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RESEARCH ARTICLE

Assessment of genetic diversity among high azadirachtin genotypes of Neem (*Azadirachta indica* A. Juss) using ISSR markers

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Abstract

Azadirachtin, a key bioactive compound derived from the neem tree (*Azadirachta indica* A. Juss), holds paramount importance in various industrial applications due to its potent pesticidal properties and versatile nature. The existence of natural variability for azadirachtin among different Neem genotypes presents an opportunity for strategic selection and breeding. The variability observed across genotypes allows for the identification and breeding of genotypes consistently high in azadirachtin content. Genetic diversity studies of *A. indica* using Inter Simple Sequence Repeats (ISSR) markers revealed that the 40 genotypes were grouped into 7 distinct clusters based on Jaccards' similarity coefficient. The dendrogram obtained at a 0.73 similarity matrix showed the highest genetic diversity between genotypes G1 and G25, in contrary to this, G32 and G37 showed the least genetic diversity. A total of 33 primers were used in the study, but only 20 primers were successfully amplified at different annealing temperatures, with the Polymorphism Information Content (PIC) values ranging from 0.201 to 0.385, averaging 0.31. Most of the primers exhibited 100 % polymorphism. The number of bands generated ranged from 6–14. Among all the bands generated, 13 unique bands were identified across 12 primers in 14 genotypes, with G16 and G25 showing the highest number of unique bands. These findings provide valuable insights for selecting superior genotypes as candidate trees for future improvement programs, contributing to better management and commercial utilisation of neem resources. This study also plays a crucial role in advancing the genetic enhancement of *A. indica.*

Keywords: *Azadirachta indica*; genetic diversity; genotypes; ISSR markers

Introduction

Azadirachta indica A. Juss, commonly known as Neem, is a member of the Meliaceae family. Believed to have originated in the Indian subcontinent (1), it thrives in Indias' dry and subtropical regions, including states like Andhra Pradesh, Telangana, Maharashtra, Gujarat, Tamil Nadu, Karnataka, Bihar, Odisha, Haryana, Punjab, Uttar Pradesh and the Shiwalik hill regions of the Himalayas (2). Neem grows well in areas with an annual rainfall of 400–1200 mm and is highly adaptable to various soil types, such as dry, clayey, sandy, sodic, lateritic and calcareous soils with a pH of 6.2 or higher (3). Each part of the tree serves a purpose in various ways. The seeds are a significant source of vegetable oil and biopesticidal compounds. The neem seed kernel contains azadirachtin and other limonoids like nimbin, salanin and meliantriol. Among these, azadirachtin is the most notable limonoid, recognised for its antifeedant, attractant, repellent, growth-disrupting and larvicidal properties against numerous pests (4).

Understanding the regional and habitat variations in azadirachtin content within neem trees is crucial for identifying region-specific elite trees and gaining insights into the factors influencing azadirachtin synthesis. The highest azadirachtin yield per seed kernel occurs in individual trees from diverse origins, rather than being confined to a single country (5). Trees growing in the desert region recorded the lowest azadirachtin content. Samples from hot sub-humid, hot arid and hot semi-arid climates with cold winters showed significantly lower azadirachtin synthesis compared to those from hot semi-arid regions with mild winters (4). Region-specific studies have shown significantly larger variations in azadirachtin content among neem kernels from different ecotypes in Tamil Nadu (6).

The extensive geographical and climatic range of neem suggests considerable variability in azadirachtin content and genetic diversity within the species, necessitating proper identification and documentation. Numerous approaches are available for evaluating genetic diversity in plant species. Traditionally, methods combining morphological characteristics with protein profiling techniques like isozymes, allozymes and seed storage proteins have been widely used (7–9). However, these traits are often affected by environmental conditions and the plants' developmental stage, making the findings from such studies less reliable as accurate indicators of genetic diversity (10).

Deoxyribonucleic acid (DNA) based markers offer valuable insights into genetic diversity and the relationships among accessions, as they are not influenced by environmental conditions or the developmental stage of plants, unlike traditional methods. These methods are based on the amplification of genomic DNA fragments by polymerase chain reaction (PCR) and have an array of applications in plant breeding programmes due to their stability, uniqueness and absence of pleiotropic and epistatic effects (11, 12). The choice of different types of DNA markers depends on the objectives of the research. For instance, usage of Random Amplified Polymorphic DNA (RAPD) markers in neem was reported previously in India, Brazil and Nigeria to study the genetic structure and variability among various accessions, whereas usage of Amplified Fragment Length Polymorphism (AFLP) markers was reported earlier to assess the genetic diversity in neem accessions (10) (13–15).

The application of Inter Simple Sequence Repeats (ISSR) markers in *A. indica* offers a precise molecular approach to assess genetic variation associated with azadirachtin content, which cannot be fully characterised by traditional morphological and biochemical markers. The ISSR analysis in the study facilitates the identification of high-yielding chemotypes and aids in linking genetic diversity with phytochemical traits. This approach provides a critical foundation for breeding and conservation programmes aimed at developing neem genotypes with enhanced bioactive compound production.

Inter Simple Sequence Repeats (ISSR) markers are widely utilised to evaluate genetic diversity in neem genotypes because they provide a dependable, efficient and reproducible approach that does not require prior genomic sequence information (16). This method produces highly polymorphic patterns, making it possible to clearly differentiate closely related genotypes and verify the genetic distinctness and consistency of superior trees. They have proven to be effective in analysing the genetic diversity of various tree species, viz. *Bruguiera gymnorrhiza* (L.) Lam., *Camellia yuhsienensis*, *Juglans regia*, *Elaeocarpus serratus*, *Pityrocarpa moniliformis*, *Santalum album*, Bamboo spp. and *Phoenix dactylifera* L. (7, 17-23). Inter Simple Sequence Repeats (ISSR) analysis is cost-effective and can be easily performed in standard laboratory settings, facilitating large-scale screening vital for breeding programs and the preservation of valuable genetic resources aimed at enhancing azadirachtin production in *A. indica*. Hence, the present study assessed the genetic diversity among 40 neem accessions from states viz. Telangana, Tamil Nadu, Karnataka and Rajasthan using ISSR methodology.

Materials and Methods

Plant materials

The 40 genotypes used in this study were surveyed, collected and assembled from different districts of Telangana, Tamil Nadu, Rajasthan and Karnataka states. The experimental site is located at 17° 728544 N to 78° 63296 E. The geographical details of the genotypes are presented in Table 1.

Selection of genotypes

The genotypes were initially screened for azadirachtin content using the method suggested by (24). The aza content in Neem seed kernels varied from 0.3–1.5 % and the average aza content reported in India was around 0.3–0.4 % (25). In the present study, all those genotypes having more than 0.4 % azadirachtin have been included for diversity studies. The Azadirachtin content of the 40 genotypes used in the study is presented in the Table 1.

