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Study on the Taxonomic Revision of The Genera Cycads (CYCADACEAE)

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ABSTRACT

Introduction : The vast majority of the species are not exclusive to island habitats but may also be found on continental landmasses.

Aim of the study : The main aim of the study is to Taxonomic Revision of The Genera Cycads (Cycadaceae)

Material and method : Gloriosa superba L., the experimental plant, was gathered from eleven different districts throughout the state of Tamil Nadu.

Conclusion : RAPD investigations conducted on the mother plant and the plant grown in vitro revealed no genetic differences.

I. INTRODUCTION

1.1 OVERVIEW

The only member of the family Cycadaceae to be found in India is the genus Cycas, which also serves as the only representative of the cycad subfamily. The approximately one hundred species that make up the genus Cycas may be found growing wild in a zone that stretches from northern Australia to southern China, all the way to Madagascar in the west and Tonga in the east (Hill, Stevenson and Osborne 2007). There are nine different species of this genus that may be found in India. These include Cycas annaikalensis Singh & Radha, Cycas circinalis L., Cycas beddomei Dyer, Cycas indica Lindstrom & Hill, Cycas nathorstiiSchust., Cycas pectinata Ham., Cycas sphaericaRoxb., Cycas swamyi Singh & Radha, and When compared to China and northern Australia, which both display large local radiations, the cycad flora of India is rather scarce in terms of both the richness of its species and the overall number of individuals. The survival of Indian cycads is mostly under jeopardy as a direct consequence of human activities. These slow-growing plants with extended reproductive cycles are on the verge of extinction as a result of human activities such as agriculture, the destruction of forest land, urbanisation, and excessive harvesting of plant parts for use in food and other sociocultural activities. Around the globe, many different conservation efforts for cycads have been explored, but only a select handful of them have proven successful. As a direct consequence of this, the populations of many species continue to dwindle in their native environments. The failure of cycad conservation programmes to raise awareness among local residents who live in close proximity to areas containing wild cycad populations has been a serious deficiency. Without the active engagement of villages and other local people, conservation efforts are far more likely to fail in the long term, as has been shown

by previous experiences. What is required is a paradigm for conservation that is capable of effectively educating and motivating individuals who live in close proximity to cycads to get active in the sustainable management of these plants. The study of relics like cycads, which have structures and developmental paths that are very ancient, gives us the opportunity to learn more about the early beginnings of seed plants and their modern-day analogues. The loss of these priceless antiques without first gaining a comprehensive understanding of their significance would be a blow to scientific research. The three families of cycads were included on the IUCN Red List of Threatened Plants in 1997 as being among the most endangered plant families in the whole world. The vast majority of the species are not exclusive to island habitats but may also be found on continental landmasses.

1.2 SIGNIFICANT TRADE CYCADS

Cycads are an ancient group of plants that are now represented by over 300 species across 11 genera and three families (Cycadaceae, Stangeriaceae, and Zamiaceae). They may be found in the tropical and subtropical regions of 58 range states around the world, including areas of North America, South America, Central America and the Caribbean, Asia, Africa, and Oceania. The loss of habitat and the commercialization of plants obtained in the wild are the two factors that pose the greatest risk to the survival of wild species. Fifty-two percent of the world's animal and plant species are in danger of becoming extinct.

According to the data collected through trade, the majority of the cycad market is comprised of specimens that have been artificially propagated. In point of fact, just 38,500 of the approximately 30 million plants that have been registered as exports during the course of the 24 years that are included by the dataset have been declared to be of wild origin. The great majority of these plants had their beginnings in Australia, which is one of the few places in the world that allows salvage harvesting. This method involves removing plants from areas where they are in danger of being destroyed as a result of land clearance. The residual trade in plants of wild origin from other range states includes plants that are transported for research reasons or for use in botanical gardens. This trade involves less than 1,500 individual plant species. There is little indication that the commercialization of wild-collected plants for scientific research or botanical gardens is being used for illegal plant trafficking. Since 1990, the commercial trading of just 458 plants of wild provenance has occurred for these uses. In addition, it is abundantly obvious that there is a general dearth of scientific knowledge on the dynamics of cycad populations as well as the influence of harvesting and management strategies on cycad populations. Because there is no other evidence available on which to establish harvest quotas, scientific authorities rely their conclusions that there is no threat to the population primarily on the number of the population. According to recent research on the dynamics of cycad populations, the harvesting procedures that are now in use may not always be acceptable. For instance, new study conducted in Australia reveals that sustainable harvesting programmes that are correctly managed may produce a better conservation result than salvage harvesting, which is presently done in a significant amount of situations.

1.3 TAXONOMIC NOTES ON ENCEPHALARTOS FEROX (CYCADALES: ZAMIACEAE), WITH THE DESCRIPTION OF A NEW SUBSPECIES FROM MOZAMBIQUE

The taxonomy of the African endemic cycad genus Encephalartos Lehmann has suffered from various taxonomic problems despite the fact that it has been stable over the last two decades, and there are presently 65 species and two subspecies that are recognised. An over-appreciation at the particular level, as maintained by Dyer (1965a) and Vorster

(2004), is attributable to their unwillingness to recognise infraspecific ranks, since solid phylogenies, demonstrating affinities, had not yet been established at the time of their research. Even molecular phylogenies are not completely determined, despite the fact that recent work has resolved species groups with sufficient support (ranging from two to seven taxa).

