

Original Research

Assessment of Genomic Integrity of *Vitex negundo* L., An Important Indian Medicinal Plant, Using RAPD Markers

Shweta Chaudhary¹, Gunjan Garg¹, Alok Bharadwaj^{2*}

Abstract

Vitex negundo is an Indian medicinal plant containing steroids, flavonoids, lignans and terpenoids that can be used as a precursor for commercial production. An efficient marker system such as Random Amplified Polymorphic DNA (RAPD) was used to assess the genetic integrity of *V. negundo*. The straightforward method RAPD can be used to evaluate genomic integrity because it uses a small amount of DNA for PCR amplification. Six out of thirteen RAPD primers generated 150 distinct bands, of which 31 were polymorphic, with an average of 5.16 polymorphic bands per primer. A maximum of up to 32 fragments were amplified, and an average of 25 per primer, and the amplicons varied in size between 100 and 2000bp. The percentage of polymorphism ranges from 12.9 to 22.5, with an average of 16.6. The PIC values ranged from 0.11 to 0.63 for RAPD primers. The study pointed out that RAPD markers evaluate the genetic fidelity in *Vitex negundo*. The UPGMA cluster analysis grouped all in vitro raised plantlets treated with different growth regulators such as BAP, DPU, TDZ, and mT. The principal component analysis also substantiates this clustering pattern. Thus, the phylogenetic relationship and a high genetic variation revealed in the present study could provide baseline data for the conservation and improvement of this plant in future. Also, the molecular marker identified in this study will be helpful in the authentication of this species to prevent adulteration in herbal medicine.

Keywords

Genetic integrity, Molecular marker, RAPD, *Vitex negundo*, Polymorphism, plant growth regulators, clustering analysis

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Ocena genomske celovitosti *Vitex negundo* L., pomembne indijske zdravilne rastline, z uporabo označevalcev RAPD

Izvleček

Vitex negundo je indijska zdravilna rastlina, ki vsebuje steroide, flavonoide, lignane in terpenoide, ki se lahko uporabljajo kot predhodnik za komercialno proizvodnjo. Za oceno genetske celovitosti *V. negundo* je bil uporabljen učinkovit markerski sistem Random Amplified Polymorphic DNA (RAPD). Preprosta metoda RAPD se lahko uporablja za ovrednotenje genomske celovitosti, ker uporablja majhno količino DNA za pomnoževanje PCR. Šest od trinajstih primerjev RAPD je ustvarilo 150 različnih pasov, od katerih je bilo 31 polimorfni, s povprečno 5,16 polimorfnimi pasovi na začetni oligonukleotid. Pomnoženih je bilo največ do 32 fragmentov, v povprečju pa 25 na začetni oligonukleotid, velikosti pomnožkov pa so se gibale med 100 in 2000 bp. Odstotek polimorfizma se giblje od 12,9 do 22,5, v povprečju 16,6. Vrednosti PIC so se gibale od 0,11 do 0,63 za primerje RAPD. Študija je poudarila, da markerji RAPD ocenjujejo genetsko zvestobo pri *V. negundo*. Analiza grozdov UPGMA je združila vse rastline, vzgojene *in vitro*, tretirane z različnimi regulatorji rasti, kot so BAP, DPU, TDZ in mT. Analiza glavnih komponent tudi utemeljuje ta vzorec združevanja v gruče. Tako bi lahko filogenetski odnos in velika genetska variacija, razkrita v tej študiji, zagotovila osnovne podatke za ohranjanje in izboljšanje te rastline v prihodnosti. Molekularni marker, identificiran v tej študiji, bo prav tako v pomoč pri avtentifikaciji te vrste, da se prepreči ponarejanje v zeliščni medicini.

Ključne besede

Genetska celovitost, molekularni marker, RAPD, *Vitex negundo*, polimorfizem, rastlinski rastni regulatorji, analiza grozdenja

Introduction

Vitex negundo L., also known as "Nishinda", is a woody, fragrant, and therapeutic shrub that is a member of the Verbenaceae family (Koirala et al., 2020). In India, this plant grows haphazardly and is frequently utilised as a hedge. The plant is erect, thin, and ranges in height from 2 to 5 metres. It can be found in China, Madagascar, Ceylon, the Philippines, India, Afghanistan, and Tropical Africa (Manokari, Priyadharshini, and Shekhawat 2021). The leaves have five leaflets in a palmate arrangement, which are lanceolate 4-10 cm long, hairy beneath and pointed at both ends. There are lots of bluish-purple blossoms. The fruit is spherical, about 4 mm in diameter, black when ripe, and succulent (Tawfeeq et al., 2023). According to a phytochemical analysis of this plant, the leaves contained the following compounds: 5,3-dihydroxy-3,6,7,4-tetramethoxylavone, hydroxyl-3,6,7,3,4-penta methoxy flavone, monoterpenes agnuside, flavonoids-casticin, chryso-sphenol and vitexin, flavonoids (vitexicarpin) (Alfarabi et al. 2022). *V. negundo* leaves have antibacterial, antitumor, astrin-

gent, febrifuge, sedative, tonic, and vermifuge properties (Vigneswari et al., 2023). Insecticidal action is observed in leaf extracts of this plant (Edwin and Jacob 2017). New leaves are burned with grass, which works as a fumigant against mosquitoes. (Alfarabi et al. 2022). According to Duke and Ayensu (1985), the fruit is also used to cure rheumatic problems, coughs, angina, colds, and other ailments. The root has febrifuge, expectorant, and tonic properties (Goswami and Roy, 2023). Medicinal plants are of great interest to researchers in the field of biotechnology as most of the drug industries depend, in part, on plants for the production of pharmaceutical compounds (Noor et al., 2022). Techniques for *in vitro* culture provide a practical means of conserving the germplasm and mass-multiplying uncommon, endangered, fragrant, and therapeutic plants (Mishra et al., 2022). Ever-increasing interest in *in vitro* culture techniques has been applied not only for the multiplication of several rare species of great importance but also for cloning elite types of plants on a larger scale. *In vitro* techniques are effectively utilised for germplasm conservation of rare, endangered, aromatic and medicinal (Priyanka et

al. 2021). Interspecific and intraspecific components make up biodiversity (Samanta et al., 2023). Because ecologists have been conducting diversity assessments for a long time, they are frequently restricted to species surveys and ignore the intra-specific components of diversity (Bublyk et al., 2020). Genetic variety is necessary for natural populations to continue as evolutionarily viable units capable of long-term adaptation to changing conditions (Iosefa et al. 2016). It is anticipated that geographic isolation will have a major impact on the population's genetic structure (Peng et al., 2021). The long-term evolutionary history of the species (distance changes, habitat fragmentation, and population isolation), mutation, genetic drift, mating system, gene flow, and selection are some of the processes whose interactions are reflected in the genetic structure of plant populations (Zhang et al. 2019).

The pharmaceutical industry uses medicinal plants as a major source of raw materials. Approximately 92% of the medicinal plants that are harvested destructively from the wild to make traditional medicines are used by industries. If biodiversity is not managed sustainably, there is a clear risk to the genetic stocks and diversity of medicinal plants (Savitikadi et al., 2020). In Indian traditional medical systems, *Vitex negundo* L. has long been employed. However, the preservation of this plant species has received little to no attention up to this point. Therefore, the goal of the current investigation was to ascertain the genetic integrity between the parent plant and treated explants that had varying concentrations of *V. negundo* plant growth regulators (Zavinon et al., 2020). Genetic variations are accumulated by geographically separated populations as they adjust to varying environmental conditions (Singer et al. 2021). Due to the impact of numerous environmental factors, genetic fidelity study based on morphological and biochemical criteria has many limitations. Because molecular markers are independent of environmental factors, they are useful for genetic diversity studies. In order to evaluate the genetic diversity of species from different phytogeographical regions, the RAPD technique has been effectively applied (Bi et al. 2021). The method was frequently used to estimate genetic links between and within species (Boomibalagan et al., 2021).

Maintaining genetic integrity is critical for conserving medicinal qualities and adaptability of *V. negundo*, as genetic fidelity ensures the stability of therapeutic compounds and allows for consistent biotechnological applications. A genetic resource management strategy for such

species needs to be based on research data examining the extent of genetic differentiation within and between populations and on understanding the processes maintaining this variation. Random Amplified Polymorphic DNA(RAPD) is a simple technique that requires a small amount of DNA for PCR amplification and can be used for genotoxicity assessment (Srinivasan et al., 2021).

There could be variations in the final DNA profiles because of band shifts, absent bands, or the emergence of new bands. These bands are assessed to assess genetic dissimilarities or similarities (Rohela et al. 2019). Furthermore, their potential to serve as the foundation for novel biomarker assays for the identification of DNA damage and mutations in the living tissues of bacteria, plants, and animals is suggested by their use in surveying genomic DNA to detect different kinds of DNA destruction and mutations (example-rearrangements, point mutations, small insert or deletion of DNA, and ploidy changes) (Dang et al. 2022). In this paper, we evaluated the use of RAPD to detect genetic integrity and gene flow of the *V. negundo*, which was conducted when explants were treated with different plant growth regulators.

The aim of the present communication was (1) to standardize a reproducible protocol, which can be used at a commercial scale, for mass propagation using nodal explants derived from the mother plant and (2) to evaluate the genetic homogeneity among the generated plants by adopting molecular technique.

Materials and Methods

Plant material and extraction of genomic DNA

We gather samples of *V. negundo* from the GBU campus herbal garden as well as through in vitro plantlets that have been treated with various concentrations of plant growth regulators and are kept in a plant tissue culture facility. All explants (nodal segments) were cultured in phytajars on a medium containing Murashige and Skoog (MS) salts, vitamins and 3% (w/v) sucrose. Depending upon the experiments, MS medium was variously supplemented with growth regulators such as Benzyl amino purine (BAP), diphenyl urea (DPU), thidiazuron (TDZ) and meta-topolin (mT) in various concentrations and combinations (Razani et al. 2020). The media were gelled with 0.8% (w/v) bacteriological grade agar, and its pH was adjusted to 5.8 using 1 N

NaOH before autoclaving at 121°C for 15 min. The cultures were maintained in a culture room illuminated by two cool white fluorescent lamps with a maintained temperature of 26±2°C (Fig. 1). For the purpose of assessing its genetic integrity, a total of six accessions were taken from the parent plant along with in vitro plantlets. (Table 1)

The Cetyl trimethylammonium bromide (CTAB) method described by Doyle and Doyle (1990) was followed with slight modification for genomic DNA isolation and purification from in vitro raised plantlets and a field-grown mother

plant. The extracted DNA was air-dried and dissolved in 100 µl of sterile mQ water and tested for purity (A260/280 ratio) on a UV visible spectrophotometer and for size, purity and integrity in 0.8% (w/v) agarose gel at 60 V for 60 min (Fig. 2).

