Asian Journal of Biotechnology and Bioresource Technology

Volume 10, Issue 1, Page 63-73, 2024; Article no.AJB2T.113740
ISSN: 2457-0125

Genetic Assessment of *Leucaena leucocephala* (Lam.) de Wit Provenances by Using ISSR Markers

P. Chezhian [a], V. Prasath [a*], K. Jayakumar [a], P. Selvakrishnan [a], P. Leena Rosy [a], T. Stalin [a] and R. Rajesh [a]

[a] Department of Plantation, Tamil Nadu Newsprint and Papers Limited, Kagithapuram, Karur- 639136, India.

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJB2T/2024/v10i1199

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/113740

Original Research Article

Received: 21/12/2023
Accepted: 26/02/2024
Published: 29/02/2024

ABSTRACT

Inter Simple Sequence Repeats (ISSR) technique was deployed for the estimation of genetic diversity in18 *Leucaena leucocephala* provenances. Seeds of these provenances were collected from across the country and planted at a single location in South India. To estimate the genetic diversity/ polymorphism, DNA was extracted through Cetyl Trimethyl Ammonium Bromide (CTAB) method, visualized and analyzed the DNA bands on UV trans- illuminator gel doc (α-imager 3.2). Totally ten ISSR primers were used in this study. Among them, eight ISSR microsatellites showed a high degree of polymorphism in all the eighteen leucaena provenances used in our study. These primers successfully amplified a total of 645 bands, of which 577 bands (89 %) showed polymorphism. On average, 14.92 bands per accession were obtained and 68.28 % provenances were distinguished through these primers. The ISSR primer $T(GT)_9$ was able to distinguish 100 % of the *L. luecocephalla* provenances. Cluster analysis based on the genotypic similarities assembled

**Corresponding author: E-mail: prasath.v@tnpl.co.in;*

the 18 leucaena accessions in to three clusters. The dendrogram generated based on the 8 ISSR primers distinctively demarcated many of the *L. leucocephalla* accessions into different clusters indicating vast genetic diversity among the provenances. The high degree of genetic variability demonstrated by the ISSR molecular markers in *L. leucocephalla* can be used as a tool for assessing genetic diversity and in future leucaena genetic improvement programs.

Keywords: Leucaena leucocephalla; ISSR; polymorphism; genetic variability.

1. INTRODUCTION

Leucaena leucocephala (Lam.) de wit is one of the long-lived and fastest growing leguminous tree species originated from Southern Mexico and widely spread across 35 countries in various continents except Antartica. It is also popularly known as Subabul in India and due to its high biomass productivity and vigorous coppicing ability become more preferable species among the tree cultivars to produce huge quantity woody biomass for various applications. This leguminous species is important and encouraged under social forestry schemes for energy, fuel and food purposes. In India around 25% of hardwood raw material for pulp and paper industry comes from *L. leucocephala* plantation [1].

In recent times, genetic improvement of *L. leucocephala* in India proved its good adaptability and suitability for biomass production as industrial raw material. The pulp quality is highly suitable for paper making. The fast growing nature of *L. leucocephalla* species can be a viable preference in the industrial supply chain mechanism to the pulp and paper industry's speedy development and large volume of raw material requirement [2]. This pulpwood material is string, light in weight, easy to work and gives more attractive finish [3]. The major research priority for industrial application of *L. leucocephala* has been focused on straight boles, fewer branches and higher proportion of wood and wider adaptability to different sites [4].

The advancement of molecular bio-technology plays a key role in genetic population studies, particularly detecting genetic variations or polymorphism among and in between *L. leucocephalla* traits cultivated across Indian subcontinent. Molecular markers have proved as a tool which is valuable in evaluation of genetic diversity between species and population. The choice of molecular marker technique depends on the mode of inheritance, reproducibility and simplicity [5]. A wide range of molecular markers such as RAPD, ISSR, SSR, AFLP and RFLP are available in fingerprinting of cultivars, germplasm identification and purity test. Among them, ISSR markers are most reliable, simple, quick and cost effective [6,7]. The ISSR method provides an alternative choice to other systems for obtaining highly reproducible markers without any necessity for prior sequence information for various genetic analyses. Because of those abundant and rapidly evolving SSR regions, ISSR amplification has the potential of revealing larger numbers of polymorphic fragments per primer than any other marker system used [8,9]. Brown-Guedira et al. [10] observed a higher level of genetic diversity with the SSR system compared to RAPD and other markers. Therefore, ISSR technique is being used extensively in several applications including molecular biology, plant breeding and conservation of germplasm [11,12].