II. LITERATURE REVIEW

Khuraijam, JS & Singh, Rita (2020) Cycas pectinata is a taxonomically convoluted and complicated organism that is found natively in Southwest China, Northeast India, Bhutan, Bangladesh, Nepal, Southeast Asia (Myanmar, Thailand, and Vietnam), and Southeast Asia (Thailand and Vietnam). A comprehensive morphometric study of the taxonomically unique vegetative and reproductive structures of Cycas populations in India found variations across populations that support their classification as two separate species: Cycas pectinata is only found in the western portion of the Indo-Burma Range (IBR), while the Southeast Asian taxon known as Cycas divyadarshanii is being described and illustrated here as a new species. Both of these regions are located in India. The new species of Cycas may be recognised from Cycas pectinata by its long and thin microsporophylls as well as the absence of a thickened protrusion on the adaxial surface of the leaf blade, which is noticeable in C. pectinata. Both species have distinct anatomical characteristics, and their pollen has a distinct structure. The connection of these species with distinct hypothesised beetle pollinators also lends credence to the classification of them as separate taxa.

Lopez-Gallego, Cristina &Calonje, Michael & Griffith, M. &Khuraijam, JS. (2020) The 10th Worldwide Conference on Cycad Biology, which was held from the 16th to the 21st of August in 2020, was very well-attended and had a genuinely international scope, with over 150 delegates coming from 17 different countries. During the course of this conference that lasted for a total of six days, four of those days were dedicated to the academic programme of presentations. These presentations consisted of fifty-five oral presentations, seven plenary speeches, and a poster session that had nine poster presentations. The discussions, which were broken up into 10 distinct sessions, covered a wide variety of issues, including horticulture, conservation, ethnobotany, ecology, genetics, and systematics, to name just a few of the many sub-fields covered. It was a privilege for me to take part in this initiative as a member of the Cycad 2015 Organizing committee. The academic agenda had a variety of presentations that were not only educational but also garnered positive feedback from attendance. CYCADS is the official newsletter of the IUCN/SSC Cycad Specialist Group, and it is our pleasure to share in this special edition of CYCADS the results of this spectacular meeting that took place earlier this year. In this special edition of CYCADS, the official newsletter of the IUCN/SSC Cycad Specialist Group, we are pleased to share the results of the spectacular meeting that took place earlier this year. We would like to extend our gratitude to J.S. Khuraijam for all of the hard work he put into bringing together this special edition of CYCADS.

Tang, William & Xu, Guang&Marler (2020) It is thought that three different kinds of beetles that live in the cones of cycads (Cycadales) in the northern hemisphere are responsible for the pollination of these plants. The primitive weevil subtribe Allocorynina of the Coleoptera family Belidae is only found in the cycad genera DioonLindl. and Zamia L., both of which are native to the New World. There is a family of weevils known as the Curculionidae that is unique to Cycas L. and seems to be a relatively new coloniser of cycads in the northern hemisphere. There are members of the beetle subfamily Pharaxonothinae (Erotylidae) living in each and every cycad genus in both Asia and the New World. There is a comparison

made between the patterns of continental drift and the phylogenies of cycads and the phylogenetic trees of these beetles, which are based on DNA analysis and backed by morphological investigations. It is believed that these groups of beetles originated in Laurasia, and that at least one of these groups dispersed to high latitudes during times when conditions throughout the world were rather warm.

Ahmed Ismail, Princedream& Hassan, Hossam &Moawad (2020) The crude extracts of the leaves of three different species of Cycas plants, Cycas armstrongiiMiq., Cycas circinalis L., and Cycas revoluta Thunb., all exhibited the presence of a variety of secondary metabolites when they were put through a metabolic profiling process. Because this is the first time that these known components have been reported for this species, a comprehensive phytochemical investigation of C. armstrongii fractions led to the separation of 15 separate classes of known components. These components were chemically characterised as follows: naringenin; dihydroamentoflavone; 2,3-dihydrohinokiflavone; amentoflavone; 2,3-dihydrobilobetin; isoginkgetin; prunin; naringin; vanillic acid; p-coumaric acid; -sitosterol; stigmasterol; -sitosterol In addition to this, the radioprotective ability of the three different species of Cycas plants was also studied. The ionising radiation experiment was carried out by subjecting the complete bodies of rats to an 8 Gy dose. By use of an intragastric tube, the extracts of all three species of Cycas were given to the animals at a dosage of two hundred milligrammes per kilogramme. According to the findings, extracts from Cycas spp. considerably reduced the damage caused by radiation to the brain and pancreas, and they also showed protection against the oxidative stress that was caused by radiation. Histopathological examination likewise provided supporting evidence for the findings.