High molecular weight genomic DNA was found in the callus of the TDZ-treated samples and the juvenile leaf tissues of all the collected samples. Ethidium bromide staining was used to assess the purity of the isolated DNA and a UV spectrum photometer set at 260 nm was used to determine its quantity.

Table 1. Six accessions for assessing genetic integrity.

Tabela 1. Šest akcesij za oceno genske celovitosti.

Accessions label	Name of Accessions label
C1	Mother plant
C2	BAP
C3	DPU
C4	Mt
V5	TDZ
V6	Callus of TDZ

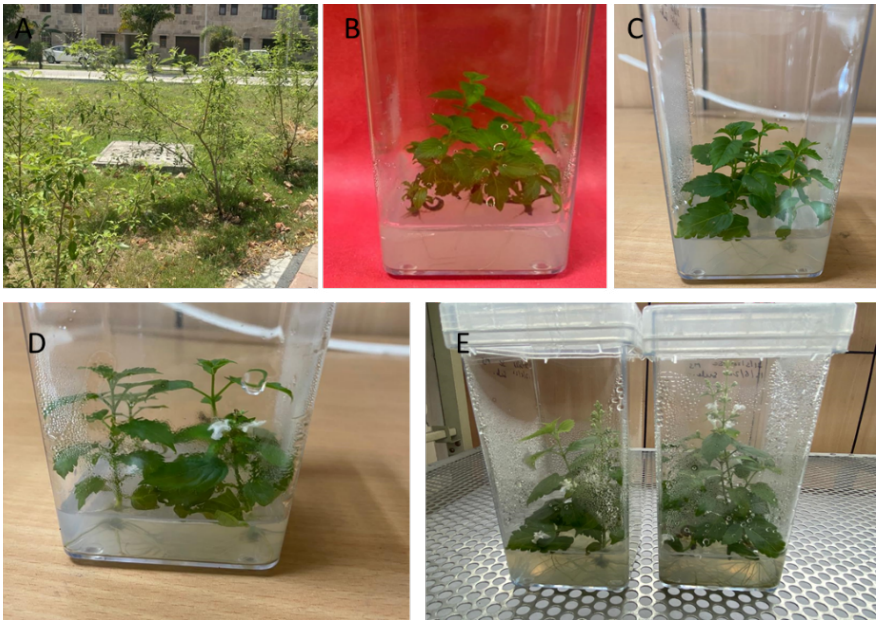


Figure 1. Samples of *V. negundo* a) Mother plant b) BAP c) DPU d) mT e) TDZ

Slika 1. Vzorci *V. negundo* a) Matična rastlina b) BAP c) DPU d) mT e) TDZ

RAPD evaluation

Genomic DNA was diluted to 50 ng/μl in order to facilitate amplification using specially designed random primers for RAPD analysis. One unit of Taq DNA polymerase, 50 ng of template DNA, 200 μM of each dNTP, 1X Taq buffer, 1.5 mM MgCl₂, and 100 pmol primer were all included in the 2.5 μl reaction mixture. PCR reactions are conducted in an Eppendorf thermal cycler, which is configured to denature for five minutes at 94°C, then anneal for one minute at 37°C, extend for two minutes at 72°C and repeat for 42 cycles. At the conclusion of the reaction, a final extension allowed was seven minutes at 72°C.

Gel separation

The 2% agarose gel in 1X TAE solution was used to resolve the amplified products, which were then stained with ethidium bromide, visualised, and recorded using a gel documentation system. Only primers that produced distinct and repeatable bands were taken into consideration for the final analysis after each experiment was run three times.

Data interpretation

Band presence in the RAPD profiles was graphically evaluated as "1" and absence as "0". Fuzzy bands were discarded, and only distinct, clear bands were scored. Using the formula $PIC = 1 - \sum (i-1)^k \cdot P_i^2$, the polymorphism information content (PIC) values for the RAPD primers were determined. P_i represents the frequency of the i th allele using the k primer. (Farahzadi et al. 2020). Pairwise similarity matrices (Jaccard 1908) were produced using Jaccard's similarity coefficient and the SimQual format for qualitative data (Tang et al. 2021), which was derived from the NTSYS-pc version 2.1 (Numerical Taxonomy and Multivariate Analysis System) (Kizilgeci et al. 2022). Using the Unweighted Pair Group Method with Arithmetic Average (UPGMA) and the SAHN module of NTSYS-pc, a dendrogram was created based on the similarity matrix (Khan et al. 2022).

Result and Discussion

RAPD Evaluation

Thirteen random primers were initially screened, with six primers producing banding patterns that could be visually

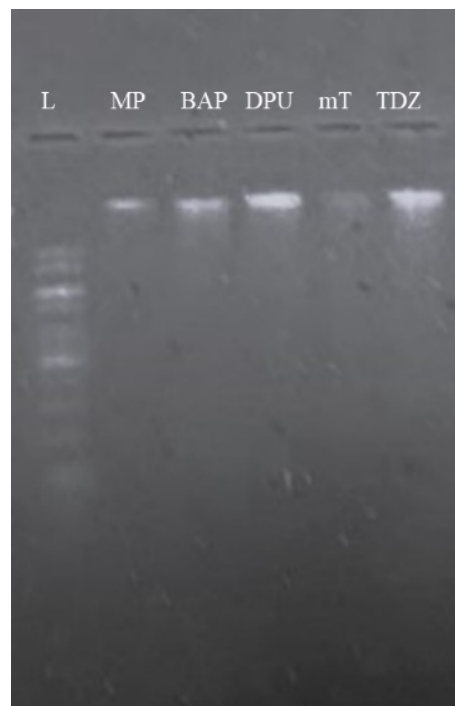


Figure 2. Image of DNA extraction on agarose gel.

Slika 2. Slika ekstrakcije DNK na agaroznem gelu.

scored, and these were selected for further analysis. Using RAPD analysis with these six primers, a total of 150 bands were successfully amplified, averaging 25 bands per primer. The amplified bands spanned sizes ranging from 100 to 2000 bp across all accessions. Out of these 150 bands, 31 were polymorphic, with a mean of 5.16 polymorphic bands per primer. The polymorphism percentage varied among primers, with OPX-20, OPX-17, and OPX-15 achieving a maximum of 100% polymorphism, whereas OPB-01 showed the lowest polymorphism percentage (Table 2, Fig. 1). The Polymorphism Information Content (PIC) values ranged from 0.11 (OPX-17) to 0.63 (OPX-20), with an average PIC of 0.27, highlighting the effectiveness of RAPD primers in detecting polymorphism among the selected accessions.

A dendrogram was constructed using the binary RAPD primer data and UPGMA clustering. The clustering represented six distinct samples of *Vitex negundo*, labelled as accessions C1, C2, C3, C4, V5, and V6. These accessions include plants grown in vitro and treated with various plant growth regulators such as BAP, DPU, TDZ, and mT, along

