International Journal of Science and Research Archive

eISSN: 2582-8185

Cross Ref DOI: 10.30574/ijsra

Journal homepage: https://ijsra.net/

(Research Article)

Check for updates

Assessment of Genetic Diversity in *Lactuca sativa* and *Lactuca taraxacifolia* Using SSR and ISSR Markers

Damilola Olofintuyi *, Agbonkpolor Blessing Ele and Ibrahim Michael

Department of Biological Sciences, University of lagos, Nigeria.

International Journal of Science and Research Archive, 2021, 03(02), 318-326

Publication history: Received on 20 August 2021; revised on 27 September 2021; accepted on 29 September 2021

Article DOI: https://doi.org/10.30574/ijsra.2021.3.2.0144

Abstract

The genus Lactuca L. is one of the economically and medicinally important genera of the Asteraceae family. This study aims to characterize and assess the genetic diversity of cultivated lettuce (Lactuca sativa) and wild lettuce (*Lactuca taraxacifolia*) using morphological, phytochemical, and molecular markers. Seven accessions of L. taraxacifolia were obtained from different towns in western Nigeria, while four accessions of L. sativa were obtained from different countries. Morphological assessment revealed variations in leaf arrangement, shape, margin, depth of incision, and texture. Phytochemical analysis showed that both species have high phenolic and saponin content, with L. taraxacifolia having higher concentrations. Molecular characterization using three SSR markers (LSSA14, LSSA43, LSSB28) and one ISSR marker (UBC816) revealed 100% polymorphism across all markers. The SSR markers showed successful transferability from L. sativa to L. taraxacifolia, demonstrating their utility for genetic diversity studies. Cluster analysis based on morphological and molecular data revealed distinct groupings between the two species. This study provides valuable insights into the genetic diversity of Lactuca species and establishes a foundation for conservation and breeding programs.

Keywords: *Lactuca sativa; Lactuca taraxacifolia;* Genetic diversity; SSR markers; ISSR markers; Morphological characterization

1. Introduction

Since the onset of life on earth more than three billion years ago, Mother Nature has generated a plethora of diverse life forms (Park et al., 2009). Plant genetic resources comprise all agricultural crops and their wild relatives of valuable traits. The genus Lactuca L. is one of the economically and medicinally important genera of the Asteraceae family, subfamily Cichorioideae, tribe Lactuceae (El-Esawi and Sammour, 2014; Dziechciarkova et al., 2004). The genus Lactuca comprises around 100 species of annual or perennial plants that are distributed in different regions around the world. The diversity of chromosome number may indicate that evolution is common within this genus, wheren=8 in European and Himalayan species, n=9 in Indian, Mediterranean, African, Asian and some European species, and n=17 in Northern American from Canada to Florida.The Mediterranean basin, South-Western Asia and Africa comprise centres of diversity of wild Lactuca species and can be considered as hot spots for lettuce conservation (Lebeda et al., 2004).

Lettuce (*Lactuca sativa)* is a dicotyledonous self-fertilizing diploid species with 2n = 2x = 18 chromosomes (Rauscher and Simko, 2013). It is an important member of the Asteraceae (Compositae) family and a notable vegetable crop produced worldwide (Christopoulou et al., 2015; Dario et al., 2012). The wild ancestor of lettuce is believed to be *Lactuca serriola* (Keseli et al., 1991). Cultivated lettuce was first recorded on the walls of Egyptian tombs ca. 2500 BCE, indicating that lettuce has been cultivated for more than 6000 years (de Vries, 1997). The plant often has a height of 15-30cm with colourful leaves running from bright green to red and yellow. The planthas a wide range of shapes and

*Corresponding author: Damilola Olofintuyi

texture, from the dense heads of iceberg type to the succulent leaves of romaine lettuce and from the crisp leaves of crisphead lettuce to the tender leaves of butterhead lettuce.

