JMBFS
Journal of Microbiology, Biotechnology and Food Sciences
International peer-reviewed scientific online journal
Published by Faculty of Biotechnology and Food Sciences

DIVERSIFICATION IN LASIA SPINOSA (LOUR.) THWAITES TWO MORPHOTYPES EXISTING WITHIN ASSAM BASED ON MORPHO METRIC AND ISSR MARKER ANALYSIS

*Puspita Hore**[1], Bhaben Tanti*[2]

Address(es): Puspita Hore
[1] Gauhati University, Research Scholar, Department of Botany, Jalukbari, Guwahati-781014, Assam, Ph no- 09706786366.
[2] Gauhati University, Faculty, Department of Botany, Jalukbari, Guwahati-781014, Assam.

*Corresponding author: puspitahore22@gmail.com

https://doi.org/10.55251/jmbfs.9844

ARTICLE INFO

Received 31. 1. 2023
Revised 13. 3. 2023
Accepted 21. 3. 2023
Published 1. 6. 2023

Regular article

OPEN ACCESS

ABSTRACT

Lasia spinosa or Unicorn plant is an important tropical spinous rhizomatous herb. This plant is one of the native species of Assam used as vegetable and in Ayurvedic medicines. There are two distinct morphological types of *Lasia spinosa*: Morphotype-I i.e.having dissected laminal and hastate form and Morphotype- II i.e. dissected laminal form. The current study revealed that the physical and anatomical parameters assent with the genetic diversity revealed through molecular data analysis. The vein type, however, was discovered to be jumbled craspedodromous and semi-craspedodromous of *Lasia spinosa* morphotypes (I, II), respectively. Least variance was discovered in the morphological forms observing the leaf vein architecture. There are small changes in the petioles' and roots' internal architecture between the two morphotypes. To do a phylogenetic analysis between the two morphotypes of *Lasia spinosa*, the ISSR-PCR product revealed approximately 56% of polymorphisms (i.e32 out of 57 bands which are distinct to each other).

Keywords: unicorn, Ayurvedic, dissected laminal, *Lasia spinosa*, parameters, phylogenetic, architecture

INTRODUCTION

Morphological and anatomical features are routinely used for identification of different plant species since ancient days. Nature provides a complete storehouse for remedies of almost all ailment of mankind. *Lasia spinosa* being seasonally dormant, has immense medicinal values. *Lasia spinosa* is a spinous perennial herb **(Hiong *et al.*, 2009)**, known to be monotypic genus found mainly in Southern Asia. It grows frequently along the sides of rivers, in ditches, swamps, and other damp areas of tropical and subtropical forests. Many southern Asian populations use its shoots as a traditional cuisine. Rhizomes are used for treating various diseases such as in snake and insect bite, lung inflammation **(Deb *et al.*, 2010)**. *Lasia spinosa* from *araceae* family and order Alismatales has long creeping and stoloniferous stem. This herb is about 1-2 cm tall with erect stem of diameter 2.5 cm. The petioles are laxly prickly with height ranges from 32-125 cm andpulvinusaculeate,16-37 mm. Dissected laminal to hastate leaf blades range in size from 34 to 64 by 21 to 61 cm. In contrast to the posterior lobes, which are oriented downward and subspreading, the anterior lobe is complete or pedate to close the midrib. Leaves possesses slightly curved prickles abaxially, lateral primary veins are strong, secondary are thinner, higher order veins are numerous. Peduncle is 47 cm. The colour of spathe varies from dull orange to black-red outside and interior side varies from dull yellow to rarely dull crimson with width ranges from 3-10 cm wide and length 18-35 cm with caudate part, spadix obscure and cylindric, 3-5cm. Flowering time is mainly during December to May. Authors recommended for uses in treating colic, rheumatism, stomachache and intestinal disease (**Kongkachuichai, 2015)**. Antihelminthic, anticestodal **(Tangpu *et al.*, 2004)**, antinematodal, and antioxidant properties are all known to exist in the plant (**Temjenmongla and Yadav, 2003, 2005; Yadav and Temjenmongla, 2006; Shefana and Ekanayake, 2009**). Based on the shape of the leaves, Bangladesh had three different varieties of *Lasia spinosa* (**Sultana *et al.*, 2006**). These come in three different forms: sagittate, dissected laminal, and a combined form of the two **(Alam *et al.*2012)**. Taxonomically recognized these variations as "ecophenic variations" **(Hore and Tanti, 2014).** However, Assam has two distinct species of *Lasia spinosa*, having two different leaf type in Morphotype I and the partitioned laminal type in Morphotype- II **(Hore and Tanti, 2018)**.
For the vast majority part, a species' leaf venation pattern is genetically predetermined. Leaf venation pattern stands as a taxonomic tool to sort out the venation pattern in primary, secondary, tertiary degree etc. Root anatomy presents difficulties since there is little information of it and because the features of the stem and the root may differ. Root anatomy is very helpful tool inorder to study the anatomical features of non-woody spieces **(Basconsuelo *et al.*, 2011)**. In order to determine evolutionary history, **Zietkiewicz *et al.*, 1994** and many researchers are increasingly adopting ISSR or inter-simple sequence repeat - PCR with dinucleotides, tetranucleotides, or pentanucleotides repeat primers. Since microsatellites evolve at a somewhat faster rate than other forms of DNA, polymorphism is higher. In terms of output data in both quantity and quality, ISSRs were superior to RFLP and RAPD for the examination of diversity in the various genus plants (**Salimath** *et al*., 1995.) In several species of *Lagenandra* (*araceae*), an aquatic plant collected from various geographic locations in Kerala State, India, **Prakashkumar** *et al*., 2015 discovered genetic differences. ISSR markers were used in a molecular study. There were 66 scoreable polymorphic markers produced from the 18 primers that were evaluated.
In the current examination, an attempt has been made to compare the morphological, anatomical, and ISSR marker data in hopes of evaluating the differences between two morphological variants of *Lasia spinosa*. Future research may use this discovery to distinguish *L.spinosa* from species that are closely related and other variants of this species. Moreover, information about the pattern and type of variances between ecological forms can be obtained.