In our present study, we examined the genetic diversity in eighteen provenance sources of *L. leucocephala* corresponding to industrial application i.e. raw material for pulp and paper industry by using ISSR markers.

2. MATERIALS AND METHODS

2.1 Provenance Sources and Trial Details

Eighteen *Leucaena leucocephala* accessions were planted in plantation research block of Tamil Nadu Newsprint and Papers Limited, Karur District in Tamil Nadu state (N11°06.0748', E 077° 98.6061': 459 ft a.s.l). The details of accessions studied in the present study are given in Table 1. Fifty ramets of each accession were planted in red sandy loam soil having the pH range of 7-8. This site falls under semi-arid climate with mean rainfall of 745 mm per annum.

2.2 DNA Extraction

DNA was isolated from juvenile leaf tissues collected from 24 months old seedlings by the CTAB method described by Doyle and Doyle [13] as reported by Rawat et al. [14]. Total genomic DNA was extracted from 100 mg of fresh, non-microbial young leaves collected in five

replications of 18 provenances (90 individuals). The RNA was removed from the DNA extract by treating it with RNase A (1 mg ml^{-1}) for 30 minutes at 37°C. The resultant DNA was dissolved in TE buffer and its concentration was estimated by agarose gel (0.8%) electrophoresis with α DNA (Bangalore Genei Ltd., India) as the standard.

2.3 DNA Quantification and Analysis through ISSR Molecular Markers

Quantification of DNA was done by reading the absorbance at 260 and 280 nm in a spectrophotometer. The purity of DNA was checked by running the samples on 0.8% agarose gel in 1X TAE buffer (pH 8.0) stain containing 2 ml of 0.3% Ethidium Bromide and visualized on UV trans illuminator gel doc (Alfa imager 3.2) to analyze the DNA bands.

Ten ISSR primers were screened for analysis and DNA amplification was carried out in 10 ml reaction volume containing genomic DNA 0.80 ml (30 ng/ml), 10 mM 1ml primers, 1 ml PCR buffer, 0.40 ml dNTPs, 0.40 ml $MgCl_2$ (0.3 u), 0.10 ml Taq polymerase and 6.30 ml RNase free double distilled water. Amplification cycle consists of an initial 5 minutes denaturation at 94°C, 35 cycles for 1 minute at 50°C, 2 minutes 72°C and final extension step for 7 minutes at 72°C. The ISSR primers used in this study for PCR amplification is given in Table 2, which were obtained from University of British Colombia and Centre for DNA fingerprinting and Diagnostics, Hydrabad, India and are widely used for genetic assessment of pulpwood tree crops in specific. There were totally 18 primers initially screened for polymorphism of *L. leucocephalla*. Of these 10 primers which were shown polymorphism were chosen to study and remaining 8 monomorphic primers were excluded.

The amplified product loaded with 2 µl 6x loading buffer were size fractionated by electrophoresis on a 2% for ISSR agarose gel with 1 kb ladder DNA using inter chelating agent ethidium bromide and visualized on UV transilluminator to determine the amplified clear bands to validate the DNA quality and suitability for PCR reactions.

2.4 ISSR Amplification

ISSR amplifications were performed in 10µl reactions volumes consisting 1 µl of 2.5 µMol/L primers, 0.80µl of DNA template of *L. leucocephala* species samples, 1µl 10X PCR buffer, 2.5 mM $MgCl_2$, 40 µM dNTPs mix, 0.3 U Taq DNA polymerase and finally 6.30 ml of ddH_2O. The PCR condition was Initial