Aluri, Jacob Solomon Raju &Kunuku (2019) In the places where they are distributed, Cycas sphaerica and C. beddomei are historically used as essential sources of food and medicinal. They are exploited without discrimination in a way that is the farthest thing from scientific, and as a consequence, their surviving numbers have been reduced. The traditional applications of these two species are discussed in this work with the intention of persuading the researchers to investigate scientific approaches to using them in an appropriate manner.

III. MATERIALS AND METHODS

3.1 RANDOM AMPLIFIED POLYMORPHIC DNA (RAPD)

3.1.1 Plant material

Gloriosa superba L., the experimental plant, was gathered from eleven different districts throughout the state of Tamil Nadu.

Places of sample collection in different locations of Tamil Nadu

Sl. No.	District	Placeofcollection	Code
1.	Dharamapuri	Keelasangapadi	GS1
2.	Erode	Malamedu	GS2
3.	Tiruchirappalli	Pachamalai	GS3
4.	Nilgiris	Gudalur	GS4
5.	Coimbatore	Marudhamalai	GS5

6.	Nagapattinam	Vedaranyam	GS6
7.	Ariyalur	Jayakondam	GS7
8.	Namakkal	Chinnavelur	GS8
9.	Salem	Edapadi	GS9
10.	Dindugal	Oddanchatram	GS10
11.	Pudukkottai	Narthamalai	GS11

In a similar manner, both the in vitro plant and the in vivo plant were used in order to compare and contrast the genetic fidelity of the in vitro and in vivo plants. GTC is the code that has been allotted for the in vitro plant, while GWD is the code for the in vivo plant.

3.1.2 DNA isolation

The DNA was isolated from the leaves of the experimental plant Gloriosa superba L that had been frozen for thirty days. In order to evaluate the performance of each extraction technique, we employed three distinct approaches: the Modified CTAB Method, the CTAB Method with Some Minor Modifications, and the Phenol-Chloroform Method with Some Minor Modifications.

3.1.3 Quantification of DNA

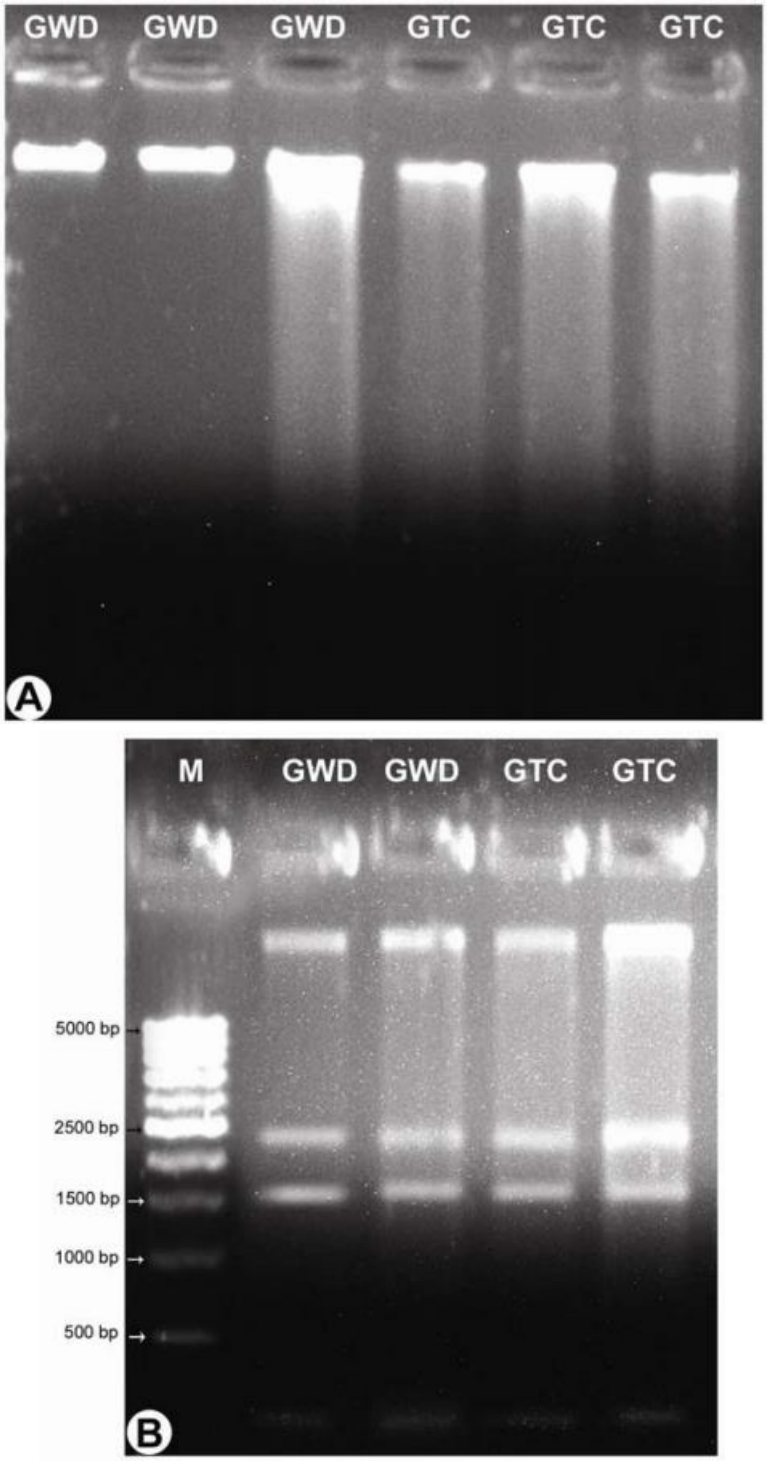
Quantification of the DNA was achieved by the use of a nano-drop spectrophotometer (Thermofisher Scientific, USA) in conjunction with the ND-1000 Ver.3.6, 2008 software. The number of DNA samples was evaluated by calculating the ratio of absorbance at A260/280. The results showed that the ratio ranged from 1.6 to 2.0, which indicates that the DNA samples were of a quality that was pretty satisfactory and were electrophorized on an agarose gel containing 0.8%.

IV. RESULTS

4.1 POLYMORPHIC STUDIES USING RANDOM AMPLIFIED POLYMORPHIC DNA MARKERS

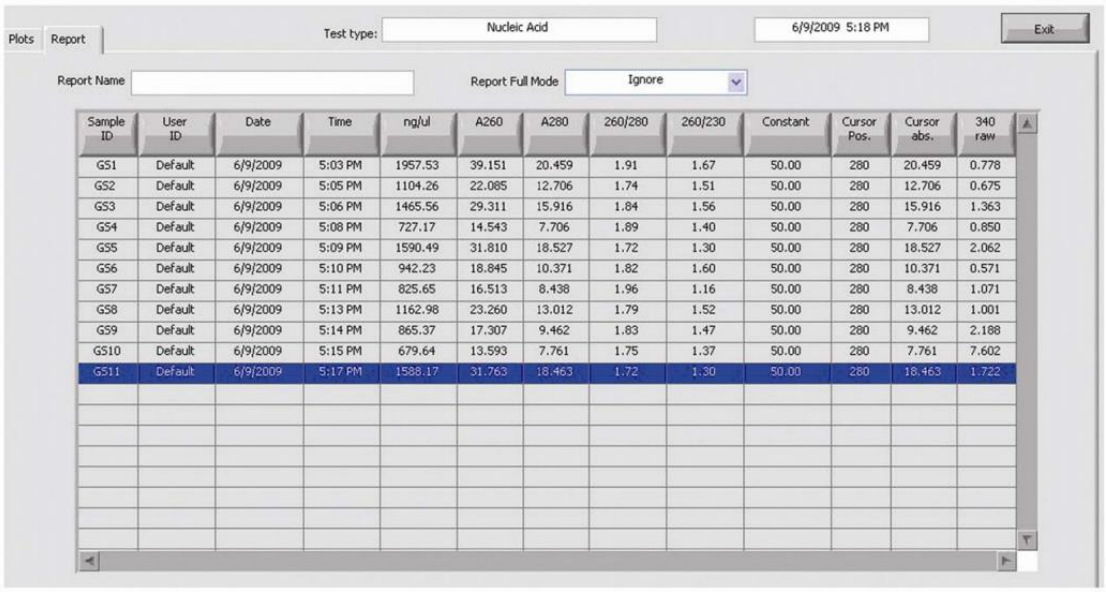
4.1.1 DNA extraction

Plants used in medicine often have a greater number of secondary metabolites. Because of these secondary metabolites, the process of isolating DNA might be difficult, and the DNA that is obtained in this manner is not appropriate for PCR amplification. In this work, the genomic DNA was extracted from the frozen leaves of G.superba that were gathered from various locations in Tamil Nadu by adhering to the techniques given by Murray and Thompson (1980), Doyle and Doyle (1987), and Bell et al. (1981). Both the Murray and Thompson approach and the phenol-chloroform method produced a meagre quantity of DNA, which resulted in inadequate PCR amplification. The Cetyl Trimethyl Amino Bromide (CTAB) technique devised by Doyle and Doyle (1987) proved appropriate for the extraction of DNA with high purity and high quantity for both in vivo grown plants and in vitro.



A. Electrophoretic analysis of genomic DNA isolated from leaves of *in vivo* and *in vitro* plants.
B. RAPD electrophoretic profile of *in vivo* and *in vitro* plants.
GWD : *In vivo* leaf ; GTC : *In vitro* leaf M : DNA Marker

Plate 1



Sample ID	User ID	Date	Time	ng/ul	A260	A280	260/280	260/230	Constant	Cursor Pos.	Cursor abs.	340 raw
GS1	Default	6/9/2009	5:03 PM	1957.53	39.151	20.459	1.91	1.67	50.00	280	20.459	0.778
GS2	Default	6/9/2009	5:05 PM	1104.26	22.085	12.706	1.74	1.51	50.00	280	12.706	0.675
GS3	Default	6/9/2009	5:06 PM	1465.56	29.311	15.916	1.84	1.56	50.00	280	15.916	1.363
GS4	Default	6/9/2009	5:08 PM	727.17	14.543	7.706	1.89	1.40	50.00	280	7.706	0.850
GS5	Default	6/9/2009	5:09 PM	1590.49	31.810	18.527	1.72	1.30	50.00	280	18.527	2.062
GS6	Default	6/9/2009	5:10 PM	942.23	18.845	10.371	1.82	1.60	50.00	280	10.371	0.571
GS7	Default	6/9/2009	5:11 PM	825.65	16.513	8.438	1.96	1.16	50.00	280	8.438	1.071
GS8	Default	6/9/2009	5:13 PM	1162.98	23.260	13.012	1.79	1.52	50.00	280	13.012	1.001
GS9	Default	6/9/2009	5:14 PM	865.37	17.307	9.462	1.83	1.47	50.00	280	9.462	2.188
GS10	Default	6/9/2009	5:15 PM	679.64	13.593	7.761	1.75	1.37	50.00	280	7.761	7.602
GS11	Default	6/9/2009	5:17 PM	1588.17	31.763	18.463	1.72	1.30	50.00	280	18.463	1.722

Plate 2 quantification of DNA isolated from 11 accessories of Gloriosa Superba L. by Nanodrop spectrophotometric method using ND-1000 version 3.5.2

When employing the CTAB approach, the amount of DNA that could be extracted from in vivo plant leaf samples (GWD) and in vitro plant leaf samples (GTC) varied from 331.16 ng/µl to 581.91 ng/l. The proportion of A260 to 280 was anywhere between 1.64 and 1.93. The DNA yield of the plant samples that were obtained from 11 different locations in Tamil Nadu showed a variance that varied from 679.64 ng/µl to 1957.53 ng/l. It may be deduced from the fact that the absorbance values (A260/280) varied from 1.72 to 1.91 that the DNA samples were of an acceptable grade.

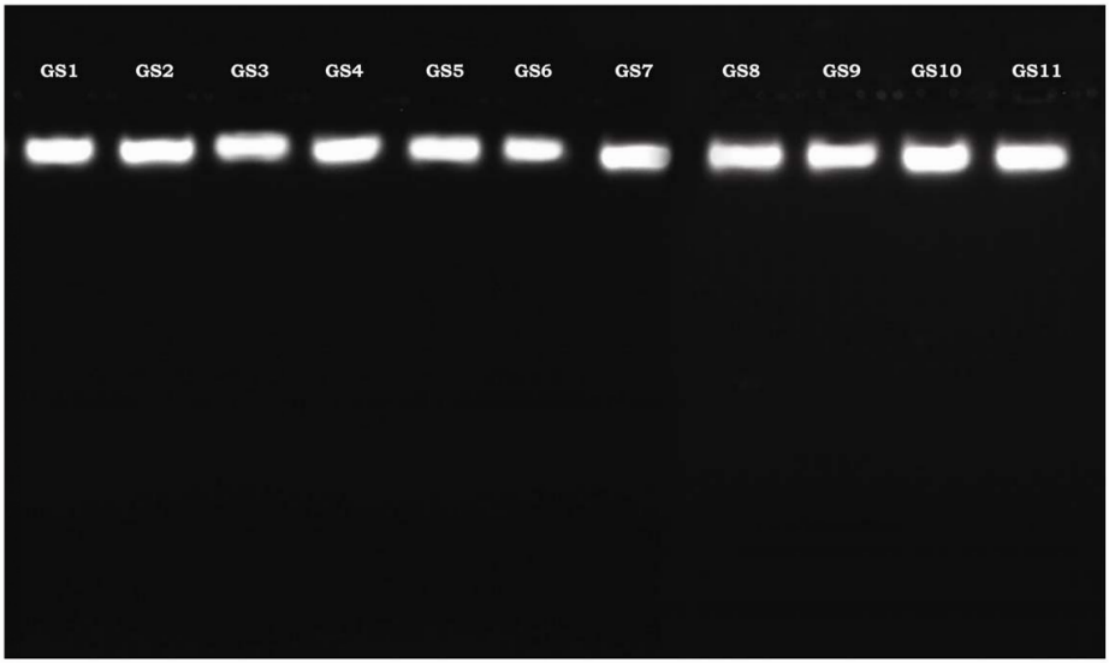


Plate 3 electrophoretic analysis of genomic DNA of 11 accessions of Gloriosa Supreba L.

The CTAB approach, with just a few tweaks here and there, produced satisfactory results for G.superba. It's possible that this is because of the changes that were made to the protocol. The concentrations of certain components were the primary factor that differentiated the CTAB approach from those of other methods. It produced a quantity of DNA suitable for use in PCR amplification. The CTAB method, with some minor modifications, produced the best DNA quality from the leaves of G.superba, despite the fact that the protocol

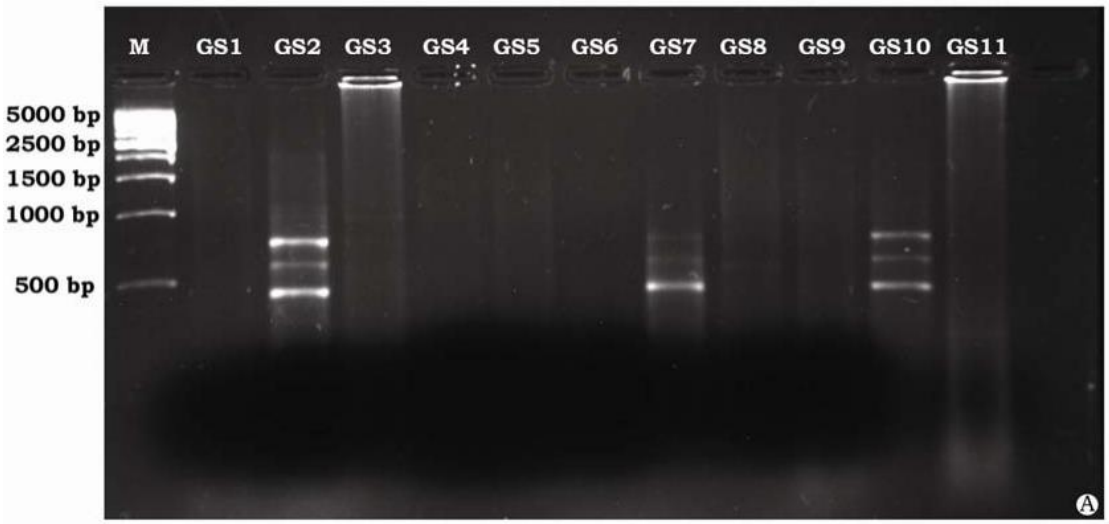
was labor-intensive because it required more quantities of solutions and also time-consuming. This conclusion was reached based on a comparison of three different protocols for the isolation of DNA. It was found that the procedure produced sufficiently clean DNA for use in PCR amplification, which was a positive result.

Seemanti Ghosh et al. (2008) conducted polymorphism research on Gloriosa superba using the CTAB technique. For those investigations, they extracted DNA from fresh and immature leaf tissues from 15 plants from each population of Gloriosa superba. Our findings are comparable to those of Seemanti Ghosh et al. (2008). Kumar et al. (2007) employed the young and healthy leaves of Senna sulfurea DC. ex Collad. and Senna surattensisBurm. The CTAB technique, with some minor adjustments, was utilised to isolate DNA from a total of 38 different accessions. The findings show that RAPD markers are capable of distinguishing between S.surattensis and S.sulfurea in an accurate and consistent manner. Ana Maria Waldschmidt and colleagues (1997) tested three different procedures for DNA extraction in Melipona quadrafasciata. These procedures differed in the concentrations of specific substances in the extraction buffer. The researchers discovered that adequate DNA can be extracted from a method with minor modifications, which highlights the need for modification in an existing method.

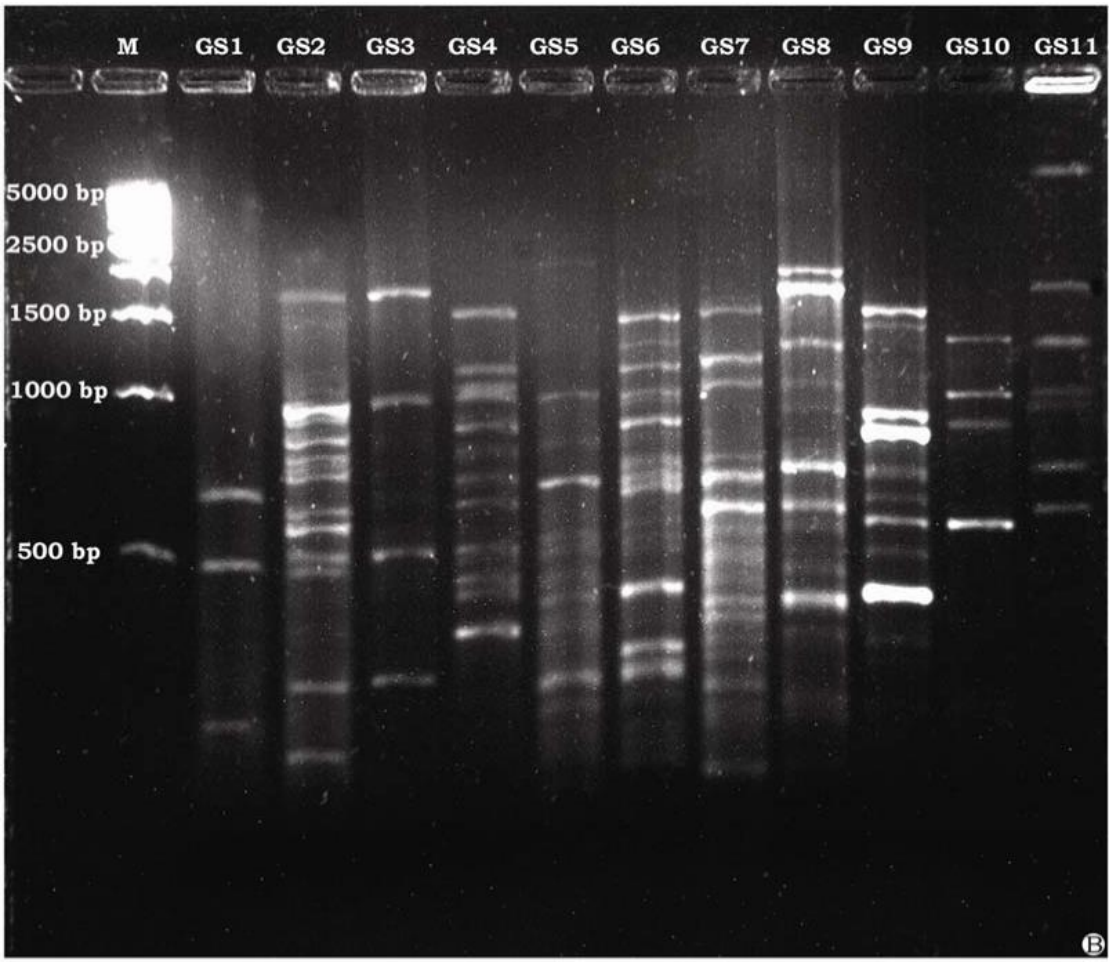
For the purpose of RAPD and restriction digestion, Abdin et al. (2007) established a procedure for the separation of genomic DNA from dry and fresh roots of medicinal plants. When compared to DNA extracted using the techniques of Dellaporta et al., (1983) and Doyle and Doyle, the approach used a modified version of the CTAB protocol, which resulted in the production of a significant amount of very pure DNA (1990). There were significant quantitative and qualitative variations found in the DNA samples that were acquired using the three distinct approaches. According to the findings that we obtained, the CTAB approach (Doyle and Doyle, 1987) with some slight adjustments was a better suitable choice for the extraction of DNA from Gloriosa superba L.

4.1.2 RAPD analysis

In this study, 11 accessions of G.superba that were obtained from various regions of Tamil Nadu were used to test a total of 12 operon- and random-generated single-stranded primers that were each 10 bases long. Only three of the twelve primers that were employed in the amplification produced distinct bands: OPA 1, OPA 8, and OPA 9. The existence of bands allowed for the identification of all of the accessions. Primers OPA 8 and OPA 9 amplified DNA in every one of the 11 accessions they were tested on. The OPA8 test found 128 polymorphic bands, the OPA9 test revealed 66, and the OPA1 test revealed 37 polymorphic bands. The remaining nine primers did not show any bands that could be scored. On average, the OPA1 provided 3.08 bands, whereas the OPA8 provided 10.66 bands and the OPA9 provided 5.5 bands. There was a total of 340 RAPD products that were scored (an average of 28.33 bands per primer), with the number of bands per primer ranging anywhere from 2 (OPA7) to 128 (OPA8); 206 of these products were polymorphic. The observed amount of polymorphism averaged out to be sixty percent. The OPA8 and OPA9 primers produced the greatest level of polymorphism, one hundred percent, compared to any other primers used.

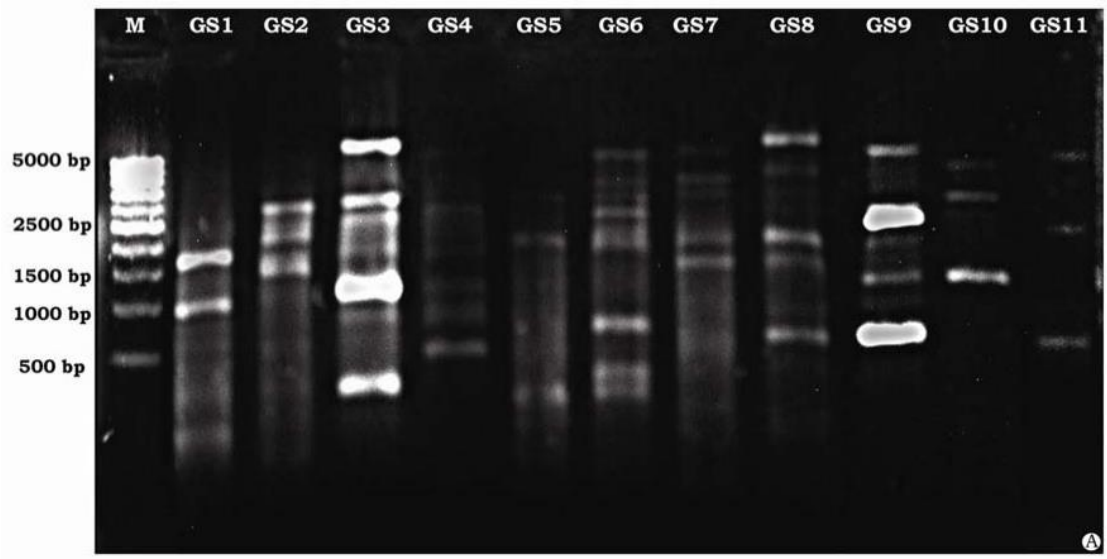


RAPD profile of 11 accessions of Gloriosa Superba L. after amplification with OPA1 primer