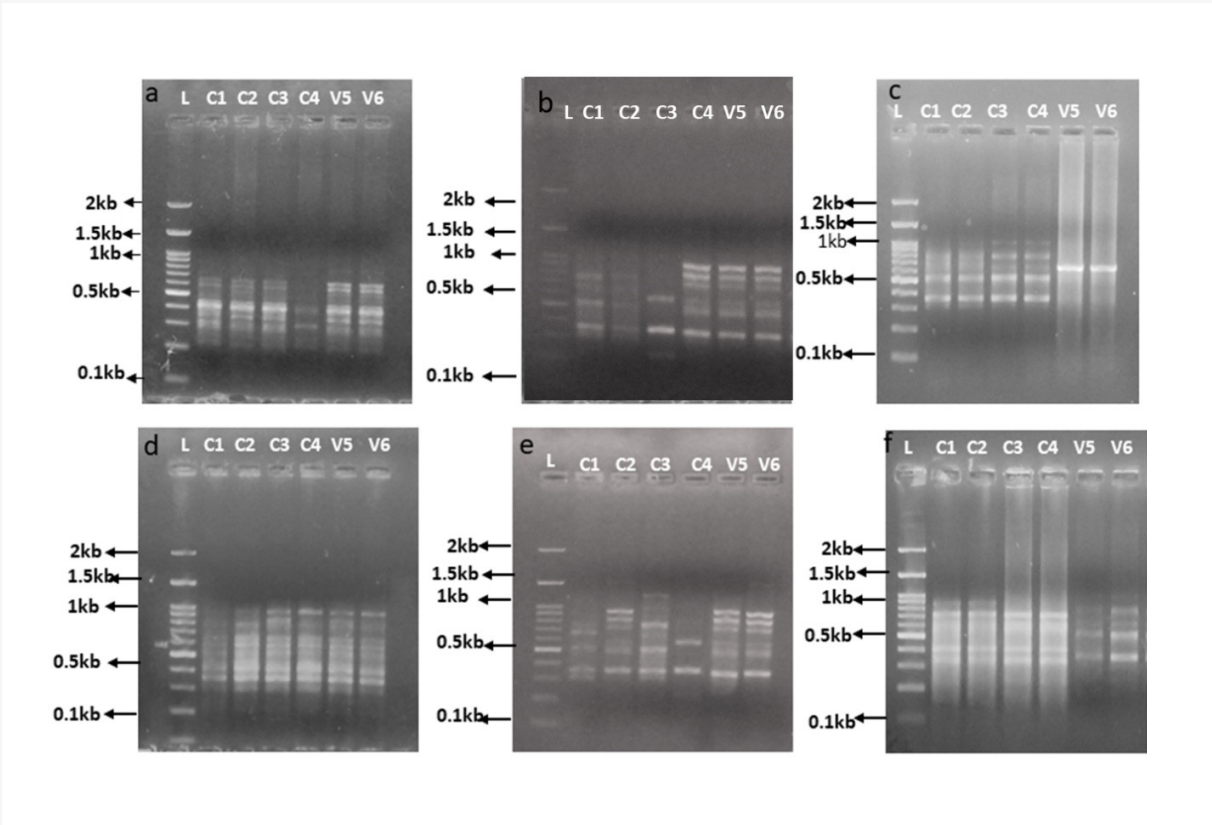


Figure 3. RAPD profiles of mother plant and *in vitro* raised plantlets of *V. negundo*. Banding pattern attained from a OPB-02, b OPX-17, c OPX-07, d OPB-012, e OPX-15, f OPX-20. L: DNA ladder, C1: DNA banding profile of mother plant, C2, C3, C4, V5: DNA banding profile of *in vitro* raised plantlets from nodes treated with different PGRs (BAP, DPU, Mt), V6: DNA banding profile of *in vitro* raised callus from node treated with TDZ.

Slika 3. Profili RAPD matične rastline in *in vitro* vzgojenih rastlin *V. negundo*. Profil pridobljen iz OPB-02, b OPX-17, c OPX-07, d OPB-012, e OPX-15, f OPX-20. L: DNA lestev, C1: profil DNA pasov matične rastline, C2, C3, C4, V5: profil DNA pasov *in vitro* vzgojenih sadik iz vozlišč, obdelanih z različnimi PGR (BAP, DPU, Mt), V6: profil DNA pasov *in vitro* dvignjen kalus iz vozla, zdravljenega s TDZ.

Table 2. The amplification pattern, percentage of polymorphism and PIC value of *V. negundo* accessions analysed by using 6 RAPD primers..

Tabela 2. Vzorec pomnoževanja, odstotek polimorfizma in vrednost PIC akcesij *V. negundo*, analiziranih z uporabo 6 primerjev RAPD.

Primers	Primer sequences (5'-3')	Size range of amplicons bp	Total no. of bands	Total no. of polymorphic bands	Total no. of polymorphic bands	% of polymorphism	PIC value
OPX-20	GGACCCTTAC	220-800	20	1	4	12.9	0.63
OPX-07	TGGCAACGCA	350-920	18	0	5	16.1	0.27
OPX-17	CAGACAAGCC	120-900	30	1	6	19.3	0.11
OPX-15	CTACTGGGAC	300-900	28	0	7	22.5	0.19
OPB-02	TGATCCCTGG	300-620	24	1	5	16.1	0.27
OPB-012	CCTTGACGCA	370-900	30	2	4	12.9	0.55

with one mother plant. Pairwise similarity matrix values, calculated with Jaccard's coefficient, ranged from 0.51 to 0.86. Specific accessions include C1 (mother plant), C2 (BAP-treated in vitro plant), C3 (DPU-treated sample), C4 (mT-treated sample), V5 (TDZ-treated in vitro plant), and V6 (callus sample). The dendrogram produced by the UPGMA clustering method is shown in Fig. 4, and it reflects the genetic relationships among the different accessions treated with various growth regulators.

RAPD method reproducibility

Genetic relationship in *Vitex negundo* L. in different in vitro plantlets treated with different growth regulators has been carried out using RAPD markers. Genetic variation in in vitro plantlets treated with different growth regulators is measured by the heterozygosity or the degree of polymorphism. For the conservation of a species, genetic variability is of the utmost importance to preserve. Genetic variability among all samples is important to maintain since it represents the 'blueprint' for all the living things on earth. The result obtained was analysed, and the dendrogram

was obtained. In order to confirm the true-to-type nature, Random Amplified Polymorphic DNA (RAPD) analysis was carried out in a selected micro-propagated plant of *Vitex negundo*. Of the thirteen selected primers, six primers generated well-resolved and reproducible banding patterns.

RAPD Method Reproducibility

The genetic relationships within *Vitex negundo* accessions treated with different growth regulators were examined using RAPD markers. Genetic variation among the in vitro plantlets, as influenced by treatment with different growth regulators, was measured by heterozygosity and the degree of polymorphism. Genetic variability is critical to conserving species diversity, as it preserves the foundational genetic blueprint for living organisms. The generated dendrogram (Fig. 4) provides a reliable assessment of genetic relationships across *V. negundo* accessions, confirming the true-to-type nature of these samples through Random Amplified Polymorphic DNA (RAPD) analysis. Of the thirteen primers tested, six produced well-resolved and reproducible banding patterns.