Table 1. Details of *Leucaena leucacephala* accessions examined in this study

Accession No	Accession Name	Source of the Accessions	Total trees and tree identity
1	BAIF1	BAIF Development Research Foundation, Pune, Maharastra, India	50 (B23)
2	BAIF2		50 (B17)
3	CRIDA1	Central Research Institute for Dryland Agriculture, Hydrabad, Andra Pradesh, India	50 (CR6)
4	CRIDA2		50 (CR33)
5	CRIDA3		50 (CR49)
6	FCRI1	Forest College and Research Institute, Mettupalayam, Tamil Nadu Agricultural University, Tamil Nadu, India	50 (TNAU26)
7	FCRI2		50 (TNAU4)
8	FCRI3		50 (TNAU12)
9	FCRI4		50 (TNAU17)
10	FCRI5		50 (TNAU43)
11	NAV1	Navsari Agricultural University, Navsari, Gujarat, India.	50 (NAU36)
12	NAV2		50 (NAU25)
13	NAV3		50 (NAU44)
14	NAV4		50 (NAU36)
15	CFRHRD1	Center for Forestry Research and Human Resource Development, Chhindwara, Madya Pradesh, India	50 (CFMP16)
16	CFRHRD2		50 (CFMP5)
17	CFRHRD3		50 (CFMP43)
18	CFRHRD4		50 (CFMP29)

Table 2. Characteristics of ISSR primers used for PCR amplification

Sl. no.	Name of the primer	Nucleotide sequence	Tm (°C)	Amplification
1	$R(CA)_7$	GRTRCYGRTRCACACACACACACA	72	Polymorphic
2	$T(GT)_9$	CRTAYGTGTGTGTGTGTGTGTGT	72	Polymorphic
3	$TA(CAG)_4$	ARRTYCAGCAGCAGCAG	72	Polymorphic
4	$RA(GCT)_6$	AYARAGCTGCTGCTGCTGCTGCT	72	Polymorphic
5	$(GA)_8R$	GAGAGAGAGAGAGAGARGY	72	Polymorphic
6	UBC810	GAGAGAGAGAGAGAGAT	72	Polymorphic
7	UBC842	GAGAGAGAGAGAGAGAYG	72	Polymorphic
8	$(CAG)_5$	CAGCAGCAGCAGCAG	72	Polymorphic
9	$(GACA)_4$	GACAGACAGACAGACA	72	Polymorphic
10	$(AG)_8TC$	AGAGAGAGAGAGAGAGTC	72	Polymorphic

Y=(C, T); R=(A, G)

denaturation at 94°C for 5 minutes followed by 35 cycles of 1 minute at 94°C, 1 minute at 50°C, 2 minutes at 72°C and final extension of 7 minutes at 72°C. PCR was executed in "Techne 5000" 96 well Thermal Cycler". Totally ten ISSR primers (Table 2) were tested initially. Eight primers amplified DNA well with polymorphic bands and were selected for further use.

2.5 Cluster Analysis

Each distinct band in the gel was assigned an identification number based on its position and scores in visual observations i.e. present (1) or absent (0) for each of the 90 individuals with all the primers used in this study. The scores of all primers were combined and data matrix was created. The data matrix was used to construct UPGMA (Un weighted Pair Group Method of Arithmetic means) dendrogram among the *L. leucocephalla* provenances.

3. RESULTS AND DISCUSSION

Genetic assessment through DNA fingerprinting of 18 *L.leucocepahlla* provenances using ISSR markers namely R $(CA)_7$, $T(GT)_9$, $TA(CAG)_4$, $RA(GCT)_6$, $(GA)_8R$, UBC 810, UBC842, $(CAG)_5$, $(GACA)_4$ and $(AG)_8TC$ revealed high intensity bands with no smearing except the primers $R(CA)_7$ and $(GACA)_4$.

3.1 Band Size

The amplified band size for 18 leucaena provenances ranged from 100 to 2000 bp (Table 3). The highest band size range of 200-2000bp was recorded with the primer $(CAG)_5$ and the narrow band size of 250-1100 bp was observed with $T(GT)_9$. Differences in samples and size of alleles contribute to the variation in the band sizes of all the primers [15].

3.2 Number of Bands, Polymorphism and Provenances Distinguished with ISSR Markers

The total number of bands scored, number of bands showing polymorphism, visible number of bands per provenance and per cent provenance distinguished with the respective primers are depicted in Table 3. The respective profiles of all 18 *L. leucocephalla* provenances using the primer UBC 842 (which showed the highest percent of polymorphism) is shown in Fig. 1. A total of 645 loci were amplified from 90 individuals belonging to the 18 provenances and total number of amplified loci per ISSR primer varied from 74 (with RA $(GCT)_6$) to 89 (with T $(GT)_9$ and TA $(CAG)_4$). Polymorphism with a range of 97 % (UBC842) to 79 % ($(CAG)_5$) was recorded in the present estimation. The primer $T(GT)_9$ showed 85 polymorphic bands and lease number of 61 polymorphic bands was observed with $(CAG)_5$. The highest mean 16.47 bands were visible with the primer $T(GT)_9$, followed by $TA(CAG)_4$ with recording mean number of bands as 16.45. All other primers recorded within the range of 13.69 to 14.80 bands per provenance. Among all, the primer $T(GT)_9$ was able to distinguish all the 18 provenances of *L.leucocephalla* (100 %), followed by $TA(CAG)_4$ with 97.5 % of corresponding parameter. The primer $RA(GCT)_6$ performed the least (43.50 %) in differentiating the leucaena provenances. The remaining five primers ranged from 57.50 to 62.75 % in this regard.

