult.

Table 3The effect of plant growth regulators on the direct shoot elongation and in vitro tuberization of Gloriosa derived from shoot apex explants

PGRs Type	Conc. (mg/l)	% growth frequency	Mean No. of Shoots	Mean Shoot length (cm)	Mean No. of in vitro tuber	Mean in vitro tuber length(cm)
BAP	1.0	40.00±3.17g h	1.0±0.0d	4.36±0.11g	-	-
	2.0	52.00±2.00ef	1.0±0.0d	5.94±0.12d	-	-

	3.0	86.00±4.01b	1.0±0.0d	7.68±0.09bc	-	-
	4.0	98.00±2.00a	1.0±0.0d	9.9±0.05a	-	-
Kin.	1.0	28.00±3.75ij	1.0±0.0d	2.7±0.23j	-	-
	2.0	38.00±3.75h	1.0±0.0d	3.74±0.12h	-	-
	3.0	52.00±2.00ef	1.0±0.0d	5.42±0.07e	-	-
	4.0	80.00±3.17bc	1.0±0.0d	8.04±0.08b	-	-
Kin.+ NAA	0.5+0.2	58.00±3.75ef	3.4±0.41a	7.5±0.08c	-	-
	1.0+0.2	50.00±3.17ef	2.36±0.32c	5.46±0.14e	6.8±.38b	1.26±0.08c
	2.0+0.2	64.00±2.45d	1.0±0.0d	4.86±0.10f	11.6±.51a	2.44±0.16a
	3.0+0.2	36.00±4.01hi	1.0±0.0d	3.52±0.12h	4.4±0.51c	1.34±0.08c
BAP+NAA	0.5+0.2	24.00±2.45j	1.0±0.0d	3.14±0.10i	3.0±0.32d	1.2±0.12c
	1.0+0.2	48.00±2.00fg	2.0±0.25a	5.38±0.14e	7.4±0.4b	2.22±0.08b
	0.5+0.5	56.00±2.45def	1.0±0.0d	5.74±0.12de	-	-
	1.0+0.5	72.00±4.01c	1.0±0.0d	7.72±0.23bc	-	-

Plate 3 The effect of plant growth regulators on the elongation of direct shoots and the development of numerous shoots from shoot apex explants of Gloriosa

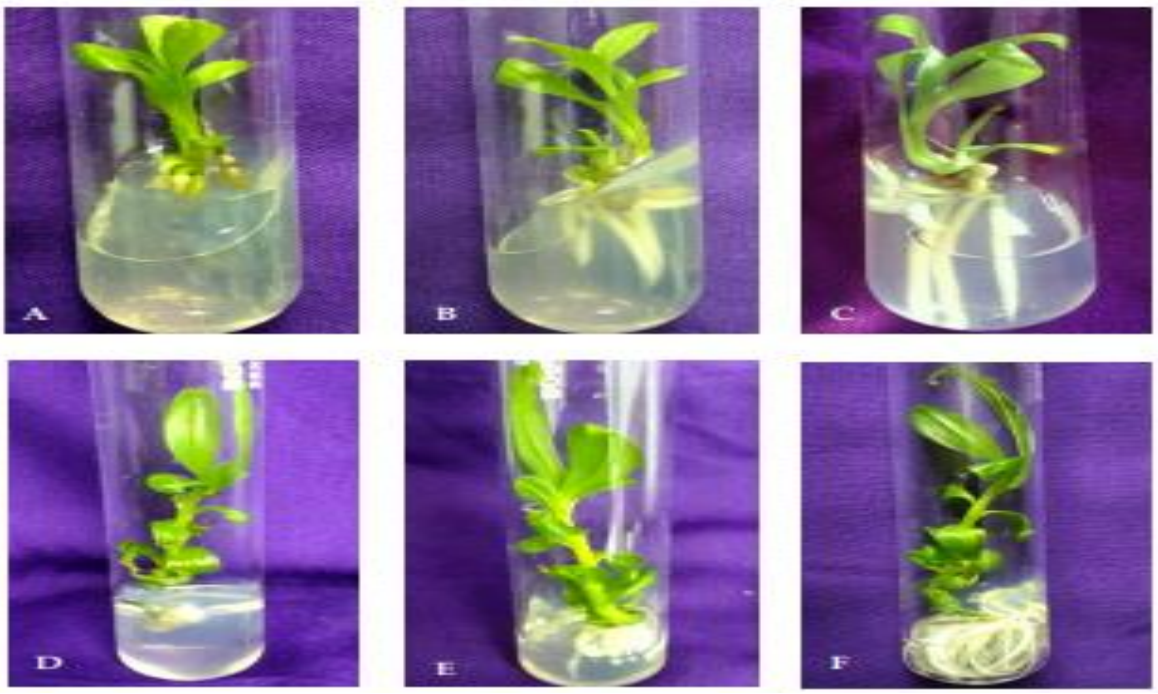


A-C: Shoot elongation in MS+4.0 mg/l Kin.

D-F: Shoot elongation in MS+4.0 mg/l BAP

G-I: Multiple shoot formation in MS+0.5 mg/l Kin.+0.2 mg/l NAA

Plate 4 The effect of plant growth regulators on the elongation of the shoot and the in vitro tuberization of Gloriosa shoot apex explants was investigated



A-C: Direct shoot elongation and multiple shoot with roots/tubers in MS+ 2.0 mg/l Kin.+0.2 mg/l NAAD-F: Direct shoot elongation with multiple roots/tubers in MS+ 1.0 mg/l BAP+ 0.2 mg/l NAA

CONCLUSION

Their increase in in vivo as well as in vitro grown plant sections was demonstrated byquantitative measurement of total flavonoid and phenol levels. The HPTLC fingerprintinganalysis indicated the presence of a number of chemicals in the form of bands in every invivo and in vitro sample from both plants; however, more standardisation is required beforeparticular kinds can be confirmed. Because the HPTLC quantification of a specific standardcomponent in each plant demonstrated that the compound was present in in vitro growntissues and parts, the plant may be exploited for the manufacture of secondary metabolitesfrom it rather than being destroyed in its natural environment. Overall, the effort will bebeneficial to the conservation of valuable medicinal plants through the establishment of anoptimum technique. Additionally, in vitro tissues are a possible source of numerous bioactivesubstances that can be useful to pharmaceutical enterprises.

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