DNA isolation

Fresh, healthy young leaves were collected from greenhouse-grown seedlings of each genotype. For each genotype, approximately 400 mg of fresh leaf tissue was randomly collected and pooled from five individual seedlings to obtain a composite sample representing that genotype. Genomic DNA was extracted from the pooled leaf tissue using the modified Cetyl trimethyl ammonium bromide (CTAB) method (26). The technical replicates of each pooled DNA sample were processed in a random sequence for PCR amplification and electrophoresis to reduce procedural or instrumental bias.

Primers used for the study

The leaf samples of high aza genotypes were used to study the genetic relationships using 33 ISSR primers at the Biotechnology laboratory, Forest College and Research Institute (FCRI), Mulugu, Hyderabad (Table 2).

PCR reaction conditions

ISSR reactions were performed on DNA samples from 40 Neem genotypes. These reactions consisted of three main steps: denaturation, annealing and extension, which are detailed in Table 3.

ISSR analysis

Clear bands of ISSR markers were scored based on the presence or absence of corresponding DNA fragments among the genotypes on each gel. The score 1 indicates the presence and the score 0 indicates the absence of bands. After analysing visually, the binary matrix was generated with the help of this data. The molecular weight was determined with a 100 bp DNA ladder as a reference. The Numerical Taxonomy System (NTSYs) package was used for further genetic analysis.

Percentage of polymorphism (PP)

Percent polymorphism was calculated by counting the total number of bands amplified by each primer among the 40 genotypes as given below:

$$PP = \frac{\text{Number of polymorphic band}}{\text{Total number of bands}} \text{ X } 100 \qquad \text{(Eqn. 1)}$$

Polymorphic information content (PIC)

Jaccards' similarity index

The binary matrices were read using NTSYs pc-version 2.02 with Jaccards' similarity coefficients and estimates of genetic distances for all pair wise comparison between genotypes were determined using Similarity for Qualitative Data (SIMQUAL).

Dendrogram construction

Dendrograms were constructed based on pooled marker data using Unweighted pair group method with arithmetical averages (UPGMA).

Results and Discussion

Out of 33 primers tested, 20 primers have shown reproducible amplification banding patterns at different annealing temperatures. Most of the primers have exhibited 100 % polymorphism across all the genotypes. Maximum polymorphism (100 %) was obtained in ISSR 03, ISSR 04, ISSR F2, ISSR M5, ISLA(CT)8TG, UBC 809, UBC 810,

Table 1. Geographical description of genotypes of *Azadirachta indica*

Sl No.	Name of genotypes	Locality	District	State
1	G1	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
2	G2	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
3	G3	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
4	G4	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
5	G5	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
6	G6	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
7	G7	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
8	G8	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
9	G9	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
10	G10	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
11	G11	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
12	G12	FC and RI Mettupalayam	Coimbatore	Tamil Nadu
13	G13	Moinabad	Rangareddy	Telangana
14	G14	Mahabubnagar	Mahabubnagar	Telangana
15	G15	Aliyabad	Medchal Malkajgiri	Telangana
16	G16	Vikarabad	Vikarabad	Telangana
17	G17	Bhuvanagiri	Yadadri Bhuvanagiri	Telangana
18	G18	Thandoor	Vikarabad	Telangana
19	G19	Vantimamidi	Siddipet	Telangana
20	G20	Jangaon	Jangaon	Telangana
21	G21	Jangaon	Jangaon	Telangana
22	G22	Hanamkonda	Warangal urban	Telangana
23	G23	Alamkhanipet	Warangal rural	Telangana
24	G24	Kamareddy	Kamareddy	Telangana
25	G25	Kalvakurthi	Nagarkurnool	Telangana
26	G26	Kishtapoor	Medak	Telangana
27	G27	Korukondapally	Mahabubabad	Telangana
28	G28	Thurkapally	Siddipet	Telangana
29	G29	Kamareddy	Kamareddy	Telangana
30	G30	Boranakoppalu	Hassan	Karnataka
31	G31	Kesamudram	Mahabubabad	Telangana
32	G32	Kondagattu	Jagtial	Telangana
33	G33	Kodimatha	Hassan	Karnataka
34	G34	Karimnagar	Karimnagar	Telangana
35	G35	Karimnagar	Karimnagar	Telangana
36	G36	Sircilla	Rajanna Sircilla	Telangana
37	G37	Sircilla	Rajanna Sircilla	Telangana
38	G38	Cheelanhally	Hassan	Karnataka
39	G39	Bageshpura	Hassan	Karnataka
40	G40	Jodhpur	Jodhpur	Rajasthan

Table 2. Annealing temperatures of different ISSR primers used

Sl. No.	Primer	Primer sequence 5'-3'	Temperature
1	ISSR 03	$(CT)_8$-GC	49
2	ISSR 04	$(CA)_6$-AC	35
3	ISSR F2	$(AG)^8$-CG	54
4	ISSR M5	$(GA)^9$-C	44
5	ISLA(CT)8TG	$(CT)_8$-TG	41
6	UBC 809	AGAGAGAGAGAGAGAGG	41
7	UBC 810	GAGAGAGAGAGAGAGAT	40
8	UBC 811	GAGAGAGAGAGAGAGAC	44
9	UBC 823	TCTCTCTCTCTCTCTCC	44
10	UBC 826	ACACACACACACACACC	51
11	UBC 840	GAGAGAGAGAGAGAGAYT	51
12	UBC 841	GAGAGAGAGAGAGAGAYC	43
13	UBC 847	CACACACACACACACARC	43
14	UBC 855	ACACACACACACACACYT	60
15	UBC 857	ACACACACACACACACYG	59
16	UBC 864	ATGATGATGATGATGATG	50
17	UBC 880	GGAGAGGAGAGGAGA	47
18	UBC 888	BDBCACACACACACACA	44
19	UBC 889	DBDACACACACACACAC	40
20	UBC 891	HVHTGTGTGTGTGTGTG	52

Table 3. Details of the steps involved in the PCR amplification of the ISSR reaction

Sl. No.	Steps	Temperature	Time	Cycles
1	Initial denaturation	94 °C	4 min	-
2	Denaturation	94 °C	45 sec	30 cycles
3	Annealing	T_a	1 min	30 cycles
4	Extension	72 °C	2 min	30 cycles
5	Final extension	72 °C	8 min	-
6	Stand by	4 °C	Infinity	-

UBC 811, UBC 823, UBC 826, UBC 841, UBC 847, UBC 855, UBC 857, UBC 864, UBC 880, UBC 888 and UBC 891, followed by UBC 889 (87.5 %) and UBC 840 (85.71 %) (Fig. 1-10). Primer UBC 857 has recorded the highest value of polymorphic information content (PIC), i.e., 0.385, followed by UBC 823 (0.379), ISSR M5 (0.377), UBC 891 (0.37), UBC 864 (0.352) and ISSR 03 (0.348) (Table 4). The number of bands produced by each primer ranged from 6–14, with UBC 841 yielding the most (14 bands) and UBC 811 and UBC 857 producing the least (6 bands). Amplified product sizes varied from 100 bp to 3100 bp. Twelve primers generated 13 unique bands across fourteen genotypes, with G16 and G25 showing the highest number of unique bands (Table 5). These results could aid in genotype identification and differentiation and further studies with additional primers are recommended for broader genomic representation. Polymorphism in different tree species was documented by several authors, viz. *Gmelina arborea* Roxb., *Camellia oleifolia*, *Elaeocarpus serratus* L., *Pityrocarpa moniliformis*, *Dalbergia sissoo* Roxb., *Melia dubia* Cav., *Pinus roxburghii* (Sarg.), *Myrciaria tenella* O. Berg and *Tectona grandis* L.f. (19, 27-34).

Clusters revealed tremendous genetic variation among *A. indica* genotypes without a clear link to geographical locations. Cluster analysis carried out by UPGMA method divided the total 40 genotypes into 8 distinct clusters at 0.76 Jaccards' similarity coefficient (ranged from 0.58–0.82) (Fig. 11). The first cluster consisted of 1 genotype i.e., G25, second cluster consisted of 3 genotypes i.e., G19, G22, G28, third cluster comprised of 1 genotype i.e., G2, fourth cluster comprised of 3 genotypes i.e., G16, G18, G30, fifth cluster consisted of 5 genotypes i.e., G14, G33, G38, G39, G40, sixth cluster consisted of 22 genotypes i.e., G3, G4, G5, G6, G7, G8, G10, G11, G12, G13, G15, G17, G20, G21, G23, G27, G29, G31, G32, G34, G35, G36, G37, seventh cluster comprised of G24, G26 and eight cluster comprised of G1 and G9 (Fig. 11).

An attempt was made to interpret the results of diversity about the Telangana region alone, as more than half of the genotypes used in the study are from Telangana, by merging the clusters according to the agroclimatic zones (North, Central and South Zones) of Telangana. The merging of smaller clusters into larger ones (clubbing of clusters I, II, III, IV and clusters V, VI, VII, VIII) clearly revealed the clusters' similarity to the zones. Genotypes in clusters I, II, III and IV were more closely associated with the northern zone, while those in clusters V, VI, VII and VIII were linked to the southern zone. Genotypes from Central Telangana were found scattered between the two zones, as these areas are adjacent to both.

The current study revealed significant genetic variation among neem genotypes, linked to their broad agroclimatic divisions in Telangana. Neem, being both self-pollinated and cross-pollinated, develops natural mechanisms to maintain genetic diversity (35). These mechanisms, along with environmental adaptations, contribute to the large diversity in species (36). The present study included genotypes yielding azadirachtin of 0.4 and above, with an average yield of 0.78 %. The stability of azadirachtin biosynthesis over the environment needs to be understood further through extensive collection, progeny trials over multiple locations for species improvement, commercial viability and sustainable production. The observed variation can be attributed to the adaptation of neem to different environmental conditions, including rainfall patterns, temperature fluctuations and soil characteristics.

Similar genetic diversity studies using ISSR markers were reported earlier in *Pterocarya fraxinifolia*, *Castanea sativa* Mill. *Camellia yuhsienensis*, *Elaeocarpus serratus* L., *Aniba rosaeodora*, *Melia dubia* Cav., *Argania spinosa* L., *Myrciaria tenella* O. Berg, *Pithecellobium dulce* (Roxb.) Benth, *Tectona grandis* L.f., *Mangifera indica*, *Acacia senegal* (L.), *Hagenia abyssinica* and *Olea europaea* L. subsp. *europaea* (18, 19, 31, 33, 34, 37–45).

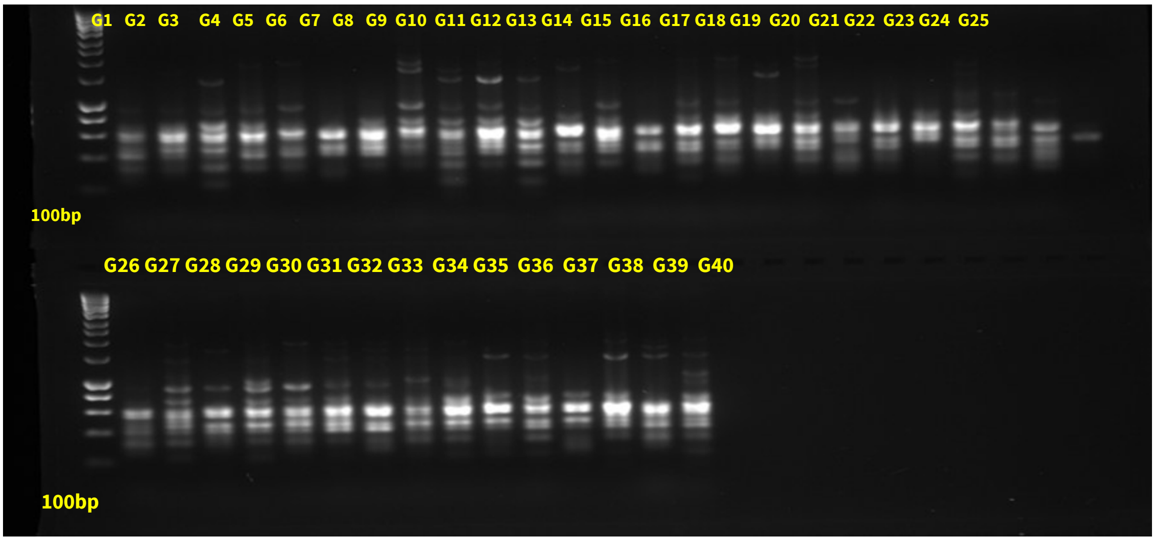

Fig. 1. Display of polymorphism by UBC 891 primer.