MATERIAL AND METHODS

Material used: *Lasia spinosa* (L.), scientifically known.
Community Name: Assamese – *'Seng-mora',* Bengali -'*Kantakochu' and many more.*
Experimental plant systematic position-
Plantae (Kingdom); Alismatales (Order); Araceae (Family); Lasiodeae (Sub-family); *Lasia* (Genus) and *L. spinosa* (the experimental plant).

L. spinosa leaf architecture study

Aqueous solution of NaOH (5%) was prepared and leaf pieces were cut and dipped in the solution for overnight. The next step was to transfer the following to hydrogen peroxide in 1:1 ratio and a chloral hydrate saturation aqueous suspension which were mixed together since 30 minutes–1 hour. Then, leaf fragments were repeatedly washed in water. 25 ml of pure ethanol and 75 ml of diluted chloral hydrate solution were added in descending order which were used to wash the leaf pieces for 20 – 30 min till green colour disappeared. The materials were then stained with safranin and observed under microscope **(Vasco *et al.*, 2014)**.

Anatomical features of petiole and root

Dissection of petiole and root

Two *Lasia spinosa* morphotypes were gathered at various phases, from juvenile to reproductive, and the petiole and roots were excised. In order to cut the petiole and root into thin transverse sections in the radial plane of a cylindrical part, sharp razor blades were used. For additional processing, fine slices and water were retained in watch glasses (**Harborne, 1998.)**

Steps of Staining

First of all, stained the selected fine pieces were to distinguish different tissue with two stains. Safranin stains the cell wall and fade green imprints soft tissues. The slices were then allowed to soak for 3-5 minutes in fifty (50) % alcohol. Further dipped in safranin for 3 min. Repeated washing in 50% alcohol was done. Each sections were followed by placing for five minutes in differential (70%, 80% and 90%) alcohol in step by step manner. Sections were then briefly submerged in light green before being thoroughly cleaned with pure alcohol for one minute. For some seconds clove oil was used to immersed the sections. Following mounting in Canada balsam or DPX and covering with cover glass, specimens were viewed under a microscope (10 and 45times magnification) and documented on camera using a Nikon Coolpix. Various chemical reagents were used to notice the behaviour of powders according to the standard procedures **(Harborne, 1998; Atif *et al.*,2015)**.

ISSR marker based phylogenetic analysis

Using ISSR-PCR based marker analysis, the subsequent steps were recommended to ascertain the genetic link between two morphological characteristics of *Lasia spinosa*.

DNA extraction

In this investigation, *Lasia spinosa*'s two morphotypes' tender leaf samples were employed. Due to the lower concentration of secondary metabolites in young, delicate tree leaves compared to adult leaves, delicate leaves were ideal source in DNA isolation. Mortar and pestle was used to pulverized leaf tissues weighing 1 g using liquid nitrogen into a thin powder. Impurities were then removed, and the DNA was drawn out with the help of modified (CTAB) cetyltrimethyl ammonium bromide or Doyle methods (**Doyle and Doyle, 1987**). Phenolic compound interruption was prevented upon addition of the component- Polyvinyl pyrolidone/ PVP and –mercaptoethanol/ -ME, both in 1% concentration to the extraction solution buffer **(Sambrook *et al.*, 1989; Tanti *et al.*, 2012)**.. After that, application of RNase done to purify the extracted DNA and eliminate the sample's RNA **(Rahman *et al.*, 2012; Barman *et al.*, 2014; Chowdhury *et al.*, 2014.)**

Measurement of DNA

Comparison was done using a DNA standard to visually quantify genomic DNA under UV light. After that, 2µ L of DNA from each sample was electrophoresed for 30 minutes on a Agarose gel (one/1 % in concentration) in buffer component of 0.5 mg/ml of Ethidium bromide/ EtBr (1X TAE)

ISSR analysis using PCR amplification

PCR amplification was used to obtain eight/8 ISSR primers panel for ISSR analysis from Operon Technologies Inc., CA (USA). Table 1 contains primers list pre-owned in the investigation that follows.