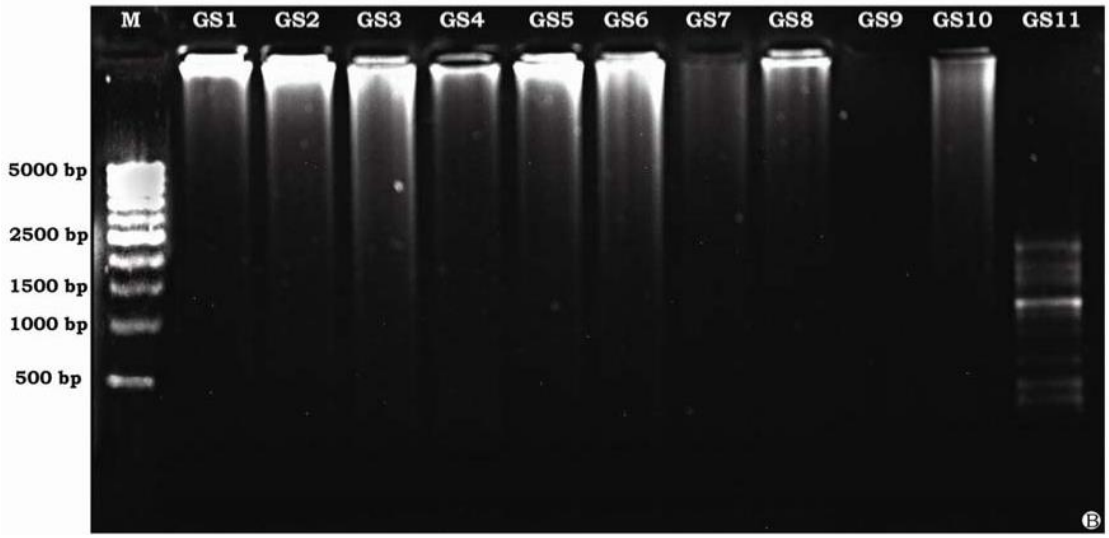


RAPD profile of 11 accessions of Gloriosa Superba L. after amplification with OPA8 primer

Plate 3



RAPD profile of 11 accessions of Gloriosa Superba L. after amplification with OPA9 primer



RAPD profile of 11 accessions of Gloriosa Superba L. after amplification with OPA10 primer

Plate 4

One population-specific RAPD marker was all that was needed to distinguish each of the eleven accessions uniquely. OPA1 produced a fragment of 2245 base pairs only in GS2; OPA4 produced a fragment of 971 base pairs only in GS 10; OPA8 produced a fragment of 106 base pairs only in GS5; and Primer OPA10 produced a fragment of 358 base pairs only in GS11. Along with the genomic DNA, the OPA 10 primer (GTGCAACGTG) was used to amplify GS11, and this resulted in the production of a single band. This band seems to be of utmost significance in the process of diagnosing GS11 accession. This might be used to delegate an identity, and it could also be utilised in the process of developing a "molecular identification" for this entry. It is clear that this species has a sizeable potential for the discovery of singular RAPD markers that are more genetically distant from one another. Nevertheless, there is a possibility that some of the one-of-a-kind features are shared by other accessions that are part of a larger group of accessions. In the research, reproducible findings were achieved by using a particular mix of primers and templates for DNA. It was ensured that none of the parameters were changed by taking the utmost caution. RAPD analyses using the OPA8 primer were performed on the DNA that was isolated from plant leaf samples both in vitro and in vivo. The findings showed

that the band intensity histogram of each gel verified the monomorphic character of the gels and excluded the possibility of any genetic differences.

V. Conclusions

RAPD investigations conducted on the mother plant and the plant grown in vitro revealed no genetic differences. These findings demonstrated that in vitro plants do not undergo any changes to their genetic make-up and that they are virtually identical to plants grown in the wild. This suggests that the in vitro techniques can be extensively used for the conservation and large-scale propagation of medicinal plants because there is an increasing need to produce a large number of plants of improved quality, which holds, good promise in this field. In addition, there is a growing demand for the propagation of medicinal plants because there is a growing demand for the use of medicinal plants. In the current study, an antibacterial evaluation of methanolic extracts of different parts of G.superba was performed. These parts included the in vivo tuber, seed, leaf, and pod, as well as the in vitro plant. The results showed that these extracts possessed measurable inhibitory action against both gram-positive and gram-negative bacteria. When compared to the other parts of the plant, the seeds and tubers of this plant offer a much greater inhibitory activity against several microorganisms. As a consequence of this, the seeds and tubers of this plant possess potent antibacterial qualities.

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