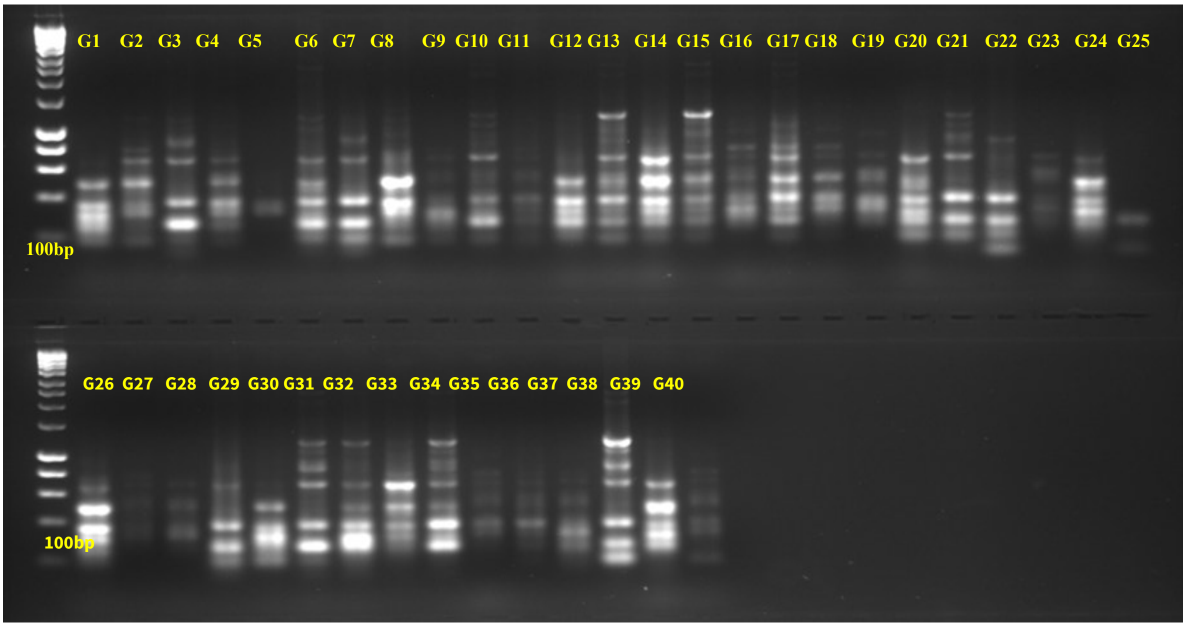

Fig. 2. Display of polymorphism by ISSR F2 primer.

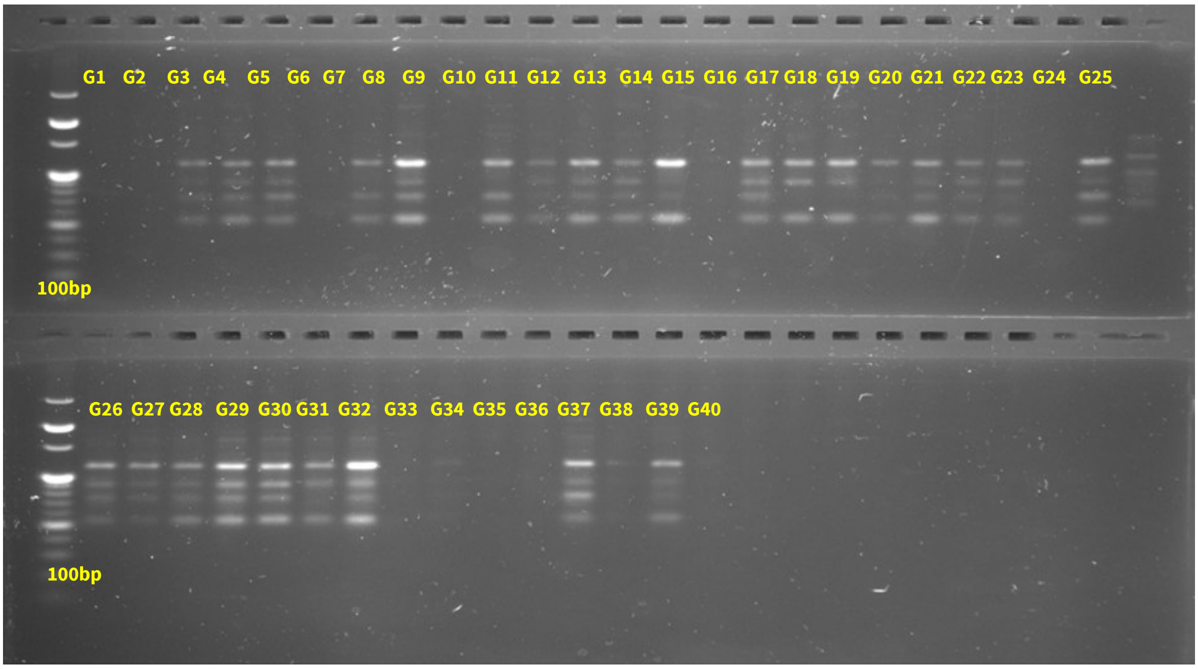

Fig. 3. Display of polymorphism by UBC 823 primer.

Fig. 4. Display of polymorphism by ISSR M5 primer.

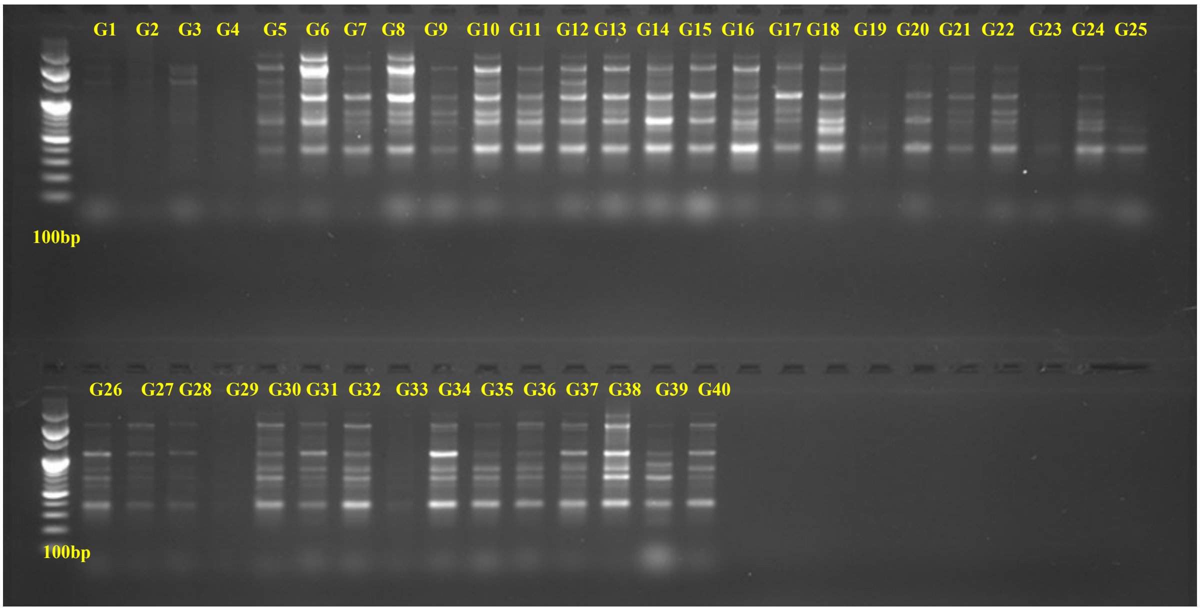

Fig. 5. Display of polymorphism by ISSR 03 primer.

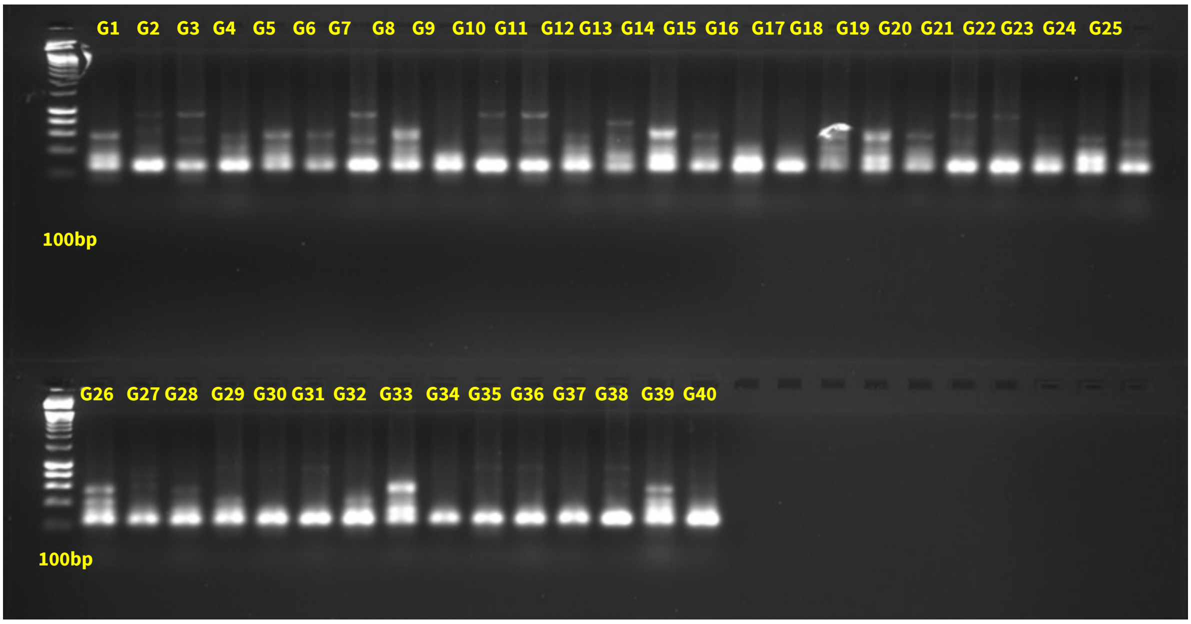

Fig. 6. Display of polymorphism by UBC 840 primer.

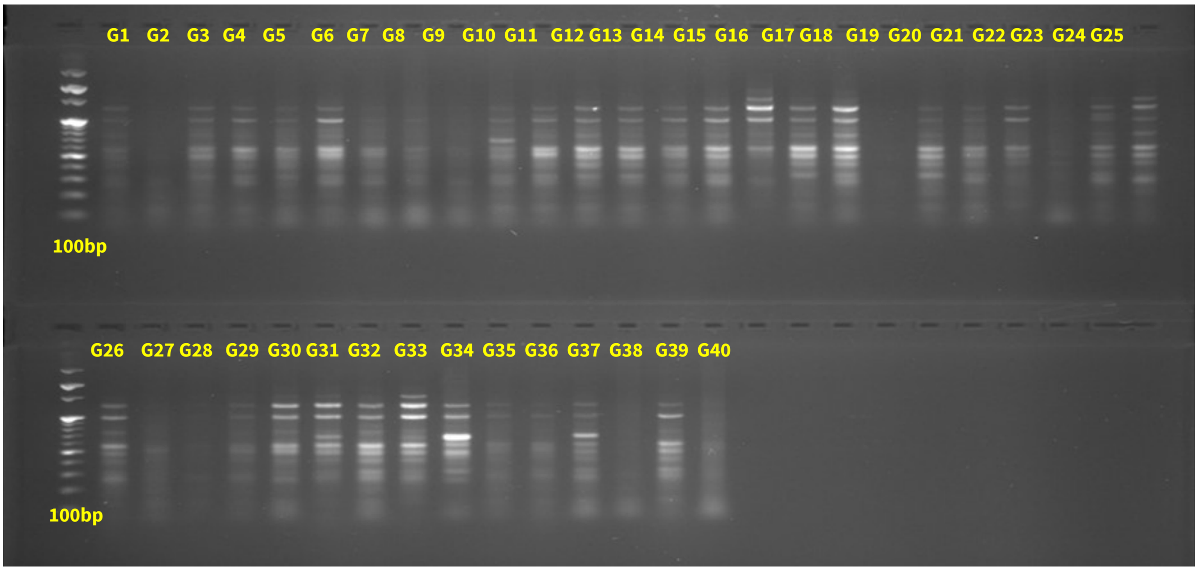

Fig. 7. Display of polymorphism by UBC 826 primer.

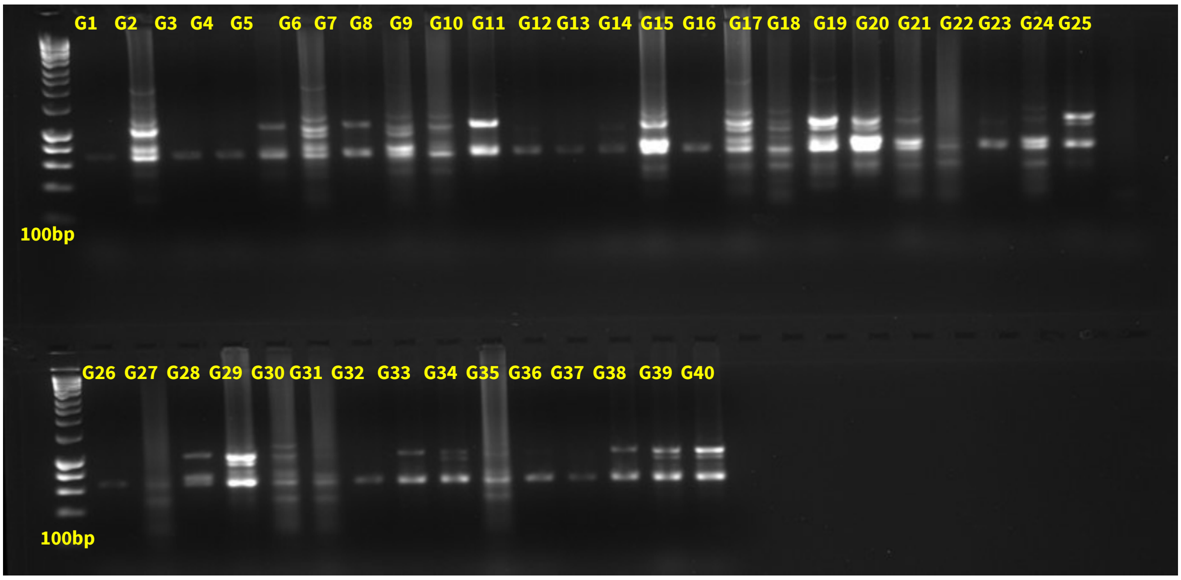

Fig. 8. Display of polymorphism by ISSR 04 primer.