Launaeataraxacifolia (Wild) Amin Ex. C Jeffrey occurs mainly in the tropics (Sakpere and Aremu, 2008). The plant is predominant in tropical African countries of Ghana, Nigeria, Senegal, Sierra Leone, Uganda, Kenya, Zimbabwe and Ivory Coast (Adebisi, 2000; Adebisi, 2004; Adebooye et al., 2003). *Lactuca taraxacifolia* is a tropical perennial plant that has a creeping root system, with its leaves at the base of an erect stem, which is about 1-3m high. The leaves are arranged in a rosette form on a stem which gets woody at the base and can be slightly hairy (Adebooye et al., 2003; Adebisi, 2004; Adedeji and Jewoola, 2008; Adegbite, 1987).The plant, which forms a rich part of the African diet, is endowed with high levels of nutrients. The leaves have been reported to contain substantial amounts of macro- and micronutrients that can compare favourably with such vegetables as spinach and cabbage (Adewale et al., 2013). The leaves of *Launaeataraxacifolia* are endowed with saponins, terpenoids, cardiac glycosides, steroids, tannins, flavonoids, leucoanthocyanins, phenolic acids, ascorbic acid, lycopene, and β-carotene (Adinortey et al., 2012; Arawande et al., 2013). The plant is used as a remedy for prevention and treatment of diseases such as measles, yaws, conjunctivitis, hyperthesion, cancer etc. It is reported to possess hypolipidaemic, antihypertensive, and anti-inflammatory properties (Ayodele, 2005; Adinortey et al., 2018). Its pharmacological properties include antibiotic, anti-venimous, anti-poison, anti-anemic, anti-inflammatory, anti-nausea, fungicide, nematocide, antalgic, febrifuge, sedative, cholesterol lowering and anti-diabetic activities (Bello et al., 2018).

Figure 1 Leaves and Flower of *Lactuca taraxacifolia* plant

1.1. Genetic Diversity and Molecular Markers

Genetic variation allows species to adjust to a changing environment, whether these changes are due to natural or human factors (El-Esawi, 2015). Genetic diversity is influenced by selection, population size, founder effects, crossing pattern and interspecific hybridization (El-Esawi et al., 2012; El-Esawi, 2012). Molecular markers have been used for the characterization of lettuce genetic diversity and germplasm collection, establishment of core collections, cultivar identification and mapping of traits (Dziechciarkova et al., 2004).

Simple Sequence Repeats (SSRs) are tandem repeats of 1-6 bp motifs which are highly polymorphic, co-dominant, multiallelic, reproducible, broadly distributed in plant genomes and are easily transferable to related species (Basu et al., 2004). Inter Simple Sequence Repeats (ISSRs) are segments of DNA sequences present in multiple copies throughout the genome, flanked at both the 3' and 5' ends by microsatellite sequences (Alansi et al., 2016). ISSR markers are dominant markers that are more reproducible than RAPD markers and do not require prior sequence knowledge.

The objectives of this study were to: (1) assess the morphological diversity of *L. sativa* and *L. taraxacifolia* accessions, (2) evaluate the phytochemical composition of both species, (3) characterize the genetic diversity using SSR and ISSR markers, and (4) assess the transferability of SSR markers developed for *L. sativa* to *L. taraxacifolia.*

2. Materials and methods

2.1. Sample Collection and Plant Materials

A total of seven (7) accessions of wild lettuce (*L. taraxacifolia*), were obtained from different towns in the western part of Nigeria (Akure, Ibadan, Lagos, Ondo and Osogbo) and four (4) accessions of cultivated lettuce (L. sativa) were obtained from different countries (Lagos, United Kingdom and Tanzania). The samples were collected in Ziploc bags and taken to Covenant University Ota, Ogun State, for nursery plantation. Details of the accessions are presented in Table 1.

Table 1 List of Lactuca accessions used in this study

S/N	Codes	Places of collection	Common names	Scientific names
1	ALI 01	Lagos	Imported lettuce	Lactuca sativa
2	ALI 02	United Kingdom	Imported lettuce	Lactuca sativa
3	ALL 03	Tanzania	Imported lettuce	Lactuca sativa
4	ALO 04	Akure	African lettuce	*Lactuca taraxacifolia*
5	LLI 05	Ibadan	African lettuce	*Lactuca taraxacifolia*
6	LTL 06	Lagos	African lettuce	*Lactuca taraxacifolia*
7	LTS 07	Ondo	African lettuce	*Lactuca taraxacifolia*
8	LTS 08	Ondo	African lettuce	*Lactuca taraxacifolia*
9	LOS 09	Osogbo	African lettuce	*Lactuca taraxacifolia*
10	LOS 10	Osogbo	African lettuce	*Lactuca taraxacifolia*
11	ALI 11	Lagos	Imported lettuce	Lactuca sativa

2.2. Morphological Assessment

Morphological characterization was carried out on mature plants using qualitative descriptors including leaf arrangement (opposite, alternate, whorled), leaf shape of blade outline (spatulate, oblong elliptic, lyrate, runcinate, ovate), leaf margin (finely serrate, coarsely serrate, cleft, double dendate and irregularly dendate), leaf depth of incision (pinnatilobed, pinnatipart, pinnatiset, pinnafid), and leaf texture (smooth, rough, hairy). These traits were scored according to standard descriptors and recorded for each accession.

2.3. Phytochemical Analysis

Fresh leaves of *L. sativa* (accession ALL 03) and *L. taraxacifolia* (accession LTS 07) were analyzed for phenol and saponin content. Phenol content was determined using the Folin-Ciocalteu method with gallic acid as standard, and results were expressed as mg gallic acid equivalent per 100g fresh weight. Saponin content was determined by gravimetric method after extraction with methanol and precipitation, and results were expressed as mg per 100g fresh weight.

2.4. DNA Extraction

Genomic DNA was extracted from young fresh leaves using the CTAB (Cetyl Trimethyl Ammonium Bromide) method. Leaf samples (0.5g) were ground in liquid nitrogen and extracted with CTAB buffer (2% CTAB, 100mM Tris-HCl pH 8.0, 20mM EDTA, 1.4M NaCl). The homogenate was incubated at 65°C for 30 minutes, then extracted with chloroform:isoamyl alcohol (24:1). DNA was precipitated with ice-cold isopropanol, washed with 70% ethanol, air-dried and resuspended in TE buffer. DNA quality was assessed by electrophoresis on 1% agarose gel, and quantity was measured using a spectrophotometer at 260nm and 280nm.

2.5. PCR Amplification

Three SSR primer pairs (LSSA14, LSSA43, LSSB28) previously developed for L. sativa and one ISSR primer (UBC816) were used for amplification. PCR reactions were performed in 25µL volumes containing 50ng template DNA, 1X PCR buffer, 2mM $MgCl_2$, 200µM each dNTP, 0.5µM each primer, and 1U Taq polymerase. SSR amplification conditions were:

initial denaturation at 94°C for 5 min; 35 cycles of 94°C for 30s, 55-60°C for 30s (depending on primer), 72°C for 1 min; and final extension at 72°C for 10 min. ISSR amplification followed similar conditions with annealing at 52°C. PCR products were separated on 12% polyacrylamide gels using SDS-PAGE and visualized by silver staining.

2.6. Data Analysis

Morphological data were scored as binary (presence/absence) or multistate characters and analyzed using NTSYS-pc software. For molecular data, bands were scored as present (1) or absent (0) to generate a binary matrix. Polymorphism information content (PIC) and expected heterozygosity were calculated for each marker. Genetic similarity was estimated using Jaccard's coefficient, and cluster analysis was performed using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). Principal coordinate analysis (PCoA) was conducted to visualize genetic relationships among accessions.

3. Results

3.1. Phytochemical Analysis

The phytochemical analysis revealed the presence of phenols and saponins in the leaves of both *L. sativa* and *L. taraxacifolia* (Table 2). Both species exhibited high phenolic and saponin content, with *L. taraxacifolia* showing higher concentrations. *L. taraxacifolia* had a phenol concentration of 112.07 mg/100g and saponin concentration of 23.30 mg/100g, while L. sativa had 103.48 mg/100g and 21.24 mg/100g for phenol and saponin concentrations respectively.

Table 2 Phytochemical composition of *L. sativa* and *L. taraxacifolia*

Samples	Phenol (mg/100g)	Saponin (mg/100g)
ALL 03 (*L. sativa*)	103.48	21.24
LTS 07 (*L. taraxacifolia)*	112.07	23.30

3.2. Morphological Assessment

Morphological assessment revealed variations in qualitative traits among the Lactuca accessions. All accessions exhibited alternate leaf arrangement. