International Journal of Scientific Research in Science and Technology
Print ISSN: 2395-6011 | Online ISSN: 2395-602X (www.ijsrst.com)
doi : https://doi.org/10.32628/IJSRST

Effect of Growth Hormones on In Vitro Seed Germination of Gloriosa Superba

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ARTICLEINFO

Article History:

Accepted: 20 Feb 2023
Published: 05 March 2023

Publication Issue
Volume 10, Issue 2
March-April-2023

Page Number
50-65

ABSTRACT

Introduction: Elmer D. Merrill, a botanist from the United States, sent a letter to a colleague in 1931 in which he discussed the presence of a rare species of cycad that had been gathered on Culion Island, which is located in the province of Palawan.

Aim of the study: The main aim of the study is to Effect of growth hormones on in vitro seed germination of Gloriosa superba

Material and method: For the purpose of the investigation, the MS basal medium that was developed by Murashige and Skoog (1962) and the SH medium were used.

Conclusion: The Pachamalai Hills are a mountain range that belong to the Eastern Ghats and can be found in the middle of the state of Tamil Nadu in India. The hills include an abundance of medicinal plants as well as a diverse plant life.

I. INTRODUCTION

1.1 OVERVIEW

Elmer D. Merrill, a botanist from the United States, sent a letter to a colleague in 1931 in which he discussed the presence of a rare species of cycad that had been gathered on Culion Island, which is located in the province of Palawan. Merrill penned the following in his letter: "Among the plants sent in for identification from Culion is a very excellent fruiting specimen of the Cycas with narrow leaflets of which I collected sterile specimens in 1902; this is undoubtedly an undescribed species, and I have tentatively named it Cycas herrei." The unique traits of this plant set it apart from the other cycads that had been gathered in the past. According to Merrill, the species had been sighted for the very first time during a "single day's expedition" to a huge open grassy region that was locally known as the cogonal grande or the pataggrande and had been considered as a prospective location for the Culion leper colony. When Merrill compared the little information, he had with comparable specimens kept at the Kew Herbarium, he came to the conclusion that the cycad was "a peculiar species." After informing his colleague in 1931 of what seemed

to be a new species, Merrill and his companions stationed in the Philippines examined the plant in further detail and separated it from prior incorrect identifications. In 1936, the cycad was presented to the community of botanists all over the world for the first time in the Philippine Journal of Science. The species was given the name Cycas wadei in honour of Herbert W. Wade, who served as a pathologist and physician at the leper colony from 1922 until his death in 1968. Merrill had initially suggested that it be named in honour of Albert W.C.T. Herre, an ichthyologist with the Philippine Bureau of Science; however, the name was later changed to honour Herbert W. Wade.

1.2 DIVERSITY, EXPLOITATION, CONSERVATION AND CURRENT STATUS OF INDIAN CYCAS

There are around one hundred different species of the dioecious genus Cycas L. found all over the globe. The Western Ghats, the Eastern Ghats, and the Northeastern areas of India are home to the majority of the country's 18 species that belong to this genus. Cycadaceae, Stangeriaceae, and Zamiaceae are the names of the three living families that are categorised as belonging to the order Cycadales. Even though some of the exotic Cycads, especially Cycas revoluta Thunb. (Native to Japan), and other species of Zamia (Zamiaceae) have been cultivated in the gardens/houses in India, Cycas is the sole genus that occurs naturally in the country. Because they are composed of one-of-a-kind compounds that are both neurotoxic and carcinogenic, the seeds of cycads are often thought to be hazardous not just to humans but also to cattle. In today's world, medicinal plants have been overharvested to satisfy the need of crude drug businesses including pharmaceutical firms, local medicine men, and traditional medical practitioners. These industries include:

- Historic perspectives of Cycads
- Characteristic features of the genus Cycas
- Diversity of Indian Cycas
- Exploitations of Indian Cycas
- Conservation of Indian Cycas
- Current status of Indian Cycas

II. LITERATURE REVIEW

Ramana, Munigela (2022) Andhra Pradesh is home to the Cuddapah, Chittoor, and Nellore districts, which is where the Cycas beddomei Dyer species was first discovered. Its natural habitats are only found in the southern Eastern Ghats in certain districts and at certain heights. Concerns about its identification, typification, range, conservation, and position with CITES are discussed here. Its position on the IUCN red list is currently being reviewed. It was estimated that there were 2.05 lakh people living there, the AOO was 36.10 square kilometres (3610 hectares), and the EOO was 1768 square kilometres. The species has just been given the "Endangered" status by the government. With its well-established subterranean stem, which is not observed in the other cycads, the species has successfully adapted to the numerous forest fires that occur in the area.

Meng, Yi-Yi & Xiang, Wei & Wen, Yin & Huang (2021) A detailed study on the structural and functional characteristics of the leaves (leaflets) of cycads was carried out by us. The purpose of this research was to provide light on the

functional distinctions that exist between cycads and angiosperms, as well as the earlier-originating Cycadaceae and the later-differentiated Zamiaceae families. Methods We examined the leaf structure, economic features, mechanical resistance (Fp), and leaf water potential at the turgor loss point (tlp) of 20 Cycadaceae species and 21 Zamiaceae species that we chose from the same cycad garden in South China. In addition, we created a dataset of geographical distribution along with environmental factors for these cycad species.

Srivastava, Ramesh & Agrawal, P &Ramabharathi, V & Patil, Sameer (2021) The purpose of this work is to make an effort to present a glimpse of the native and imported Gymnosperms of India, together with their revised nomenclature and categorization according to the most recent molecular classification. The information that is currently available regarding the ethnobotany and economic significance of gymnosperms was reviewed and discussed with the intention of assisting the teachers and students of botany in their curriculum in terms of nativity, introductions, and current taxonomy; to benefit the researchers of economic and medicinal plants on the one hand and the pharmacological researchers on the other; in addition, the Pharmaceutical industries which are collecting and using these taxa were also mentioned.

Mankga, Ledile&Yessoufou, Kowiyou&Mugwena (2020) In terms of biogeography, cycads originally occupied a broad range of latitudes and longitudes; now, however, they are only found in tropical and subtropical climates. They first appeared less than 300 million years ago and had a recent re-diversification approximately 12 million years ago; the genus Cycas is the lineage that has quickly diversified and is widely dispersed. However, we do not yet have a complete understanding of the processes that were responsible for the diversification and biogeography of the species. In this study, we began by collecting and analysing DNA sequences from GenBank (nuclear: PHYP, RPB1, HZP, AC3, F3H, SAMS and GTP; chloroplasts: plant barcode trnH-psbA, trnL-trnF, trnS-trnG and psbM-trnD) in order to construct a fully annotated and dated After that, we used the Bayesian Binary Method to reconstruct the historical biogeography of the extant Cycas, and then, using the Bayesian approach for diversification analysis, we explored the evolutionary events that might have shaped the rapid diversification and large distribution of Cycas across the Pacific Islands.

Sethi, Poonam & Gupta, Himanshu & K, Ellakiya& Kumar, Bharat (2020) Cycas, an ancient gymnosperm, is on the verge of extinction due to the fact that the stem of the plant generates a starch-like material known as sago, which is also known as sago palm. Cycads are classified as xerophytes because they can survive with very little water and are resistant to drought. In this work, the micromorphological characteristics of the leaflets are compared as a taxonomic tool to independently identify two species of Indian cycad. Cycas.circinalis and Cycas.revoluta. Investigations of the patient's anatomy were conducted out and presented. An investigation on the powdered leaflet's phytochemicals and physicochemical properties was also carried out.

III.MATERIALS AND METHODS

3.1 TISSUE CULTURE STUDIES IN G. SUPERBA

3.1.1 Procedure

3.1.1.1 Preparation of growth medium

For the purpose of the investigation, the MS basal medium that was developed by Murashige and Skoog (1962) and the SH medium were used. The chemicals that were employed in the current research were of an annular grade, and they were acquired from Hi-media labs in Mumbai. The vitamins, amino acids, and hormones were all purchased from a company in the United States called Sigma. From Glaxco Laboratories in Mumbai, we were able to acquire bacteriological-grade agar of the highest possible quality.

3.2 PHYTOCHEMICAL STUDIES

3.2.1 Preliminary Phytochemical

Screening Plants may be thought of as a "factory" that produces a variety of biochemical substances such as alkaloids, flavanoids, terpenoids, and tannins, all of which have physiological as well as medicinal effects. These chemicals can be found in many plants. Performing a comprehensive analysis of the plant material to determine its phytochemical behaviour is comprised of a number of processes, including:

ϖ Collection of raw materials.

ϖ Extraction, purification and characterization of bio-active constituents of the plant.

Using a Soxhelet apparatus, dried powdered material of plant components (leaves, seeds, tubers, and pods) and in vitro plant were individually extracted with methanol. These extracts were concentrated, and a preliminary phytochemical screening was carried out using them.

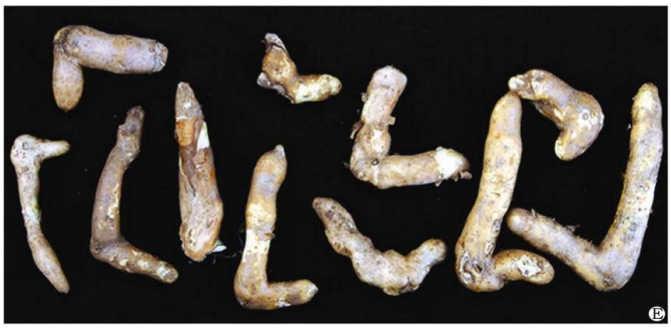
IV.RESULTS

4.1 TISSUE CULTURE IN G. SUPERBA

Because of its enormous significance in complementary and alternative medicine, Gloriosa superba L. was selected for use as a test subject in the current investigation. Once upon a time, the slopes of Pachamalai were home to a plentiful supply of this magnificent plant. The plant was mercilessly harvested from the Pachamalai hills by local healers due to the enormous medical capabilities it had. Additionally, the plant is extensively used in contemporary medicine, which contributes to the depletion of the species and causes it to become endangered. Because of this, we felt compelled to preserve this plant using various in vitro procedures.

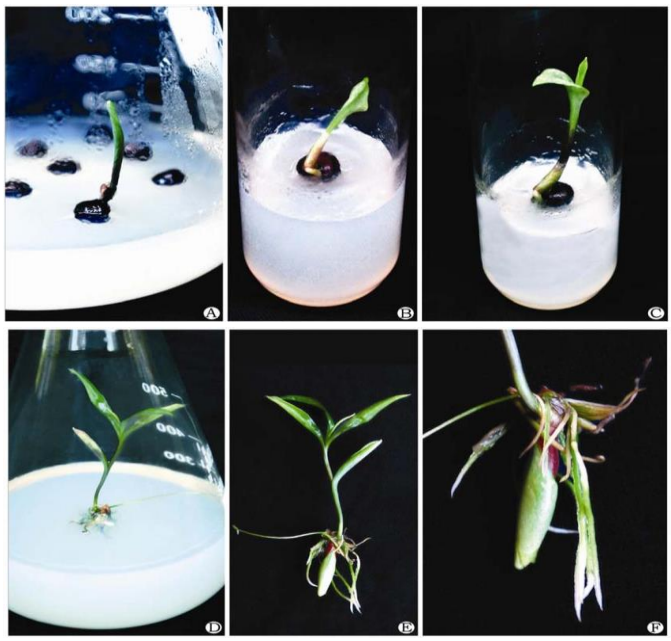
a) In vitro seed germination and tuberization

The seeds of Gloriosa superba are very vulnerable to being eaten by insects and have a very low chance of successfully germinating into new plants. The seeds were gathered from wild plants in the Pachamalai hills (Plate-7D), subjected to surface sterilisation, and then planted on seed germinating medium consisting of MS basal salts with GA3, BA, 1% sucrose, and 0.8% agar. The medium that had MS baseline salts and was supplemented with 0.5 mg/l GA3 and 1.0 mg/l BA was found to have the highest germination percentage (72.5%). This was shown in Plate 8 A-C. (Table 4.2).



A. Entire plant of G. superba L.
B. A flower of G. superba L.
C. Fruits of G. superba L.
D. Seeds of G. superba L.
E. Tubers of G. superba L.

Plate 7 gloriosa superba L.



A & B. Germinating seeds of G. superba L.

C. 20 days old seedling
D. transfer of seedling into corm-producing media
E. in vitro tuberization with plantlet
F. in vitro tuber

Plate 8 In vitro seed germination and tuberization of Gloriosa superba L.

Table 4.1 : Effect of growth hormones on in vitro seed germination of Gloriosa superba

GrowthHormone(mg/l)		No. of seedsinoculated/tube	No. of seedsgerminated(Mean±S.D)	Percentage ofseedgermination
BA	GA_3			
0.5	0.5	4	1.1±0.95	27.5
1.0	0.5	4	2.9±0.94	72.5
1.5	0.5	4	1.3±1.15	32.0
2.0	0.5	4	0.9±0.7	22.0
2.5	0.5	4	No germination	-

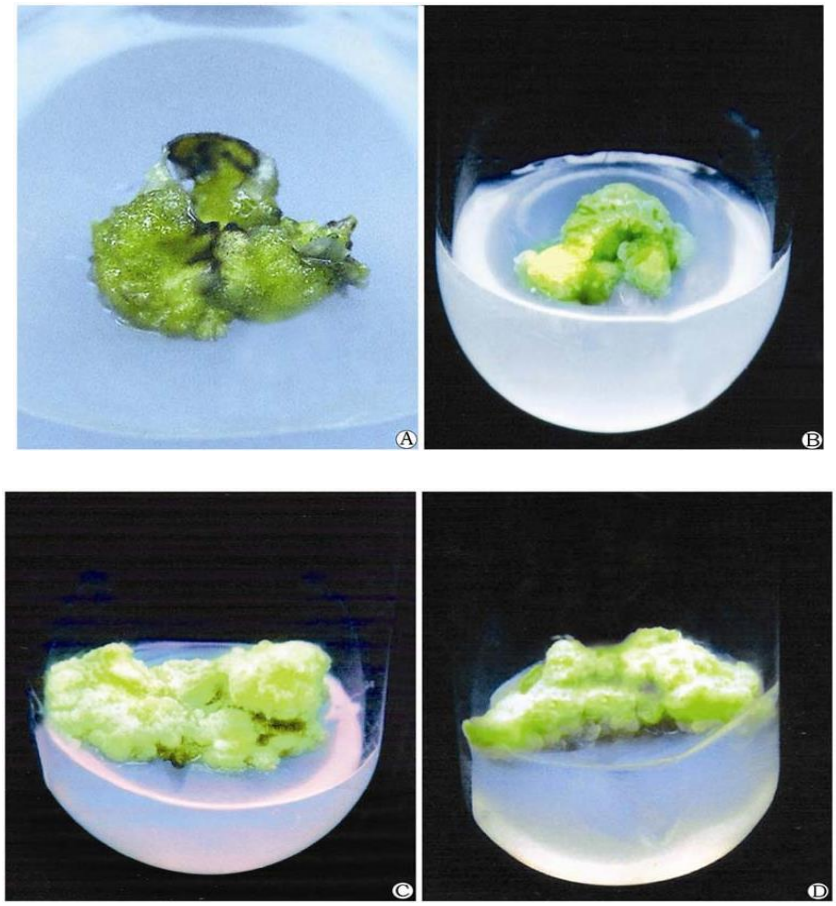
Table 4.2: Effect of growth regulators on in vitro tuberization when incorporated in MS medium supplemented with 6% sucrose

HormonalConcentration			Percentage oftuberization	Lengthofrhizome(Mean± S.D)	No. of rootsformed inrhizomes
BAP(mg/l)	GA_3(mg/l)	NAA(mg/l)			
0.50	0.05	9.1	25	1.2±0.1	3-4
0.75	0.05	9.2	65	2.5±0.7	9-11
1.00	0.05	9.3	90	3.5±0.3	10-15
1.25	0.05	9.4	50	1.5±0.5	20-25
1.50	0.05	9.5	Notuberization	-	-

After the seeds had germinated, they were moved to a medium designed to produce corms (Plate 8D) that included MS basal salts along with BAP, GA3, and NAA. When the MS medium was supplemented with 1.0mg/l BAP, 0.05mg/l GA3, and 9.3mg/l NAA with 6% sucrose, the maximum percentage (90%) of in vitro tuberization (3.5cm length) and rooting was found (Plate 8 E&F). When the culture media was supplemented with 1.25 mg/l BAP, 0.05 mg/l GA3, and 9.4 mg/l NAA, there was a 50% success rate of in vitro tuberization with a tiny rhizome (1.5 cm) and 25 roots. This was found when the in vitro tuberization process was carried out. It was deduced that an increase in BAP and NAA concentration led to a reduction in tuberization and an increase in root induction (Table 4.2).

b) Callus induction from in vivo leaf explants

The leaf explants that were removed from the wild plants and placed on MS media that had been enriched with varying amounts of 2,4-D (0, 0.5, 1.0, 1.5, 2.0, and 2.5 mg/l) were injected with the pathogen. Following an inoculation period of fifteen days, the explants had a bloated appearance, and callus formation started at the cut end. The callus that developed from the explants had a yellowish-green coloration. When MS basal salts were supplemented with 2.0 mg/l 2,4-D within 40 days, the highest percentage of callus induction was seen (78.57), as can be shown in Plate 9. (Table 4.3). When leaf explants were inoculated on SH media containing growth regulators such 2,4-D and BA, the callus proliferation was increased within a short period of time. Within 35 days of incubation with 1.0 mg/l of 2,4-D and 0.5 mg/l of BA in SH medium, the maximum percentage of callus was found (85.71). (Table 4.4).



1. Callus induction on MS medium containing 2,4-D
2. Proliferation of the callus on MS medium containing 2,4-D
3. & D. proliferation of the callus on MS medium containing 2,4-D and BA

Plate 9 callus induction from leaf explants of Gloriosa superba L.

Table 4.3 : Effect of concentrations of 2,4-D in MS basal salts on callus induction from leaf explants of Gloriosa superba L.

S.No.	Concentrationo f2,4-Dinmg/l	No of tubesinocul ated	No of tubesrespon ded	Rateofcallu s (%)inducti on	Dayst ocallu s	Degree ofcallus
1.	0.0	14	-	-	-	-
2.	0.5	14	2	14.28	53	+
3.	1.0	14	6	42.85	53	++
4.	1.5	14	9	64.28	49	++
5.	2.0	14	11	78.57	40	+++
6.	2.5	14	6	42.85	56	++

Table 4.4: Effect of concentrations of 2,4-D and BA in SH media on callus induction from leaf explants of Gloriosa superba L.

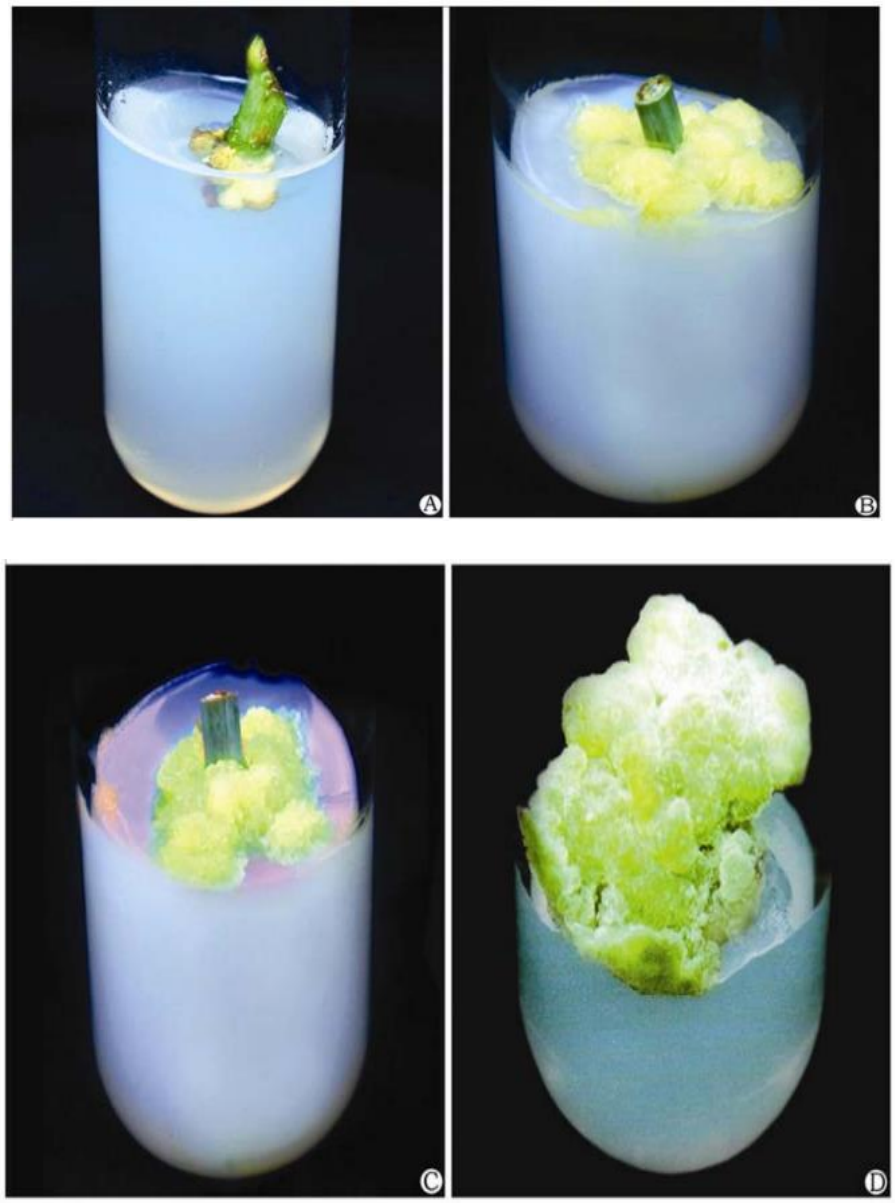
S.No.	Concentrationsof 2,4-D(mg/l)	Concentrationof BA(mg/l)	No. oftube s Inoculated	No.oftubes responded	Rateofcall usinductio n(%)	Dayst ocallu s	Degree ofcallus
1.	0	0.5	14	-	-	-	-
2.	0.5	0.5	14	7	50.00	42	++
3.	1.0	0.5	14	12	85.71	35	+++
4.	1.5	0.5	14	6	42.85	56	++
5.	2.0	0.5	14	4	28.57	60	+
6.	2.5	0.5	14	2	14.28	63	+

The formation of callus cultures is an essential step in the process that leads to the regeneration of complete plants. The need of growth regulators for the beginning of the callus formation process has been adjusted in accordance with the nutritional state of the medium. In the current experiment, the MS medium supplemented with 2,4-D separately or the SH medium fortified with 2,4-D and BA was shown to be the most effective growth regulator among the other growth regulators examined individually or in combination. It was discovered that the SH medium that had been reinforced with 2,4-D and BA was the most effective for the development of callus from G.superba leaf and nodal explants. Sivakumar et al., (2003), who reported that solid cultures of embryonic calli were formed from leaf explants on SH medium containing either 2,4-D combined with 2iP in the range of 4.52 – 9.84 M and kept in darkness, provide support for our findings. They stated that the calli were formed on SH medium containing either 2,4-D combined with 2iP in the range of 4.52 – 9.84 M. Similar findings were found by Jawahar et al. (2003) in Solanum nigrum from leaf explants grown on MS medium with the addition of IAA, BAP, and GA3. On MS medium with 2.0 mg/l BAP and 0.01 mg/l GA3, the highest frequency of green compact callus and numerous shoots was found.

a) Callus induction from nodal explants

The nodal explants of the plant that was grown in vivo were inoculated on SH media that had been enriched with 0–2.5 mg/l of 2,4-D and 2.0 mg/l of 2iP. The greatest response of callus was seen (71.42%) when the SH medium was added with 2.0 mg/l of both 2,4-D and 2iP. Under completely

dark circumstances (Plate 10), all of the cultures were kept alive (Table 4.6). In plant tissue culture, the selection of appropriate explants is an additional factor that contributes to the success of any experimental system. In the current research, the most sensitive G. superba explants were found to be the nodal explants, followed by the leaf, internodal, and shoot tip explants. The frequency of initial callus formation and subsequent development was shown to vary depending on the explants and hormones used. During the lag phase that occurs before the induction of active development, the growth of the callus might be unpredictable.



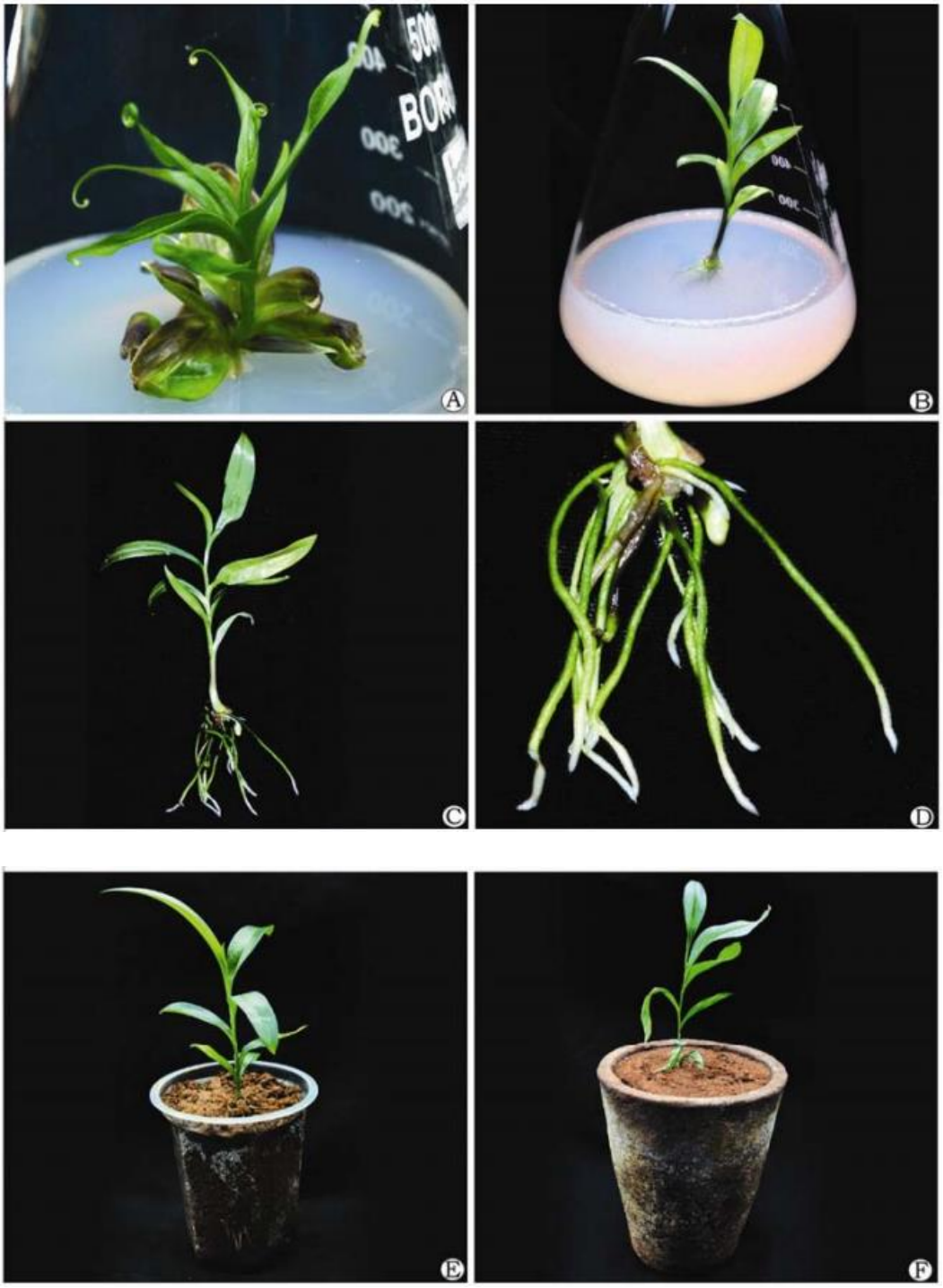
A. initiation of callus
B & C. proliferation of callus
D. fully proliferated callus
Plate 10 callus induction from nodal explants of Gloriosa superba L.

Table 4.5 Effect of different concentrations of 2iP and 2,4-D in SH medium on callus induction from nodal explants of G. superba L.

S.No	Concentrationof2,4-D (mg/l)	Concentrationof2iP(mg/l)	No.oftubesinoculated	No.oftubesresponded	Rateofcallus (%)	Daysto callus	Degree ofcallus
1..	0.0	2.0	14	-	-	-	-
2.	0.5	2.0	14	1	7.14	40	+
3.	1.0	2.0	14	4	28.57	42	+
4.	1.5	2.0	14	8	57.14	37	++
5.	2.0	2.0	14	10	71.42	31	+++
6.	2.5	2.0	14	6	42.85	41	++

b) Multiple shoot induction from nodal explants

G.superba nodal explants were inoculated onto MS media that had been enriched with several doses of BAP (3.5, 4.0, 4.5, 5.0, 5.5, and 6.0 mg/l) and 0.5 mg/l IAA. The medium was then incubated. Following a period of three weeks, the explants produced many new shoots. When MS medium was fortified with 5.0 mg/l BAP and 0.5 mg/l IAA, the perfect concentration for inducing the largest percentage of multiple shoots was achieved (Plate 11A), which resulted in the highest number of multiple shoots (8.9) being produced (Table 4.6). On MS medium that had been supplemented with a variety of growth regulators in varying concentrations, either individually or in combination, the possibility of direct regeneration from different explants, including nodal and shoot tip explants of G.superba, was investigated. The results of this investigation were evaluated. The combination of BAP and IAA was shown to be effective for the induction of a good number of shoots with a greater frequency in nodal explants. Similar findings were found by Arockiasamy et al. (2002) in Solanum trilobatum, where they cultured nodal explants on LS media that was enriched with varying amounts of BAP and IAA. These findings validated the previous findings. After a period of three weeks, a number of shoots immediately developed from the explants. The combination of 5 mg/l BAP and 0.5 mg/l IAA produced a positive response. According to a study conducted by Chaplot et al. (2006), the use of MS media with 4.4 mg/l of BA and 1.4 mg/l of IAA was effective in eliciting the greatest number of shoots (12 multiple shoots) from nodal explants in Chitrak (Plumbago zeylanica Linn.).



A. initiation of multiple shoots from nodal explants
B. A shoot inoculated in the rooting medium
C &D. well developed rooting
E. hardened plantlet
F. transferred to in vivo conditions

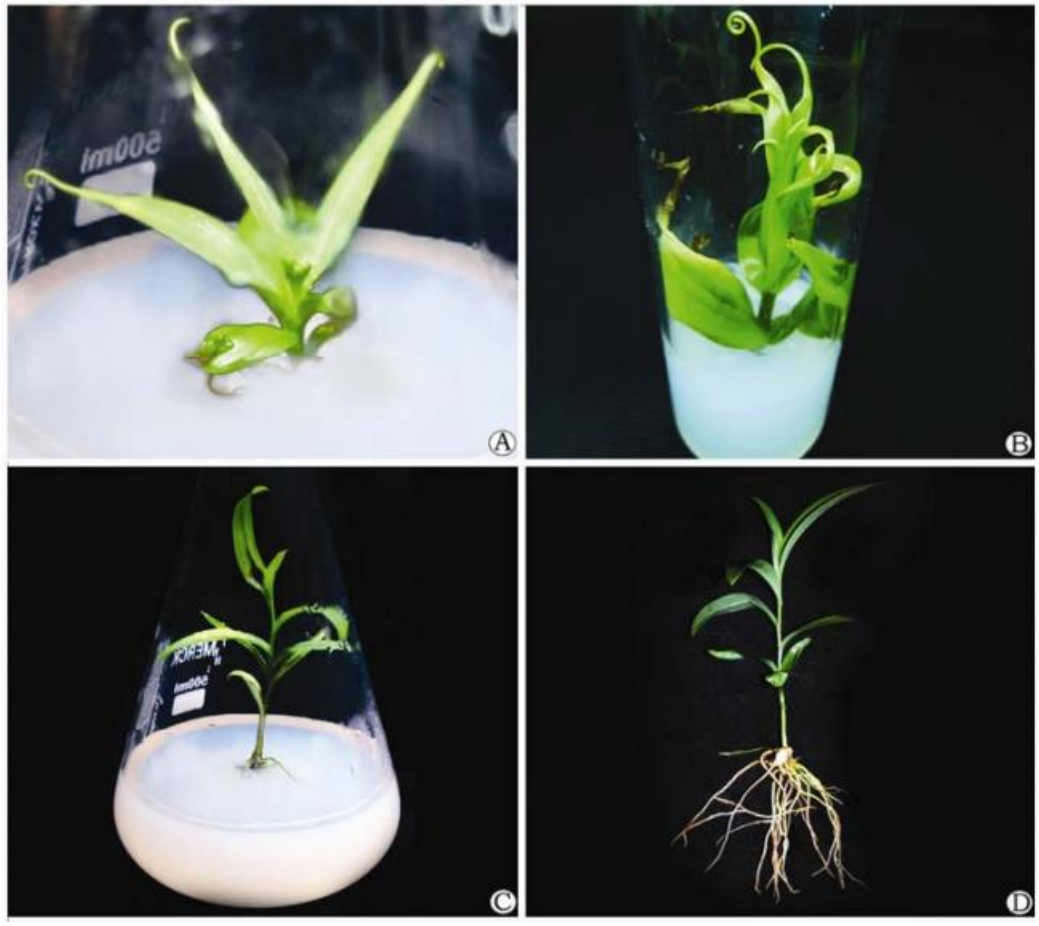
Plate 11 multiple shoot induction and in vitro regeneration from nodal explants of gloriosa superba L.

Table 4.6 Effect of BAP and IAA for multiple shoot initiation from nodal explants of G. superba L.

S.No.	Hormonalconcentration(mg/l)		No.oftubesinoculated	No.oftubes responded	Percentageof multipleshoot proliferation	Numberofshoots/explant (Mean±SD)
	BAP	IAA				
1.	3.5	0.5	14	1	7.1%	1.1±1.3
2.	4.0	0.5	14	5	35.7%	6.1±0.5
3.	4.5	0.5	14	7	50.0%	7.4±0.7
4.	5.0	0.5	14	12	85.7%	8.9±1.1
5.	5.5	0.5	14	9	64.2%	7.5±0.9
6.	6.0	0.5	14	6	42.8%	6.9±0.4

c) Shoot proliferation from shoot tip explants

After surface sterilisation, the shoot tip explants of G.superba were inoculated into MS medium containing 2iP and BA. When MS medium was enriched with 2.0 mg/l 2iP and 0.5 mg/l BA (Plate 12 A&B), the level of shootlet proliferation was reported to be at its highest percentage (78.57%). (Table. 8). According to the findings of this experiment, the optimal concentrations of 2iP and BA for the production of numerous shoots are 2.0 mg/l and 0.5 mg/l respectively. When shoot tip explants of G.superba were cultured on MS medium supplemented with BAP in the range of 0.444 M- 4.44 M and 2iP in the range of 4.92 M - 9.84 M with or without Kin, the researchers found that the results supported the findings of Sivakumar et al., (2000). Sivakumar et al., (2000) reported that the results of their study were supported by the findings of When it came to the creation of shoots, 2iP proved to be more successful than BAP. Similarly, Hassan and Roy (2005) found that 92% of the cultures of apical and axillary buds of immature sprouts from naturally growing Gloriosa superba L. species were able to regenerate four shoots per culture when cultivated in MS basal medium that was supplemented with 1.5 mg/l BA and 0.5 mg/l NAA. Rapid shoot multiplication was seen after repeated subculturing in the same media, with eight new shoots emerging from each culture. The number of shoots produced by each culture rose to a maximum of 15 with the addition of 15% (v/v) of coconut water (CW) and 2 g/l of activated charcoal.



A. Development of shoot tip
B. Developed shootlet
C. Shootlet transferred to rooting medium
D. Well developed roots
E. Hardened plantlet
F. Transferred to *in vivo* conditions

Plate 12 in vitro regeneration from shoot tip explants of gloriosa superba L.

Table 4.7 Effect of Different concentrations of 2iP and BA for shoots proliferation from shoot tip explants G. superba L.

S.No.	Hormonalconcentration(mg/l)		No.oftubesinoculated	No.oftubesresponded	Percentageofshootletproliferation	Numberofshoots/shoottipexplant (mean±SD)
	2iP	BA				
1.	0.5	0.5	14	1	7.14%	2.56±0.25
2.	1.0	0.5	14	4	28.57%	3.02±0.17
3.	1.5	0.5	14	9	64.28%	4.32±0.28
4.	2.0	0.5	14	11	78.57%	5.12±0.15
5.	2.5	0.5	14	8	57.14%	3.50±0.29
6.	3.0	0.5	14	6	42.85%	1.52±0.31

V. CONCLUSION

The Pachamalai Hills are a mountain range that belong to the Eastern Ghats and can be found in the middle of the state of Tamil Nadu in India. The hills include an abundance of medicinal plants as well as a diverse plant life. A study of medicinal plants in these hills was carried out, and a database listing the existence of medicinal plants was tabulated along with their status. One of the rare medicinal plants found in these hills, Gloriosa superba L. was chosen for in vitro research because of its potential benefits. This plant is often referred to as a "Glory lily," and it has an incredible amount of therapeutic benefits. Because of its pungent, bitter, acrid, hot, antihelminthic, laxative, alexiteric, and abortifacient properties, the tuber of the plant is widely recognised in the Ayurvedic and Yunani medical systems. It is used extensively in the treatment of a broad variety of conditions, including but not limited to ulcers, leprosy, piles, inflammations, stomach aches, intestinal worms, thirst, bruising, infertility, and skin issues. In vitro seed germination, induction of callus and numerous shoots, as well as tuberization from a variety of explants were all performed according to defined procedures.

When the MS medium was supplemented with 1.0 mg/l of BA and 0.5 mg/l of GA3, the in vitro seed germination rate was reported to be at its highest percentage (72.5). After the seedlings had germinated, they were transferred onto a substrate that produced corms for in vitro tuberization. When MS medium

was enriched with 1.0 mg/l BAP, 0.05 mg/l GA3, and 9.3 mg/l NAA, and 6% sucrose was added, the maximum percentage (90) of in vitro tuberization and rooting was achieved. When MS media was added with 2.0 mg/l of 2,4-D, we were able to successfully induce callus formation from in vivo leaf explants. There was an increase in the percentage of calli proliferation when SH medium was supplemented with 1.0 mg/l 2,4-D and 0.5 mg/l BA. When the SH medium was added with 2.0 mg/l of both 2,4-D and 2iP, the greatest response of callus induction from a nodal explant was seen. When MS media was supplemented with 5.0 mg/l BAP and 0.5 mg/l IAA, the greatest number of multiple shoots were produced as a result of the induction process.

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Cite this article as :

Ravi Yadav, Ashok Singh, "Effect of Growth Hormones on In Vitro Seed Germination of Gloriosa Superba", International Journal of Scientific Research in Science and Technology (IJSRST), Online ISSN : 2395-602X, Print ISSN : 2395-6011, Volume 10 Issue 2, pp. 50-65, March-April 2023.

Journal URL : https://ijsrst.com/IJSRST2310194