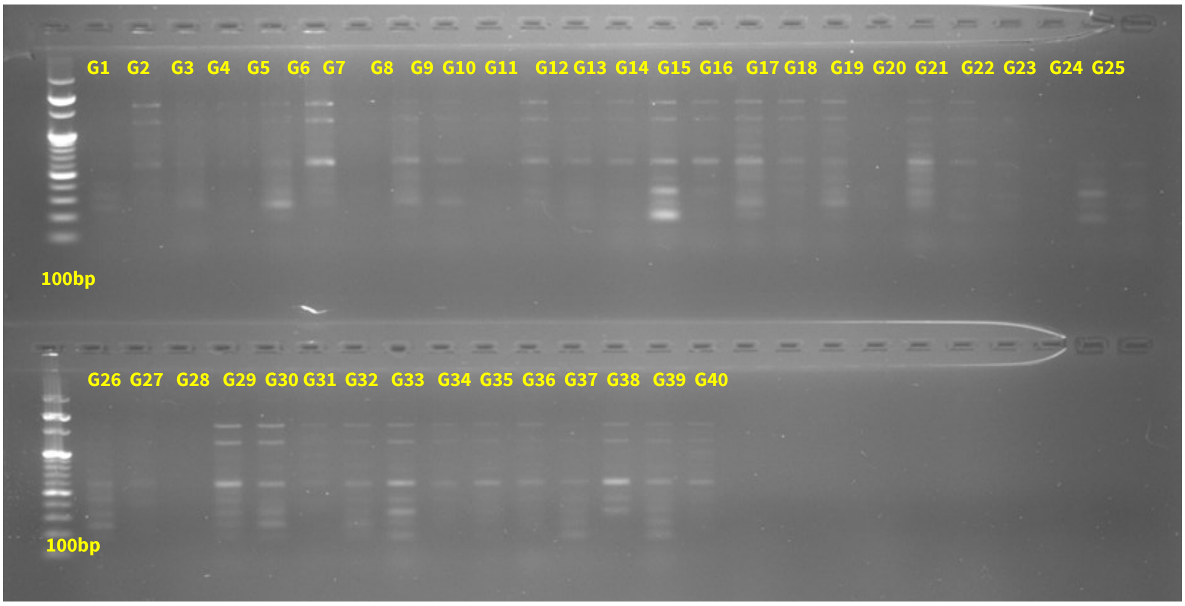

Fig. 9. Display of polymorphism by UBC 855 primer.

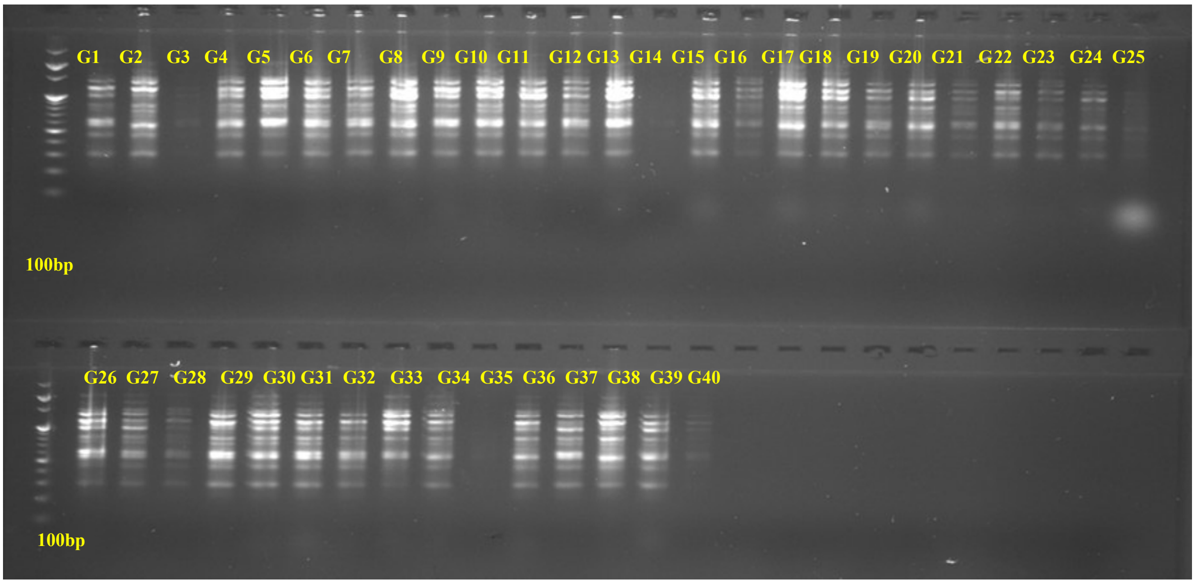

Fig. 10. Display of polymorphism by UBC 864 primer.

Table 4. Primers used for DNA amplification in the ISSR marker

Sl. No	Primer	Primer sequence 5’-3’	Percent polymorphism	PIC values
1	ISSR 03	(CT)8-GC	100	0.348
2	ISSR 04	(CA)6-AC	100	0.326
3	ISSR F2	(AG)8-CG	100	0.337
4	ISSR M5	(GA)9-C	100	0.377
5	ISLA(CT)8TG	(CT)8-TG	100	0.239
6	UBC 809	AGAGAGAGAGAGAGAGG	100	0.223
7	UBC 810	GAGAGAGAGAGAGAGAT	100	0.323
8	UBC 811	GAGAGAGAGAGAGAGAC	100	0.335
9	UBC 823	TCTCTCTCTCTCTCTCC	100	0.379
10	UBC 826	ACACACACACACACACC	100	0.293
11	UBC 840	GAGAGAGAGAGAGAGAYT	85.71	0.353
12	UBC 841	GAGAGAGAGAGAGAGAYC	100	0.229
13	UBC 847	CACACACACACACACARC	100	0.268
14	UBC 855	ACACACACACACACACYT	100	0.333
15	UBC 857	ACACACACACACACACYG	100	0.385
16	UBC 864	ATGATGATGATGATGATG	100	0.352
17	UBC 880	GGAGAGGAGAGGAGA	100	0.312
18	UBC 888	BDBCACACACACACACA	100	0.256
19	UBC 889	DBDACACACACACACAC	87.50	0.201
20	UBC 891	HVHTGTGTGTGTGTGTG	100	0.37

Table 5. Unique bands of different primers among 40 genotypes

Sl. No.	Primer	Genotype	Unique band (base pairs)
1	UBC 891	G33	1600
2	UBC 889	G23	550
3	UBC 864	G15	2800
4	UBC 855	G20	500
5	UBC 840	G14	700
6	UBC 826	G16	1800
7	UBC 823	G21 G25	1250 600
8	ISSR M5	G25	550
9	ISSR F2	G19	350
10	ISSR 04	G2 G38	700 400
11	ISSR 03	G6 G16	3000 1000
12	UBC 809	G27 G30	850 600