PCR reaction mix

The Thermal cycler/PCR amplifier from Bio-Rad (USA) was used in PCR amplification of each primer listed in Table 1 in a reaction volume (25 mL with DNA template of 50 ng Assay Buffer (1X) of 2.5µl, dNTPs (0.2 mM), *Taq* DNA polymerase (1 U) and $MgCl_2$ (1.5 mM) and primers (5pmol) (**Williams *et al.*, 1990**). Thermo- Technologies provided the PCR components.

Table 1 Sequences of ISSR primers that were used.

No.	ISSR Primers	Sequences in (5′-3′) direction of Primer
I	UBC (812)	GA GA GA GA GA GA GA GAA (5′-3′)
II	UBC (814)	CT CT CT CT CT CT CT CT CTA (5′-3′)
III	UBC (818)	CA CA CA CA CA CA CA CAG (5′-3′)
IV	UBC (836)	AG AG AG AG AG AG AG AG XA (5′-3′)
V	UBC (840)	GA GA GA GA GA GA GA GA XT (5′-3′)
VI	UBC (842)	GA GA GA GA GA GA GA GA XG (5′-3′)
VII	UBC (843)	CT CT CT CT CT CT CT CT CT PA (5′-3′)
VIII	UBC (848)	CA CA CA CA CA CA CA CA PG (5′-3′)

Legend: *A- adenine, G- guanine, T- thymine, C-cytosine, (here* X = C, T; P= A/G)

Condition for PCR reactions

The standard PCR amplification conditions were maintained: a denaturation step for 5 minutes (at 94°C), acocompanied by running cycles of thirty-five/35 considering 30 seconds at 94°C, 45 seconds at 42°C and 45 seconds at 72°C, and a last expansion step for 10 minutes (at 72°C). Resultant by-products of amplification were isolated in agarose gel (1.5% in concentration).

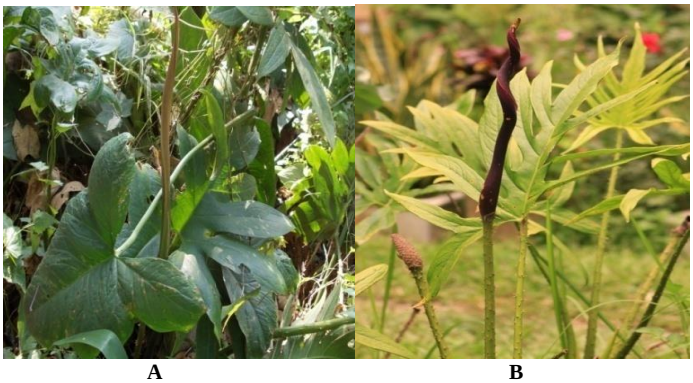
Genetical diversity and data evaluation

Bands of ISSR carefully assessed before binary data conversion to as either "1" (if bands are present) or "0." (if bands are absent). In this research, only distinctive and translucent bands were displaying similarity taken into consideration. Numerous band characteristics, including values for each marker, polymorphism percentages, and polymorphic information contents (PIC), were evaluated and measured (**Roldan-Ruiz *et al.*, 2000**).

RESULTS AND DISCUSSION

Description of the experimental plant

Material plant: *Lasia spinosa* (L.) Thwaites; Native Name of the Studied plant: Assamese- *'Seng-mora'* , Bengali- '*Kantakochu'* , Telagu -'*Malasari'* .
Lasia spinosa, a tropical herb with thick creeping rhizome has tremendous utility in folklore medicine, found usually by the side of marshy areas, muddy streams and swampy ground **(Hossain *et al.*, 2021)**. Erect or ascending evergreen marshy herb length ranges from 1.5-2.0 m tall. Stoloniferous creeping rhizome with stem in 4 – 6.5 centimeters diameter and spines that are 1 cm long covered thoroughly in stout internodes.
The leaf, which can occasionally be sagittate-hastate or pinnatifid, is held by a long petiole measuring 110 to 190 centimeters. Lobes of the leaves are acuminate with 9 to 12 lobes per lamina and length of leaves varies from 37 – 40 cm. Peduncle are green with stout prickles,1 - 1.5 cm in dia and 30 - 40 cm in length. The colour of spathe varies from dull orange to black - red outerside and towards interior colour is dull yellow to rarely dull crimson. Sapthe is usually spirally twisted in the upper side over the spadix. Length of spathe is 26 – 32 centimeter and 4.5 – 6.5 centimeter wide with cylindrical spadix of length 4 – 5 centimeter. Bisexual flower and fruits are clustered of berries.
Assam is home to two distinct morphological types of *Lasia spinosa*, known as the mixed form having two different forms of leaves i.e. Morphotype I and partitioned laminal type i.e. Morphotype II (Fig. 1. A-B).



A **B**

Figure 1 Material plants; A**:** Mixed type having hastate- sagittate and dissected laminal leaf or Morphotype-I and B**:** Dissected laminal leaf or Morphotype-II of *L. spinosa.*

Following table2 was documented and morphological variations were recorded in both the *Lasia spinosa* morphotypes.

Table 2 Distinguishing morphological variation of *L. spinosa* morphotypes

Observed features	***L. spinosa* (Mixed type or Morphotype I)**	***L. spinosa* (Dissected laminal type or Morphotype II)**
Leaf nature	Hastate- sagittate leaf and dissected-lamina or pinnatifid, so mixed type.	One type i.e pinnatifid or dissected laminal
Size of leaf lobes	6 - 9 cm	2 - 5 cm
Length of petioles	80 - 110 cm	65 - 85 cm
Colour of spathes	Fade orange	Purplish
Size of peduncles	40 - 60cm in length, 0.8 - 1.7cm diameter	30 - 40cm in length, 0.8 - 1.4cm diameter
Colour variation in flowers	Crimson	Purple

Legend: cm- centimeter

***L. spinosa* morphotypes leaf architecture**

Leaf architecture of both the morphotypes depicts certain differences in certain venation pattern. Overall leaf blade type is hastate, apex is acuminate, sagittate sometimes hastate base. The leaf texture is coriaceous, margins are lobed and lamina is convex. Venation pattern of morphotype–I is mixed craspedodromous and morphotype-II is semi-craspedodromous in *Lasia spinosa*. Randomly reticulate degree of venation. Venation pattern ranges from primary vein radiation is basal (*i.e.,* at the leaf base) up to 5° in both the forms. Primary and secondary veins are clothed with spines and more or less venation patterns are same (Fig. 2). The differences were tabulated in the following Table 3.

Table 3 Noticeable features of *L. spinosa* morphotypes according to vein orders.

No. Of Vein orders	**Mixed type or Morphotype I**		**Dissected laminal or Morphotype II**
	Hastate –Saggittate type	**Dissected laminal or Pinnatifid**	**Dissected laminal or Pinnatifid**
1◦- veins	Veins were branched, straight and massive.	As like hastate-saggitate	As like hastate-saggitate
2◦- veins	The majority of the 2◦ vein ends at the edge, the tips are only abruptly bent, joins superadjacent 2◦ vein at an obtuse angle and actue (w.r.t. 1◦ vein).	As like hastate- saggitate, veins are actue (w.r.t. 1◦ vein), abruptly bent, superadjacent 2◦vein are joining at an obtuse angle.	As like dissected laminal in Morphotype - I
3◦- veins	Veins are mostly alternative arrangement, forked, percurrent, oblique and connected with 1◦ vein.	As like hastate type	As like Morphotype I
4◦- veins	Veins are random fringed orientation and thin.	As like hastate type	As like Morphotype I
5◦- veins	Curved and simple.	As like hastate type	As like Morphotype I

Legend: 1◦-primary, 2◦-secondary, 3◦-third, 4◦-fourth, 5◦-fifth, w.r.t- with respect to

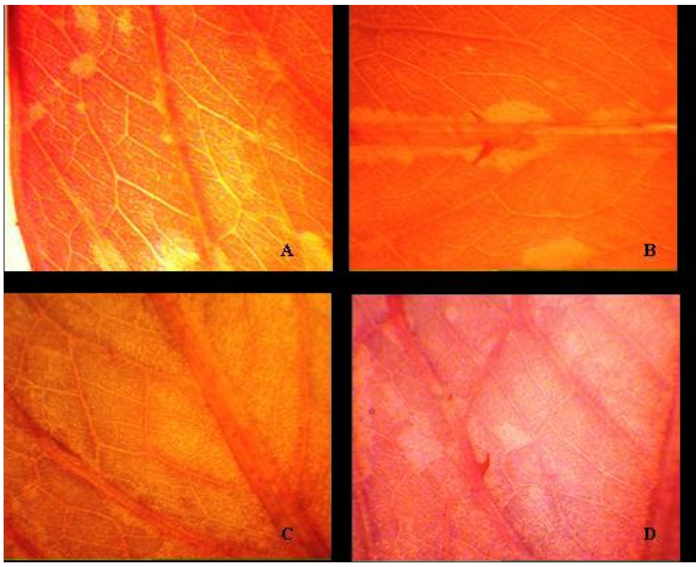


Figure 2 Leaf architecture under 45X magnification: Mixed form or Morphotype I (A,B) and Dissected laminal or Morphotype II (C,D) of *L. spinosa*

Anatomical features of *L. spinosa* morphotypes of petiole and root

Internal structure of petiole and root showed a distinct difference between two morphotypes. In petiole- the epidermis is uniseriate with closely packed cell and unbranched hair is present. Below the bands of single layered parenchyma cells numerous air spaces are present. In ground tissue, vascular bundles are surrounded with parenchyma cells. It bears distinct xylem and phloem. The majority of the characteristics of the two morphotypes are similar, however the Morphotype II parenchyma cells has more of the light-stained crystals of raphid in the leaf petiole. (Fig. 3E.) The xylem cell count was also found to be high and to be concentrated around the phloem cells.

In root - the epidermis is thin-walled with a row of closely-set cell and uniseriate. Parenchyma cell and air chambers are present in the cortex part. In the ground tissue-endodermis, pericycle, vascular bundle and pith are present. Differences in the vascular bundles between the two morphotypes were observed. Both the morphotypes has radial orientation of vascular bundles. Protoxylem is observed to be exarch, which means that metaxylem is directed toward the centre while protoxylem is directed toward the periphery. Thus, this could serve as a framework for taxonomic differentiation and accurate morphotype placement of *L. spinosa* (Fig. 4A- 4D.)

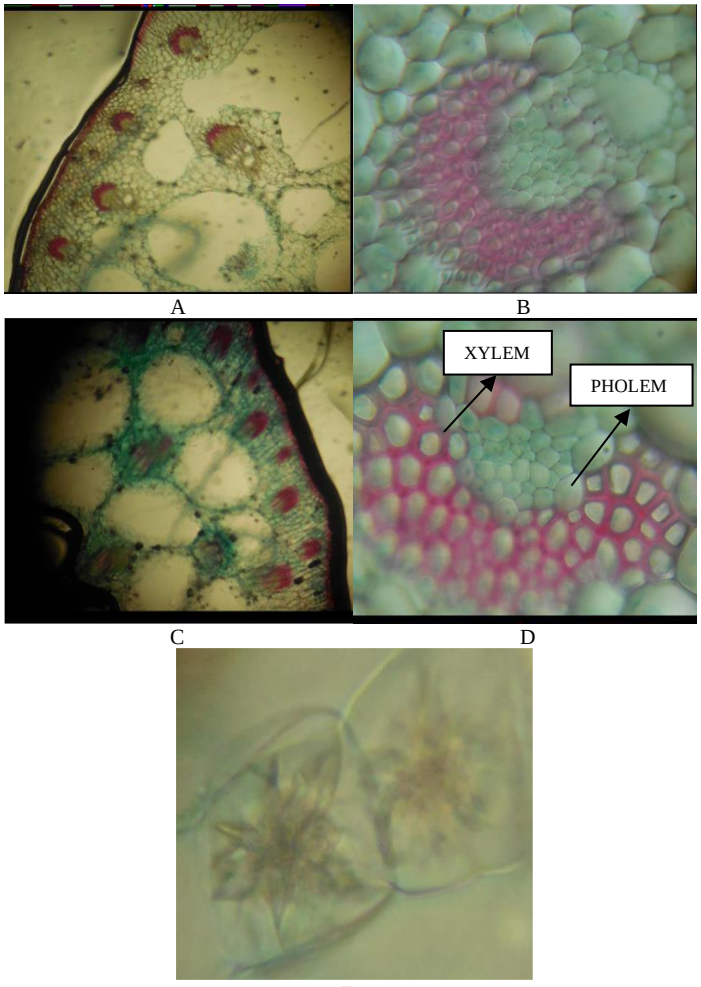


Figure 3 *Lasia spinosa* morphotypes: Under magnification 45(X) – (A-B) Mixed type or Morphotype I and (C-D) Dissected laminal or Morphotype II, anatomical characters of petiole, (E) Raphids under 45X magnification observed in parenchyma cell

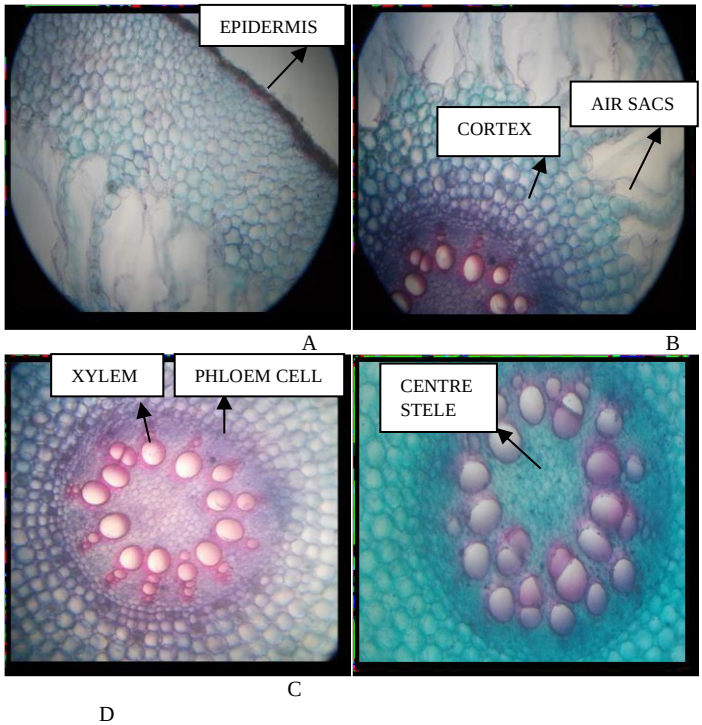


Figure 4 *L. spinosa:* Root TS under magnification 45X*;* A-B Both mophotypes possess epidermis, cortex and air-sacs; C-D **R**espective vascular bundles-Morphotype I and Morphotype II.

ISSR-PCR-based analysis to distinguish between two *Lasia spinosa* morphotypes

ISSR markers were assessed to investigate genetical diversification and connection between both *Lasia spinosa* morphotypes. For ISSR fingerprinting, eight primers are included in Table 3 below.

To assess the quality of the DNA sample, a tiny aliquot of isolated genomic DNA was run on Tris-acetate EDTA/ TAE (0.8% in concentration) agarose gel using uncut lambda DNA as a reference. The chosen samples had displayed a band of intact high molecular weight genomic DNA (Fig. 5.). For measuring DNA, a UV-Vis spectrophotometer with a range of 550–650 g/gm was utilised.

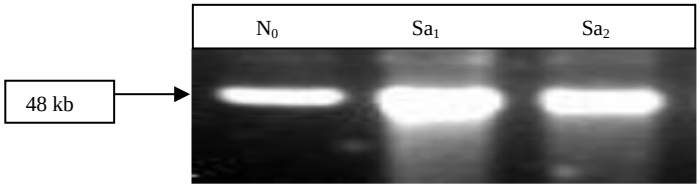


Figure 5 *Lasia spinosa* morphotypes genomic DNA in agarose gel. **N_0:** Lambda DNA (uncut) 100 ng **Sa_1:**Morphotype – I, **Sa_2:** Morphotype – II *L. spinosa*

Through ISSR marker analysis, a total of 57 amplified fragments were counted after the genomic DNA of two morphotypes of *Lasia spinosa* was amplified. The remaining 25 were monomorphic in nature, with 32 of them being polymorphic (Fig. 5. and 6). There were 04 to 13 amplified fragments total, ranging in size from 200 - 1100 bp (UBC 836-848) bands. 56.14 % i.e. 32 bands were similar, yielding 7.12 similar fragment per primer in an average, with a range of 25% to 87.50% in primers UBC 836 and UBC 842 respectively (Table 4). The primers with the most bands (13 bands) were centered on poly CA having PG at 3′, whereas poly AG having XA at 3′ bring about the fewest bands i.e. 4 bands. Required mean PIC (polymorphism information content) was calculated 0.5 as only two samples were used for analysis. The eight primers were all useful in determining polymorphism and differentiating both *Lasia spinosa* morphotypes by roughly 56% on genetical level, according to the results of the analysis shown above.

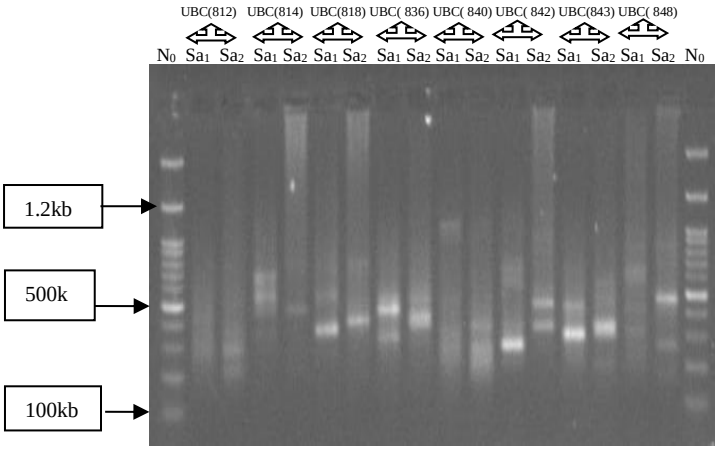


Figure 6 *Lasia spinosa* morphotypes: Amplification products of ISSR in agarose gel (1.0% in conc.). N_0 = ladder sequence 100 bp, **Sa_1:** Morphotype I, **Sa_2:** Morphotype II

Table 4 PCR was performed to compile a list of the primers used in amplified fragments and polymorphic bands (in number) taking eight/8 ISSR primers panel.

Reqired Primer	No of fragment	Band (Polymorphic)	% of Similarity/ Polymorphism	PIC(in average)
UBC (812)	5	2	40.00	0.5
UBC (814)	7	4	57.14	0.5
UBC (818)	6	2	33.33	0.5
UBC (836)	4	1	25.00	0.5
UBC (840)	6	3	50.00	0.5
UBC (842)	8	7	87.50	0.5
UBC (843)	8	6	75.00	0.5
UBC (848)	13	7	53.84	0.5
Sum	57	32	56.14	0.5

With the help of physical, anatomical, and molecular markers, our current study has been conducted to differentiate between the two morphotypes. Because environmental factors primarily control how genes are expressed, genetic differences may not always manifest in the phenotypic.

Sultana *et al.*, 2006 identified morphological form of *Lasia spinosa* only observing external leaf character. Our information provides the fundamental morphological variations across the morphotypes based on leaf characteristics and colour of the inflorescence. Morphotype, I possess lamina – mixed form and morphotypeII possess dissected laminal form. In morphotype I and morphotype II, it was found that the leaf vein architecture was jumbled craspedodromous and semi-craspedodromous, respectively. Root anatomy shows differences in arrangement of vascular bundles. Alternate patches of xylem and phloem was distinct in morphotype I whereas xylem and phloem were intermixed in mophotype II. Along with the two morphotypes of *Lasia spinosa*, internal root architecture characteristics match. In order to construct a local key, a transverse section must be compared to a known root sample since anatomical root features within a root system might change dramatically due to different environment types (**Rewald *et al.*, 2011, 2012**).

Most plant species, including crop plants, have benefited from the use of ISSRs to quantify the degree of genetical diversity at the level of intra and inter specific (**Reddy *et al*.,2000**). Genetical diversity in Cocoa was evaluated using ISSR markers (**Charters and Wilkinson, 2000**). The relationships and genetical diversity in between both *Lasia spinosa* morphotypes occurring in Assam, India, have been evaluated in this work using a panel of 8 ISSR primers. These two morphotypes shared 25 of the 57 bands available. The two morphotypes shared a homoplasy evolutionary history because they belonged to the common species. The rest 32 bands show divergence in the morphotypes. Thus, between the two morphotypes of *Lasia spinosa*, there were roughly 56% similarity/polymorphisms that were unique to each other. With the help of 18 primers and an ISSR marker-based study, 66 scorable polymorphic markers for the genus *Lagenadra* were created. *Lagenadra* samples obtained from various locations in Kerala revealed an intriguing pattern of genetic variation. The population's genetic identity and the genetic distance showed remarkable diversification (**Prakashkumar** *et al*.,2015.) Our research demonstrates unequivocally that these two morphotypes, despite belonging to the same species, are genetically 56% distinct from one another, resulting in two phenotypic variances.

CONCLUSION

Our study's goal was to distinguish between the two morphological variants of *Lasia spinosa*'s anatomy, molecular makeup, and morphology. We can conclude that though most of the anatomical features between the two morphotypes are similar yet they are identical. Our findings uphold both classical method and molecular biotechnology using ISSR markers to justify the plant has two morphological forms. Our findings can be used to distinguish *L. spinosa* from closely related species and species with differing morphologies. A single species can be divided into various morphotypes using anatomical characteristics as taxonomic criteria. For these two morphotypes of *Lasia spinosa*, ISSR based markers offer as potent tool for prospective fingerprinting markers generation. This information will in addition be useful for phylogenetical evaluation, which will assist identify significant rare and endemic groups of medicinal plants to use for bioprospecting.

Acknowledgement: It is extremely well respected to have received a research scholarship from the UGC-BSR (Basic Scientific Research) of the Indian government.

REFERENCES

Alam, F., Haque, M., Sohrab, H., Monsur, M. A., Hasan, C. M., & Ahmed, N. (2011). Antimicrobial and cytotoxic activity from *Lasia spinosa* and isolated lignan. *Lat Am J Pharm*, *30*(3), 550-553.*pdf*

Alam, S. S., Munira, S., Habib, M. A., & Sultana, S. S. (2012). Karyotype and RAPD analysis in common and rare forms of *Lasia spinosa* (L.) Thwaites. *Cytologia*, *77*(4), 499-505.https://dx.doi.org/10.1508/cytologia.77.499

Atif, M., Azharuddin, M., & Mahmood, S. B. (2015). Gastroprotective potential of *Lasia spinosa* in albino rats. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*(3), 254-257.

Basconsuelo, S., Grosso, M., Molina, M. G., Malpassi, R., Kraus, T., & Bianco, C. (2011). Comparative root anatomy of papilionoid legumes. *Flora-Morphology, Distribution, Functional Ecology of Plants*, *206*(9), 799-807.https://dx.doi.org/10.1016/j.flora.2011.04.002

Barman, P., Handique, A. K., & Tanti, B. (2014). Tagging STMS markers to Fusarium wilt race-1 resistance in chickpea (*Cicer arietinum* L.). http://nopr.niscpr.res.in/handle/123456789/29618

Charters, Y. M., Robertson, A., Wilkinson, M. J., & Ramsay, G. (1996). PCR analysis of oilseed rape cultivars (*Brassica napus L. ssp. oleifera*) using 5′-anchored simple sequence repeat (SSR) primers. *Theoretical and Applied Genetics*, *92*(3), 442-447.https://dx.doi.org/10.1007/BF00223691

Chowdhury, U., Tanti, B., Rethy, P., & Gajurel, P. R. (2014). Analysis of genetic diversity of certain species of piper using RAPD-based molecular markers. *Applied biochemistry and biotechnology*, *174*(1), 168-173.https://dx.doi.org/10.1007/s12010-014-1053-5

Deb, D., Dev, S., Das, A. K., Khanam, D., Banu, H., Shahriar, M., ... & Basher, S. A. M. K. (2010). Antinociceptive, anti-inflammatory and anti-diarrheal activities of the hydroalcoholic extract of Lasia spinosa Linn.(Araceae) roots. *Latin American Journal of Pharmacy*, *29*.*pdf*

Doyle, J. J., & Doyle, J. L. (1987). A rapid DNA isolation procedure for small quantities of fresh leaf tissue (No. RESEARCH).*pdf*

Harborne, A. J. (1998). Phytochemical methods a guide to modern techniques of plant analysis. springer science & business media.ISBN 0412572605(HB) and 0412572702(PB)

Hore, P., & Tanti, B. (2014). Karyomorphological studies of two morphotypes of *Lasia spinosa* (Lour.) Thwaites available in Assam, India. *Annals of Plant Sciences*, *3*(08), 792-796.*pdf*

Hore, P., & Tanti, B. (2018). Regeneration of plantlets from rhizome bud explants of *Lasia spinosa* (Lour.) Thwaites-A medicinal plants of Assam. *International Journal of Life Science and Scientific Research*, *4*, 1736-1743.http://dx.doi.org/10.21276/ijlssr.2018.4.3.1

Hossain, R., Quispe, C., Herrera-Bravo, J., Islam, M., Sarkar, C., Islam, M. T., ... & Cho, W. C. (2021). *Lasia spinosa* chemical composition and therapeutic potential: a literature-based review. *Oxidative Medicine and Cellular Longevity*, *2021*.https://dx.doi.org/10.1155/2021/1602437

Kongkachuichai, R., Charoensiri, R., Yakoh, K., Kringkasemsee, A., & Insung, P. (2015). Nutrients value and antioxidant content of indigenous vegetables from Southern Thailand. *Food chemistry*, *173*, 838-846.https://dx.doi.org/10.1016/j.foodchem.2014.10.123

Prakashkumar, R., Anoop, K. P., Ansari, R., Sivu, A. R., Pradeep, N. S., & Madhusoodanan, P. V. (2015). Analysis of genetic diversity of *Lagenandra* spp.(Araceae) of Kerala (South India) using ISSR Markers. *Int J Sci Res*, *4*(6), 775-777.

Rahman, A., Tanti, B., Sarma, G. C., & Kalita, J. (2012). Genetic diversity of *Persea bombycina* from Goalpara district of Assam, India.http://dx.doi.org/10.4236/abb.2012.31004

Reddy, M. P., Sarla, N., Neeraja, C. N., & Siddiq, E. A. (2000, October). Assessing genetic variation among Asian A-genome *Oryza* species using inter simple sequence repeat (ISSR) polymorphism. In *Fourth international rice genetics symposium* (pp. 22-27).*pdf*

Rewald, B., Leuschner, C., Wiesman, Z., & Ephrath, J. E. (2011). Influence of salinity on root hydraulic properties of three olive varieties. *Plant Biosystems*, *145*(1), 12-22.https://dx.doi.org/10.1080/11263504.2010.514130

Rewald, B., Meinen, C., Trockenbrodt, M., Ephrath, J. E., & Rachmilevitch, S. (2012). Root taxa identification in plant mixtures–current techniques and future challenges. *Plant and Soil*, *359*(1), 165-182.https//dx.doi.org/10.1007/s11104-1164

Salimath, S. S., Oliveira, A. C. D., Bennetzen, J. L., & Godwin, I. D. (1995). Assessment of genome origins and genetic diversity in the genus *Eleusine* with DNA markers. *Genome*, *38*(4), 757-763.

Sambrook, J., Fritsch, E. F., & Maniatis, T. (1989). Molecular cloning: a laboratory manual. *Cold Spring Harbor, NY, Cold Spring Harbor Laboratory*, *11*, 31.*pdf*

Shefana, A. G., & Ekanayake, S. (2009). Some nutritional aspects of *Lasia spinosa* (kohila).*pdf*

Sultana, S., Rahman, R. B., & Alam, S. S. (2006). Karyotype analysis in three morphological forms of *Lasia spinosa* (L.) Thwaites (Araceae). *Cytologia*, *71*(4), 359-364.https://dx.doi.org/10.1508/cytologia.71.359

Tangpu, V., Temjenmongla, K., & Yadav, A. K. (2005). Anticestodal activity of *Trifolium repens*. extract. *Pharmaceutical biology*, *42*(8), 656-658.https://dx.doi.org/10.1080/13880200490902617

Tanti, B., Das, A. K., Kakati, H., & Chowdhury, D. (2012). Cytotoxic effect of silver-nanoparticles on root meristem of *Allium sativum* L. *J Res Nanobiotechnol*, *1*(1), 1-8.*pdf*

Tanti, B., Ray, S. K., & Buragohain, A. K. (2012). Differentiation of petroleum hydrocarbon-degrading *Pseudomonas* spp. based on PCR-RFLP of the 16S-23S rDNA intergenic spacer region. *Folia microbiologica*, *57*(1), 47-52.https//dx.doi.org/10.1007/s12223

Temjenmongla, Y. A., & Yadav, A. K. (2003). Filaricidal efficacy of some folklore medicinal plants against *Setaria cervi* (Nematoda: Filarioida). In *Proc Zool Soc (Cal)* (Vol. 56, pp. 57-61).*pdf*

Temjenmongla, T., & Yadav, A. K. (2005). Anticestodal efficacy of folklore medicinal plants of Naga tribes in north-east India. *African Journal of Traditional, Complementary and Alternative Medicines*, *2*(2).https://dx.doi.org/10.4314/ajtcam.v2i2.31111.

Vasco, A., Thadeo, M., Conover, M., & Daly, D. C. (2014). Preparation of samples for leaf architecture studies, a method for mounting cleared leaves. *Applications in plant sciences*, *2*(9), 1400038.https://dx.doi.org/10.3732/apps.1400038

Williams, J. G., Kubelik, A. R., Livak, K. J., Rafalski, J. A., & Tingey, S. V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucleic acids research*, *18*(22), 6531-6535.https://dx.doi.org/10.1093/nar/18.22.6531

Yong, Y. H. (2009). Antioxidant activity and total phenolic contents in *Cucurbita moschata*, *Lasia spinosa and Limnocharis flava* (Doctoral dissertation, Universiti Malaysia Sabah).*pdf*

Zietkiewicz, E., Rafalski, A., & Labuda, D. (1994). Genome fingerprinting by simple sequence repeat (SSR)-anchored polymerase chain reaction amplification. *Genomics*, *20*(2), 176-18.https://dx.doi.org/10.1006/geno.1994.1151.