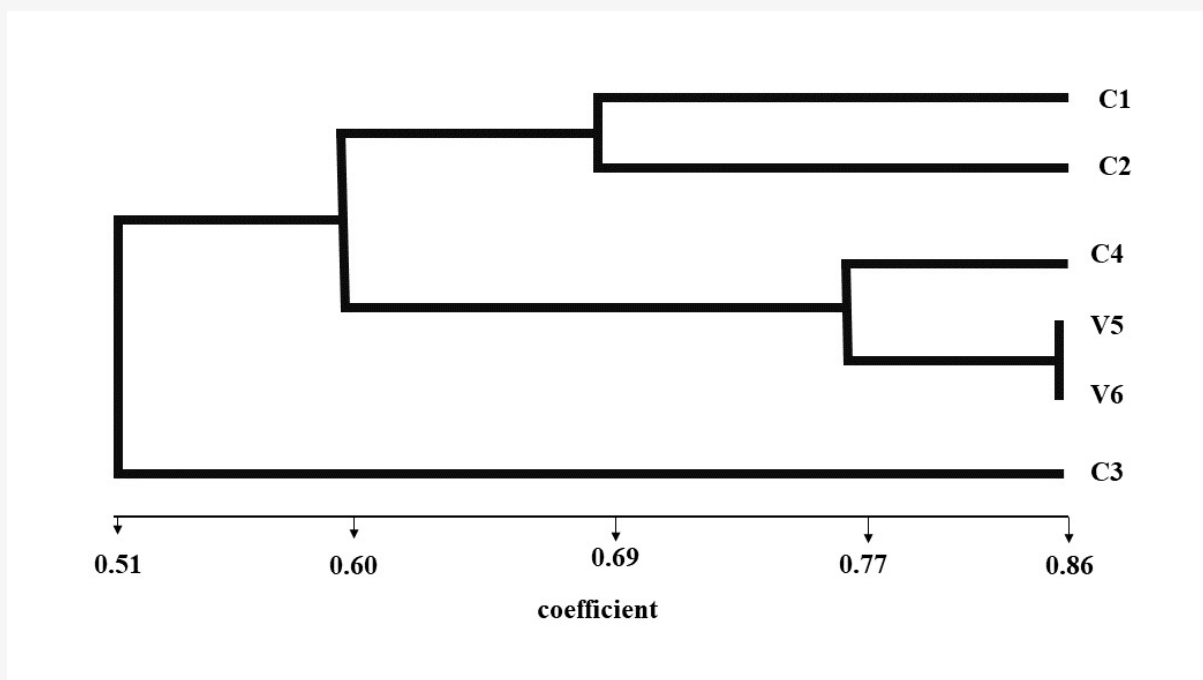


Figure 4. Dendrogram obtained by UPGMA cluster analysis based on Jaccard's coefficient of 6 *Vitex negundo* accessions using RAPD data.

Slika 4. Dendrogram, pridobljen z analizo grozdov UPGMA na podlagi Jaccardovega koeficienta 6 akcesij *Vitex negundo* z uporabo podatkov RAPD.

Genetic Integrity and Utility of RAPD Markers

The use of RAPD as a DNA fingerprinting technique effectively revealed the degree of polymorphism among six *V. negundo* accessions (Geetha and Siril 2022). Six RAPD primers yielded a total of thirteen polymorphic markers, with an average polymorphism percentage of 16.6%, which suggests a high level of genetic fidelity within the *V. negundo* accessions. The mean PIC value of 0.33, as noted by Powell et al. (1996), serves as an indicator of the usefulness of RAPD markers in detecting polymorphism across these taxa (Li et al. 2021). RAPD analysis captures a broad genetic picture by sampling from a substantial portion of the genome (Thakur et al. 2019). The clustering analysis groups the accessions according to their genetic similarity, as indicated by UPGMA analysis based on RAPD data (Seredin et al., 2022).

The dendrogram obtained from RAPD analysis of 150 PCR products demonstrates the high genetic integrity of *V. negundo* accessions. This finding is significant for selecting parent plants in breeding programs, as it supports the creation of populations useful for genome mapping and related genetic studies.

Conclusions

The molecular marker technique was helpful in determining high levels of genetic integrity and in estimating the genetic relationships between mother plant *V. negundo* accessions and in vitro-raised plantlets treated with various plant growth regulators. According to the study, RAPD markers are only slightly useful for determining the genetic diversity of *V. negundo*. The results of this work suggest that *V. negundo* in vitro plantlets treated with various growth regulators are an excellent source of genetic variety and that the identified molecular markers may be better utilised for the conservation of germplasm and genetic enhancement of this species.

Conflict of Interest

The authors declare that there is no conflict of interest.

Author Contributions

Conceptualization, S.C. and G.G.; Data collection, A.B.; Analysis and interpretation of results, A.B.; writing original draft, review, & editing, S.C. and G.G.

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References

- Alfarabi, M., Turhadi, T.S., Imaneli, N.A., Sihombing, O.P., 2022. Short Communication: Antioxidant Activity and Metabolite Profiles of Leaves and Stem Extracts of Vitex Negundo. *Biodiversitas*, 23(5), 2663-2667. doi: 10.13057/biodiv/d230550.
- Bi, D., Chen, D., Khayatnezhad, M., Hashjin, Z.S., Li, Z., Ma, Z., 2021. Molecular Identification and Genetic Diversity in *Hypericum* L.: A High Value Medicinal Plant Using RAPD Markers. *Genetika*, 53(1), 393-405. doi: 10.2298/GENSR2101393B.
- Boomibalagan, P., Subramanian, R.S., Rajasekharan, P.E., Karpakal, S., Veeranan, U., Saminathan, E., Narayanan, V., Kathiresan, D., 2021. Genetic Relationship and Polymorphism of Selected Medicinal Plants of Asclepiadaceae Using RAPD Molecular Analysis Method. *Ecological Genetics and Genomics*, 21, 100101. doi: 10.1016/j.egg.2021.100101.
- Bublyk, O., Andreev, I., Parznikoza I., Kunakh, V., 2020. Population Genetic Structure of *Iris Pumila* L. In Ukraine: Effects of Habitat Fragmentation. *Acta Biologica Cracoviensia Series Botanica*, 62(1), 51-61. doi: 10.24425/abcsb.2020.131665.
- Dang, H., Zhang, T., Li, Y., Li, G., Zhuang, L., Pu, X., 2022. Population evolution, genetic diversity and structure of the medicinal legume, *Glycyrrhiza uralensis* and the effects of geographical distribution on leaves nutrient elements and photosynthesis. *Frontiers in Plant Science*, 12, 708709. doi: 10.3389/fpls.2021.708709.
- Edwin, I.E., Jacob, I.E., 2017. Bio-insecticidal potency of five plant extracts against cowpea weevil, *Callosobruchus maculatus* (F.), on stored cowpea, *Vigna unguiculata* (L). *Jordan Journal of Biological Sciences*, 10(4), 317-322.
- Farahzadi, F., Ebrahimi, A., Zarrinnia, V., Azizinezhad, R., 2020. Evaluation of Genetic Diversity in Iranian Rice (*Oryza sativa*) Cultivars for Resistance to Blast Disease Using Microsatellite (SSR) Markers. *Agricultural Research*, 9(4), 1-9. doi: 10.1007/s40003-019-00447-1.
- Geetha, C.M., Elenjikkal, A.S., 2022. Cross-Species Transferability of Genomic SSR Markers and Genetic Diversity among *Asparagus Racemosus* Willd. Accessions. *Plant Gene*, 31, 100361. doi: 10.1016/j.plgene.2022.100361.

- Goswami, S., Biswajit, R., 2023. *Vitex Negundo* L., An Indigenous Plant: A Systematic Review on Traditional Use, Bioactives, And Pharmacological Activities. in Bioactives and Pharmacology of Lamiaceae. in Bioactives and Pharmacology of Lamiaceae, 458. <https://doi.org/10.1201/9781003346142>
- Iosefa, T.L., Hunter, D., Taylor, M., Tuia, V.S., 2016. Taro Networks and Seed Systems: Promoting the Use of Diversity for Crop Improvement. *Acta Horticulturae* 1118, 43-50. doi: 10.17660/ActaHortic.2016.1118.7.
- Khan, M.M.H., Rafii, M.Y., Ramlee, S.I., Jusoh, M., Oladosu, Y., Al Mamun, M., Khaliqi, A., 2022. Unveiling Genetic Diversity, Characterization, and Selection of Bambara Groundnut (*Vigna Subterranea* L. Verdc) Genotypes Reflecting Yield and Yield Components in Tropical Malaysia. *BioMed Research International* 2022, 6794475. doi: 10.1155/2022/6794475.
- Kizilgeci, F., Bayhan, B., Türkoğlu, A., Halilgılı, K., Yildirim, M., 2022. Exploring Genetic Diversity and Population Structure of Five *Aegilops* Species with Inter-Primer Binding Site (IPBS) Markers. *Molecular Biology Reports*, 49(9), 8567-8574. doi: 10.1007/s11033-022-07689-3.
- Koirala, N., Dhakal, C., Munankarmi, N.N., Ali, S.W., Hameed, A., Martins, N., Sharifi-Radm J., Imran, M., Arif, A.M., Hanif, M.A., Basnyat, R.C., Salehi, B., 2020. *Vitex Negundo* Linn.: Phytochemical Composition, Nutritional Analysis, and Antioxidant and Antimicrobial Activity. *Cellular and Molecular Biology*, 66(4), 1-7. doi: 10.14715/cmb/2020.66.4.1.
- Li, K., Hua, C.C., Xin, X.Y., Guan, P., 2021. Distributed Consensus Control for Nonlinear Multiagent Systems under Directed Graphs of Dynamic Frequency Switches. *IEEE Transactions on Automatic Control*, 66(2), 841-848. doi: 10.1109/TAC.2020.2987302.
- Manokari, M., Priyadharshini, S., Shekhawat, M.S., 2021. Micro-Structural Stability of Micropropagated Plants of *Vitex Negundo* L. *Microscopy and Microanalysis*, 27(3), 626–34. doi: 10.1017/S1431927621000283.
- Mishra, A.K., Tiwari, K.N., Mishra, P., Mishra, S.K., Tiwari, S.K., 2022. Germplasm Conservation of Economically Important Medicinal Plant *Nyctanthes Arbor-Tristis* L. through Encapsulation Technique and Maintenance under Slow Growth Condition. *Plant Cell, Tissue and Organ Culture*, 149(1–2), 281-293. doi: 10.1007/s11240-022-02244-1.
- Noor, F., Tahir Ul Qamar, M., Ashfaq, U.A., Albutti, A., Alwashmi, S., Aljasir, M.A., 2022. Network Pharmacology Approach for Medicinal Plants: Review and Assessment. *Pharmaceuticals*, 2022, 15(5), 572. doi: 10.3390/PH15050572.
- Peng, J., Shi, C., Wang, D., Li, S., Zhao, X., Duan, A., Cai, N., He, C., 2021. Genetic Diversity and Population Structure of the Medicinal Plant *Docynia Delavayi* (Franch.) Schneid Revealed by Transcriptome-Based SSR Markers. *Journal of Applied Research on Medicinal and Aromatic Plants*, 21, 100294. doi: 10.1016/j.jarmap.2021.100294.
- Priyanka, V., Kumar, R., Dhaliwal, I., Kaushik, P., 2021. Germplasm Conservation: Instrumental in Agricultural Biodiversity—a Review. *Sustainability (Switzerland)*, 13(12), 6743.
- Razani, M., Kayat, F., Redwan, R.M., Susanto, D., 2020. Detection of Abnormal Banana Plantlets Produced by High BAP Concentration and Number of Subcultures Using Representational Difference Analysis. *International Journal of Agriculture and Biology*, 23(3), 541-548. doi: 10.17957/IJAB/15.1321.
- Rohela, G.K., Jogam, P., Bylla, P., Reuben, C., 2019. Indirect Regeneration and Assessment of Genetic Fidelity of Acclimated Plantlets by SCOT, ISSR, and RAPD Markers in *Rauwolfia Tetraphylla* L.: An Endangered Medicinal Plant. *BioMed Research International*, 2019, 698742. doi: 10.1155/2019/3698742.
- Samanta, T., Jha, T.B., Ray, S., Jha, S., 2023. Comparative Cytogenetics and Fluorescent Chromosome Banding in Five Indian Species of Dipsacadi Medik. *Plants*, 12(13), 2534. doi: 10.3390/plants12132534.
- Savitikadi, P., Jogam, P., Rohela, G.K., Ellendula, R., Sandhya, D., Allini, V.R., Abbagani, S., 2020. Direct Regeneration and Genetic Fidelity Analysis of Regenerated Plants of *Andrographis Echoides* (L.) - An Important Medicinal Plant. *Industrial Crops and Products*, 155, 112766. doi: 10.1016/j.indcrop.2020.112766.
- Seredin, O., Liakhov, D., Kushnir, O., Lomov, N., 2022. Jaccard Index-Based Detection of Order 2 Rotational Quasi-Symmetry Focus for Binary Images. *Pattern Recognition and Image Analysis*, 32(3), 672-681. doi: 10.1134/S1054661822030403.
- Singer, S.D., Laurieriy Bilichak, J.D., Kumar, S., Singh, J., 2021. Genetic Variation and Unintended Risk in the Context of Old and New Breeding Techniques. *Critical Reviews in Plant Sciences*, 40(1), 68-108. doi: 10.1080/07352689.2021.1883826.
- Srinivasan, P., Raja, H.D., Tamilvanan, R. 2021. Efficient in Vitro Plant Regeneration from Leaf-Derived Callus and Genetic Fidelity Assessment of an Endemic Medicinal Plant *Ranunculus Wallichianus* Wight & Arn by Using RAPD and ISSR Markers. *Plant Cell, Tissue and Organ Culture*, 147(2), 421. doi: 10.1007/s11240-021-02134-y.
- Tang, M., Kaymaz, Y., Logeman, B.L., Eichhorn, S., Liang, Z.S., Dulac, C., Sackton, T.B., 2021. Evaluating Single-Cell Cluster Stability Using the Jaccard Similarity Index. *Bioinformatics*, 37(15), 2212-2214. doi: 10.1093/bioinformatics/btaa956.
- Tawfeeq, T.A., Tawfeeq, A.A., Eldalawy, R., Ibraheem, S.K., 2023. Phytochemical Analysis, GCMS Identification, and Estimation of Antioxidant Activity of Iraqi *Vitex Negundo* L. *Journal of Medicinal and Chemical Sciences*, 6(4), 876-883. doi: 10.26655/JMCHMSCI.2023.4.19.
- Thakur, V.V., Tiwari, S., Tripathi, N., Tiwari, G., 2019. Molecular Identification of Medicinal Plants with Amplicon Length Polymorphism Using Universal DNA Barcodes of the AtpF–AtpH, TrnL and TrnH–PsbA Regions. *3 Biotech*, 9(5), 188. doi: 10.1007/s13205-019-1724-6.
- Vigneswari, T., Kanthimathi, G., Muthulakshmi, L., 2023. Superparamagnetic Properties of Iron Oxide Nanoparticles Using *Vitex Negundo* Leaf Extract by Green Synthesis Method and Its Antimicrobial Activity against Wound Pathogen. *Materials Today: Proceedings*, in press. doi: 10.1016/j.matpr.2023.06.293.
- Zavinon, F., Adoukonou-Sagbadja, H., Keilwagen, J., Lehnert, H., Ordon, F., Perovic, D., 2020. Genetic Diversity and Population Structure in Beninese Pigeon Pea [*Cajanus Cajan* (L.) Huth] Landraces Collection Revealed by SSR and Genome Wide SNP Markers. *Genetic Resources and Crop Evolution*, 67(1), 1-18. doi: 10.1007/s10722-019-00864-9.
- Zhang, X., Su, H., Yang, J., Feng, L., Li, Z., Zhao, G., 2019. Population Genetic Structure, Migration, and Polyploidy Origin of a Medicinal Species *Gynostemma Pentaphyllum* (Cucurbitaceae). *Ecology and Evolution*, 9(19), 11145-11170. doi: 10.1002/ece3.5618.