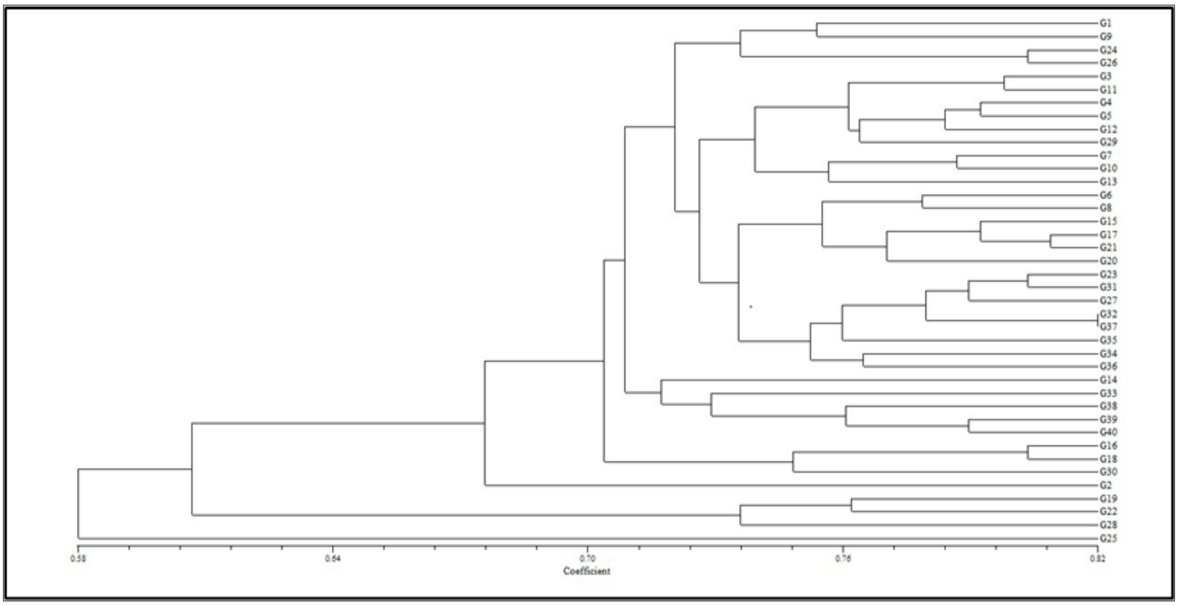

Fig. 11. The UPGMA dendrogram computed using genetic distance matrix based on ISSR data.

Conclusion

The use of ISSR markers in assessing the genetic diversity of neem (*A. indica*) represents a significant contribution to our understanding of this species. The diversity analysis demonstrated the effectiveness and reliability of ISSR markers for genetic studies in neem, highlighting their potential for use in future research. These findings offer valuable insights for neem improvement programs aimed at developing genotypes/chemotypes with higher azadirachtin production for commercial purposes. Documenting this genetic diversity will play a crucial role in species improvement, conservation efforts and the sustainable management of neem resources.

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Authors' contributions

TVBSL carried out the research as part of her postgraduate studies. RS framed the research objectives, design and methodology and provided guidance. MM supported the research execution in the field and lab. AR contributed to the study's design and supported statistical interpretations. SM participated in the design and methodology. KK conceived of the study and provided seed material and coordinated the azadirachtin extraction works for Tamil Nadu genotypes. All authors read and approved the final manuscript.

Compliance with ethical standards

Conflict of interest: Authors do not have any conflict of interests to declare.

Ethical issues: None

References

1. Sombatsiri K, Ermel K, Schmutterer H. The Thai neem tree: *Azadirachta siamensis* (Val.). In: Schmutterer H, editor. The Neem Tree. Weinheim: VCH Verlagsgesellschaft; 1995. p. 585–97.
2. Parrotta JA. Healing plants of peninsular India. New York: CABI Publishing; 2001.
3. Tomar A, Singh KK. Neem, a Treatise. New Delhi: IK International Pvt Ltd; 2019.
4. Nybom H. Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Mol Ecol. 2004;13(5):1143–55. https://doi.org/10.1111/j.1365-294X.2004.02141.x
5. Ermel K, Pahlich E, Schmutterer H. Azadirachtin contents of neem kernels. In: Proceedings of the 3rd International Neem Conference. Nairobi, Kenya; 1986. p. 171–84.
6. Kumar MG, Kumar RJ, Regupathy A, Rajasekaran B. Liquid chromatographic determination and monitoring of azadirachtin in neem ecotypes. Neem Update. 1995;1:4.
7. Frova C, Krajewski P, Di Fonzo N, Villa M, Sari-Gorla M. Assessment of genetic diversity in *Azadirachta indica* using AFLP markers. Theor Appl Genet. 1999;99(1):272–9. https://doi.org/10.1007/s001220051233
8. May B. Starch electrophoresis of allozymes. In: Hoelzel AR, editor. Molecular genetic analysis of populations. Oxford: IRL Press; 1992. p. 1–27.
9. Doll H, Brown AHD. Hordein variation in wild and cultivated barley. Can J Genet Cytol. 1979;21(3):391–404.
10. Sudhir S, Vel SV, Selvam H, Arunprasath A. Using RAPD and ISSR markers for sustainable management on Keralas' West Coast. J Appl Biol Biotech. 2024;12(1):1–7. https://doi.org/10.7324/JABB.2024.153402
11. Adhikari S, Saha S, Biswas A, Rana TS, Bandyopadhyay TK, Ghosh P. Application of molecular markers in plant genome analysis: a review. Nucleus. 2017;60(3):283–97. https://doi.org/10.1007/s13237-017-0214-8
12. Raji R, Siril EA. Genetic diversity analysis of promising Ceylon olive using ISSR markers. Curr Plant Biol. 2021;26:100197. https://doi.org/10.1016/j.cpb.2021.100197
13. Tandon R. Reproductive biology of *Azadirachta indica*. Plant Species Biol. 2011;26(1):116–23. https://doi.org/10.1111/j.1442-1984.2010.00303.x
14. Ana VCDS, Allivia RCR, Camila SA, Debora C. Genetic structure and diversity of the neem germplasm bank from Brazil northeast. Afr J Biotechnol. 2013;12(20):2822–9. https://doi.org/10.5897/AJB2013.12061
15. Awodele O, Ogunkanmi LA, Oyelakin OO. Assessment of genetic diversity of *Azadirachta indica* using RAPD markers. Niger Q J Hosp Med. 2011;21(1):41–4.
16. Desai P, Gajera B, Mankad M, Shah S, Patel A, Patil G, et al. Comparative assessment of genetic diversity among Indian bamboo genotypes using RAPD and ISSR markers. Mol Biol Rep. 2015;42(8):1265–73. https://doi.org/10.1007/s11033-015-3867-4
17. Syed M, Gupta VK, Pandey HC. Studies of phenotypic and genetic variation in Neem germplasm. Agric Sci Res J. 2013;3(3):72–8.
18. Kaushik N, Singh BG, Tomar UK, Naik SN, Vir S, Bisla SS, et al. Regional and habitat variability in azadirachtin content of Indian neem. Curr Sci. 2007;92(10):1401–7.
19. Rawat S, Joshi G, Annapurna D, Arunkumar AN, Karaba NN. Standardization of DNA extraction method from mature dried leaves and ISSR-PCR conditions for *Melia dubia* Cav. -A fast growing multipurpose tree species. Am J Plant Sci. 2016;7:437–45.
20. Felix FC, Chagas KP, Ferrari CD, Vieira FD, Pacheco MV, et al. Applications of ISSR markers in studies of genetic diversity of *Pityrocarpa moniliformis*. Rev Caatinga. 2020;33:1017–24. https://doi.org/10.1590/1983-21252020v33n417rc
21. Santana JGS, Nascimento ALS, Pereira MMG, Silva GF, Oliveira EJ. Genetic diversity in cambui tree using ISSR markers. Genet Mol Res. 2016;15(4):15048995.
22. Amom T, Tikendra L, Apana N, Goutam M, Sonia P, Koijam AS, et al. Efficiency of RAPD, ISSR, iPBS, SCoT and phytochemical markers in the genetic relationship study of five native and economical important bamboos of North-East India. Phytochemistry. 2020;174:112330. https://doi.org/10.1016/j.phytochem.2020.112330
23. Mirbahar AA, Khan S, Markhand GS, Kauser N, Saeed R. DNA fingerprinting of Pakistani date palm using ISSR markers. Pak J Bot. 2016;48(5):2005–10.
24. Kaushik N. Determination of azadirachtin and fatty acid methyl esters of *Azadirachta indica* seeds. Anal Bioanal Chem. 2002;374(7):1199–204. https://doi.org/10.1007/s00216-002-1614-2
25. Djibril1 D, Mamadou F, Gérard V, Geuye MDC, Oumar S, Luc R. Physical characteristics and chemical composition of Neem seeds collected in Senegal. Res J Chem Sci. 2015;5(7):52–8.
26. Rawat S, Arunkumar AN, Annapurna D. Genetic diversity of *Melia dubia* using ISSR markers. Int J Genet. 2018;10(9):490–4.
27. Naji AK, Trivedi PG, Prajapati KV, Panchal MB, Sindhav GM.

Evaluation of genetic diversity in *Gmelina arborea* Roxb. Plant Mol Biol Rep. 2025;43(1):154–64. https://doi.org/10.1007/s11105-024-01445-5

28. Zou Y, Ge X, Yan C, Zhong Q, Chen D, Chen Z, et al. Assessment of genetic diversity of *Camellia yuhsienensis* based on leaf structure and inter simple sequence repeat (ISSR) markers. Genet Resour Crop Evol. 2024;71:4749–62. https://doi.org/10.1007/s10722-024-01939-w
29. Ijaz S, Ul Haq I, Razzaq HA, Nasir B, Babar M. ISSR-based population genetics study for tagging *Dalbergia sissoo* in Pakistan. Appl Ecol Environ Res. 2019;17(3):5851–61. https://doi.org/10.15666/aeer/1703_58515861
30. Javaid A, Akram W, Shoaib A, Haider MS, Ahmad A. ISSR analysis of genetic diversity in *Dalbergia sissoo*. Pak J Bot. 2014;46(5):1573–6.
31. Reeja S. Seed source evaluation of *Prosopis juliflora* DC [thesis]. Mettupalayam: Tamil Nadu Agricultural University; 2004.
32. Zheng J, Su H, Pu S, Chen H, El-Kassaby YA, Yang Z. High-yield hybrid breeding of *Camellia oleifera* based on ISSR markers. BMC Plant Biol. 2024;24(1):517. https://doi.org/10.1186/s12870-024-05202-6
33. Sevindik E, Okan K, Sevindik M, Ercisli S. Genetic Diversity and Phylogenetic Analyses of *Juglans regia* L. Erwerbs-Obstbau. 2023;65:311–20. https://doi.org/10.1007/s10341-022-00713-3
34. Ansari SA, Narayanan C, Wali SA, Kumar R, Shukla N, Rahangdale SK. ISSR markers for analysis of molecular diversity and genetic structure of Indian teak (*Tectona grandis* Lf) populations. Ann For Res. 2012;55(1):11–23.
35. Varsha AP, Shubhada RT. Inter population genetic diversity analysis in *Pinus roxburghii*. Int J Biodivers Conserv. 2012;4(5):219–27. https://doi.org/10.5897/IJBC11.233
36. Sandeep C, Kumar A, Rodrigues V, Viswanath S, Shukla AK, Sundaresan V. Morpho-genetic divergence and population structure in Indian *Santalum album* L. Trees. 2020;34(5):1113–29. https://doi.org/10.1007/s00468-020-01984-y
37. Çetin B, Sevindik E, Özkara Z, Filiz E, Baş E. ISSR Marker Based Genetic Variation Analysis in *Pterocarya fraxinifolia*. Biol Bull. 2025;52(2):66. https://doi.org/10.1134/S106235902460677X
38. Beyazyuz F, Gulbahce-Mutlu E, Semsettin K, Ertrugul F. Evaluation of Genetic Diversity of Chestnut Populations Using SCoT and ISSR Markers. Biol Bull. 2025;52(4):153. https://doi.org/10.1134/S1062359024606550
39. Guizado SJ, Nadeem MA, Ali F, Barut M, Habyarimana E, Gómez TP, et al. Genetic diversity and population structure of endangered rosewood from the Peruvian Amazon using ISSR markers. Acta Amazon. 2020;50(3):204–12. https://doi.org/10.1590/1809-4392201904121
40. Louati M, Ucarli C, Arikan B, Ghada B, Salhi Hannachi A, Turgut-Kara N. Genetic, morphological, and biochemical diversity of argan tree (*Argania spinosa* L.) (Sapotaceae) in Tunisia. Plants. 2019;8(9):319. https://doi.org/10.3390/plants8090319
41. Goyal P, Jain R, Kachhwaha S, Kothari SL. Assessment of genetic diversity in *Pithecellobium dulce* using RAPD and ISSR markers. Trees. 2015;29(3):637–53. https://doi.org/10.1007/s00468-014-1142-3
42. Bajpai A, Srivastava N, Rajan S, Chandra R. Genetic diversity and discrimination of mango accessions using RAPD and ISSR markers. Indian J Hortic. 2008;65(4):377–82.
43. Josiah CC, George DO, Eleazar OM, Nyamu WF. Genetic diversity in Kenyan populations of *Acacia senegal*. Afr J Biotechnol. 2008;7(14):2333–40.
44. Feyissa T, Nybom H, Bartish IV, Welander M. Analysis of genetic diversity in the endangered tropical tree species *Hagenia abyssinica* using ISSR markers. Genet Resour Crop Evol. 2007;54(5):947–58. https://doi.org/10.1007/s10722-006-9154-z
45. Gemas VJ, Almadanim MC, Tenreiro R, Martins A, Fevereiro P. Genetic diversity in the Olive tree cultivated in Portugal. Genet Resour Crop Evol. 2004;51(5):501–11. https://doi.org/10.1023/B:GRES.0000024152.16415.0d

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