Earlier work carried out in India by Rajarajan et al. [16] in studying genetic variability among 21 *L.*

leucocephala accessions using simple SSR markers resulted in 82.57 % of polymorphism among the traits. Similar kind of analyzing genetic variations among the accessions of other pulpwood species like *Casuarina* [17,5], *Eucalyptus* [18-20] and *Populus* [21,22] were already well documented.

3.3 Percent Polymorphism Recorded in Provenances with ISSR Markers

The degree of polymorphism found in the 18 *L. leucocephalla* provenances accessed in our study with all the ISSR primers is presented in Table 4. The provenances BAIF1 and NAV3 recorded 100 % polymorphism with all the primers. Following this, the provenances CRIDA2, TNAU4, TNAU5, NAV1, CFRHRD1 and CFRHRD4 recorded > 90 % polymorphism. The provenance CRIDA2 showed the least polymorphism of 54.29 %. The remaining 9 provenances recorded the polymorphism in the range of 82.86 to 89.54 %.

Leucaena leucocephalla, being a tetraploid species (2n=4X=104) and regulated with diversified gene sequences that are responsible for different functional characteristics like tetraploid biosynthesis [23], drought tolerance [24], foliage nutrient content [25], chitinase antifungal biosynthesis [26], monolignolol biosynthesis [27], mimosine biosynthesis [28] and genes related to environmental stress response [29]. These diversified gene sequences in turn expressed as high degree of polymorphism among the various provenances of *L. leucocephalla* [30].

3.4 Cluster Analysis of *L. leucocephalla* Genetic Accessions

The dendrogram of ISSR data was able to clearly distinguish the *L. leucocephalla* accessions and established the uniqueness of the each provenances. Distinct clusters and sub-clusters were produced among the accessions, showing appreciable genetic diversity of *L. leucocephalla* species.

The application of unweighted pair group method of arithmetic means (UPGMA) resolved the 18 genotypes into three major clusters having 6, 7 and 5 germplasm in C1, C2 and C3 clusters respectively. As stated by Pande et al. [31] and Chavan and Keerthika [32], the variation in genotypic coefficient found in the dendrogram among *L. leucocephala* provenances indicates that considerable inter- genotypic variation exists and that could be a major tool for further genetic improvement in selecting site specific suitable traits under large scale planting. Further, the genetic diversity of *Leucaena leucocephalla* provides an opportunity to improve in specific applications as raw materials for various industries [33], including pulp and paper industries in India [34].

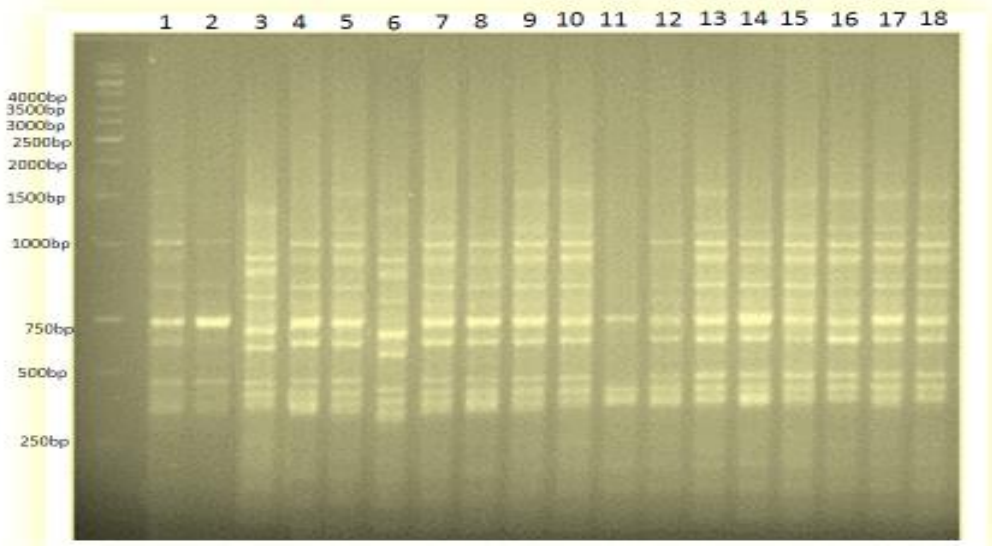

Fig. 1. ISSR profiles for 18 accessions of *L. leucocephalla* based on the primer UBC842
(lane1- BAIF1, lane2- BAIF2, lane3- CRIDA1, lane4- CRIDA2, lane5- CRIDA3, lane6- FCRI1, lane7- FCRI2, lane8- FCRI3, lane9- FCRI4, lane10- FCRI5, lane11- NAV1, lane12- NAV2, lane13- NAV3, lane14- NAV4, lane15- CFRHRD1, lane16- CFRHRD2, lane17- CFRHRD3 and lane18- CFRHRD4)

Table 3. Microsatellite primers used in this study with their corresponding scores, size range, percent polymorphism, number of bands per provenance and number of provenance distinguished in 18 *Leucaena leucocephella* accessions using ISSR primers

Primer	Loci size range (bp)	Total No of Bands Scored	No of bands		Percentage		Corresponding bands	
			Mono morphic	Poly morphic	Mono morphism	Poly morphism	Number of bands per provenance	Provenance distinguished (%)
$T(GT)_9$	250 - 1100	89	4	85	4	96	16.47	100.00
$(AG)_8TC$	250 - 1400	79	5	74	6	94	14.62	62.50
CAG_5	200 -2000	77	16	61	21	79	14.25	57.50
UBC842	100 - 1150	78	2	76	3	97	14.43	60.00
$(GA)_8R$	250 -1500	80	11	69	14	86	14.80	62.75
UBC 810	150 -1500	79	12	67	15	85	14.62	62.50
$RA(GCT)_6$	150 -2000	74	8	66	11	89	13.69	43.50
$TA(CAG)_4$	200 -1500	89	10	79	11	89	16.45	97.50
Total	-	645	68	577	-	-	-	-
Mean	-	80.63	8.50	72.13	11	89	14.92	68.28

Table 4. Degree of polymorphism found in 18 *Leucaena leucocephella* provenances using ISSR primers (values in %)

Provenance	ISSR Primers								
	$T(GT)_9$	$(AG)_8TC$	$(CAG)_5$	UBC842	$(GA)_8R$	UBC 810	RA $(GCT)_6$	TA $(CAG)_4$	Mean
BAIF1	100	100	100	100	100	100	100	100	100.00
BAIF2	100	100	100	100	60	67	100	80	89.54
CRIDA1	100	100	80	100	80	33	100	63	84.76
CRIDA2	100	100	100	100	80	80	100	100	94.29
CRIDA2	100	100	80	100	0	0	0	100	54.29
TNAU1	100	40	80	100	100	100	90	100	87.14
TNAU2	100	100	40	100	100	80	100	100	88.57
TNAU3	100	80	60	100	80	100	60	40	82.86
TNAU4	100	100	100	100	100	70	100	100	95.71
TNAU5	60	100	100	100	100	100	100	100	94.29
NAV1	80	100	100	100	100	100	100	100	97.14
NAV2	100	100	60	100	100	100	60	100	88.57
NAV3	100	100	100	100	100	100	100	100	100.00
NAV4	100	100	60	100	67	100	100	80	89.54
CFRHRD1	80	100	60	100	100	100	100	100	91.43
CFRHRD2	100	100	40	80	100	100	100	100	88.57
CFRHRD3	100	67	60	100	80	100	100	60	86.68
CFRHRD4	100	100	100	60	100	100	100	80	94.29
Mean	95.56	93.71	78.89	96.67	85.93	85.00	89.44	89.03	89.31

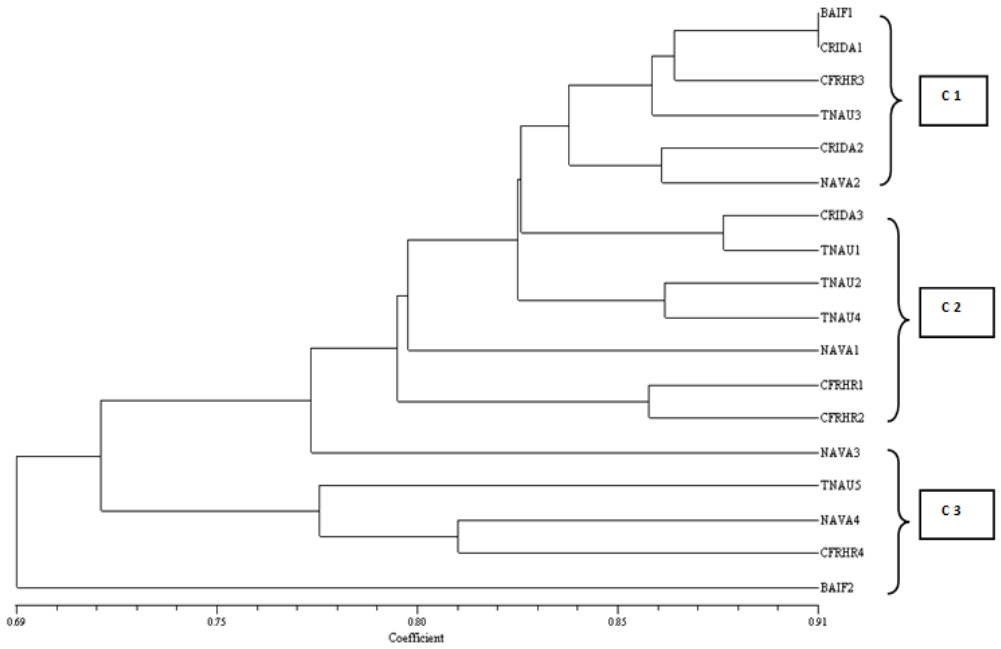

Fig. 2. Dendrogram showing genetic relationship among 18 accessions of *L. leucocephalla* based on UPGMA analysis using 8 ISSR primers

4. CONCLUSION

ISSR primers are effective in quantification of genetic variability among eighteen *Luecaena leucocephalla* provenances. The highly effective primer in the present study in distinguishing the accessions is $T(GT)_9$, which could able to distinguish all the *Leucaena leucocephalla* provenances. Therefore, this study concludes that the high degree of genetic variability among the *Leucaena leucocephalla* provenances described by the ISSR markers can be used as a basic contrivance for linkage mapping in Leucaena genetic improvement programs.

ACKNOWLEDGEMENTS

The authors obliged deepest gratitude to Tamil Nadu Newsprint and Papers Limited (TNPL), Karur for their support of this research and providing all necessary resources. The authors owe a huge debt of thanks to late Dr. R. Seenivasan, former Executive Director of TNPL, who opened up the path of clonal forestry and plantation research at TNPL.

COMPETING INTERESTS

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

REFERENCES

1. Kulkarni HD. Private farmer and private industry partnerships for industrial wood production. A case study. International Forestry Review. 2008;10:147–155.
2. Pandey VC, Kumar A. *Leucaena Leucocephalla*: An underutilized plant for pulp and paper production. Genetic Resources and Crop Evolution. 2013;60: 1165-1171.
   Available:https://doi.org/10.1007/s10722-012-9945-0
3. Chavan SB, Uthappa AR, Keerthika A, Parthiban KT, Vennila S, Kumar P, Anbu PV, Newaj R, Sridhar KB. Suitability of *Leucaena leucocephala* (Lam.) de Wit as a source of pulp and fuelwood in India. Journal of Tree Science. 2015;34(1):30–38.
4. Suraj PG, Hegde R, Varghese M, Kamalakannan R, Sahoo DR, Bush DJ, Harwood CE. Growth and pulpwood traits of *Leucaena leucocephala* and *Eucalyptus camaldulensis* at rainfed and irrigated sites in south India. Trees, Forests and People; 2024.

Available:https://doi.org/10.1016/j.tfp.2023.100482

5. Pauldasan A, Vipin P, Durai A, Nicodemus A. Characterization and identification of four species of casuarina and their interspecific hybrids using EST-SSR markers. Research Journal of Biotechnology. 2021;16(5):126-132.
6. Paglia GP, Olivieri AM, Morgante M. Towards second generation STS (sequence- tagged sites) linkage maps in conifers: a genetic map of Norway spruce (*Picea abies* K.), Molecular Genetics and Genomics. 1998;258:466-478.
Available:https://doi.org/10.1007/s004380050757
7. Kamalakannan R, Barthwal S, Chezhian P, Balasaravanan T, Yasodha R, Gurumurthi K, Ghosh M. Morphological and molecular diversity *Casuarina* and *Allocasuarina* species, Biotechnology. 2006;5(3):301-307
8. Moarefi N, Michael P, Beckett P, Nkongolo, KK. Identification of Molecular Markers Differentiating *Betula papyrifera* and *B. pumila* Populations from Northern Ontario (Canada). American Journal of Environmental Sciences. 2018;14:246-256.
Available:https://doi.org/10.3844/ajessp.2018.246.256
9. Boyd M, Panoyan MA, Michael P, Nkongolo KK. Development and characterization of species-diagnostic ISSR and SCAR DNA markers differentiating red maple (*Acer rubrum*) and Silver Maple (*A. saccharinum*). Genome. 2019;62: 527-535.
Available:https://doi.org/10.1139/gen-2019-0037
10. Brown-Guedira GL, Thompson JA, Nelson RL, Warburton ML. Evaluation of genetic diversity of soybean introductions and north American ancestors using RAPD and SSR Markers. Crop Science. 2000;40: 815-823.
Available:https://doi.org/10.2135/cropsci2000.403815x
11. Dayanandan S, Rajora OP, Bawa KS. Isolation and characterization of microsatellites in trembling aspen (*Populus* tremuloides), Theory of Applied Genetics. 1998; 96: 950-956.
Available:https://doi.org/10.1007/s001220050825
12. Moyib OK, Odunola OA. Dixon AGO. SSR markers reveal genetic variation between improved cassava cultivars and landraces within a collection of Nigerian cassava germplasm. African Journal of Biotechnology. 2007;6:2666-2674.
Available:https://doi.org/10.5891/AJB2007.000-2427
13. Doyle JJ, Doyle JL. A rapid DNA Isolation procedure for small quantities of fresh Leaf Tissue. Phytochemical Bulletin. 1987;19: 11-15.
14. 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. American Journal of Plant Sciences. 2016; 7: 437-445.
Available:http://dx.doi.org/10.4236/ajps.2016.73037
15. Hasan MK, Khan NA, Ghose AK, Mukta SP, Hossain MA, Sen A, Akter MM. Assessment of genetic diversity and fingerprinting of sugarcane varieties using simple sequence repeat (SSR) markers. Asian Journal of Research in Agriculture and Forestry. 2023; 9(4): 134-142.
Available:https://doi.org/10.9734/AJRAF/2023/v9i4240
16. Rajarajan K, Uthapa AR, Handa AK, Chavan SB, Vishnu R, Srivastava A, Handa A, Rana M, Sahu S, Kumar N, Singh AK, Ahmed S, Rana M. Genetic diversity and population structure of *Leucaena lueocephalla* (Lam.) de wit genotypes using molecular and morphological attributes. Genetic Resources and Crop Evolution; 2021.
Available:https://doi.org/10.1007/s10722-021-01203-7
17. Kullan ARK, Kulkarni AV, Kumar RS Rajkumar R. Development of microsatellite markers and their use in geneticdiversity and population structure analysis in Casuarina. Tree Genetics and Genomes. 2016;12(49).
Available:https://doi.org/10.1007/s11295-016-1009-8
18. Balasaravanan T, Chezhian P, Kamalakannan R, Yashoda R, Varghese M, Gurumurthy K, Ghose M. Identification of species- diagnostic ISSR markers for six *Eucalyptus* species. Silvae Genetica. 2006;55(3):119-122.
Available:https://doi.org/10.1515/sg-2006-0017

19. Arumugasundaram S, Ghosh M, Veerasamy S, Ramasamy Y. Species discrimination, population structure and linkage disequilibrium in *Eucalyptus camaldulensis* and *Eucalyptus tereticornis* using SSR markers. PloS One. 2011;6 (12):e28252.
Available:https://doi.org/10.1371/journal.pone.0028252
20. Lv J, Li C, Zhou C, Chen J, Li F, Weng Q, et al. Genetic diversity analysis of a breeding population of *Eucalyptus cloeziana* F. Muell. (Myrtaceae) and extraction of a core germplasm collection using microsatellite markers. Industrial Crops and Products. 2020;145: 112157.
Available:https://doi.org/10.1016/j.indcrop.2020.112157
21. Khasa DP, Nadeem S, Thomas B, Robertson A, Bousquet J. Application of SSR markers for parentage analysis of Populus clones. Forest Genetics. 2003; 10(4):273- 281.
22. Xiang X, Zhohu X, Zi H, Wei H, Cao D, Zhang Y, Zhang L, Hu J. *Populus cathayana* genome and population resequencing provide insights into its evolution and adaptation. Horticulture Research. 2024;11:uhad255.
Available:https://doi.org/10.1093/hr/uhad255
23. Ishihara KL, Honda MDH, Pham DT, Borthakur D. Transcriptome analysis of *Leucaena leucocephala* and identification of highly expressed genes in roots and shoots. Transcriptomics. 2016;4:135.
Available:https://doi.org/10.4172/2329-8936.1000135
24. Honda MDH, Ishihara KL, Pham DT, Borthakur D. Identification of drought-induced genes in giant leucaena (*Leucaena leucocephala* subsp. glabrata). Trees. 2018;32:571–585.
Available:https://doi.org/10.1007/s00468-018-1657-4
25. Honda MDH, Borthakur D. Highly expressed genes in the foliage of giant leucaena (*Leucaena leucocephala* subsp. *glabrata*), a nutritious fodder legume in the tropics. Plant Biosystems. 2019; 154(1):107-116.
Available:https://doi.org/10.1080/11263504.2019.1578283
26. Kaomek M, Mizuno K, Fujimura T, Sriyotha P, Cairns JRK. Cloning, expression, and characterization of an antifungal chitinase from *Leucaena leucocephala* de Wit. Bioscience Biotechnology Biochemistry. 2003;67(4):667–676.
Available:https://doi.org/10.1271/bbb.67.667
27. Shaik NM, Misra A, Singh S, Fatangare AB, Ramakumar S, Rawal SK, Khan BM. Functional characterization, homology modeling and docking studies of b-glucosidase responsible for bioactivation of cyanogenic hydroxynitrile glucosides from *Leucaena leucocephala* (subabul). Molecular Biology Reports. 2013; 40: 1351–1363.
Available:https://doi.org/10.1007/s1133-012-2179-6
28. Jube SL, Borthakur D. Transgenic *Leucaena leucocephala* expressing the Rhizobium gene pydA encoding a meta-cleavage dioxygenase shows reduced mimosine content. Plant Physiology and Biochemistry. 2010;48(4):273–278.
Available:https://doi.org/10.1016/j.plphy.2010.01.005
29. Negi VS, Pal A, Singh R, Borthakur D. Identification of species-specific genes from *Leucaena leucocephala* using interspecies suppression subtractive hybridization. Annals of Applied Biology. 2011;159:387–398.
Available:https://doi.org/10.1111/j.1744-7348.2011.00506.x
30. Bageel A, Honda MDH, Carrillo T, Borthakur D. Giant leucaena (*Leucaena leucocephalla* subsp..*glabrata*): A versatile tree- legume for sustainable agroforestry. Agroforestry Syatems. 2020;94:251-268.
Available:https://doi.org/10.1007/s10457-019-00392-60123458
31. Pandey VC, Kumar A. Leucaena Leucocephalla: An underutilized plant for pulp and paper production. Genetic Resources and Crop Evolution. 2013;60: 1165-1171.
Available:https://doi.org/10.1007/s10722-012-9945-0
32. Chavan S, Keerthika A. Genetic Variability and Association Studies among Morphological traits of *Leucaena leucocephala* (Lam.) de Wit. Genetic Resources. Research Journal of Agriculture and Forestry Sciences. 2013; 1(8):23- 29
33. Real D, Revell C, Han Y, Li C, Castello M, Bailey CD. Successful creation of seedless (sterile) leucaena germplasm developed from interspecific hybridization for use as

forage. Crop and Pasture Science. 2022; 74(7-8):783-796.
Available:https://doi.org/10.1071/CP22281

34. Khanna NK, Shukla OP, Gogate MG, Narkhede SL. Leucaena for paper industry in Gujarat, India: Case study. Tropical Grasslands-Forrajes Tropicales. 2019;7: 200–209.
Available:https://doi.org/10.17138/tgft(7)200-209

Peer-review history:
The peer review history for this paper can be accessed here:
https://www.sdiarticle5.com/review-history/113740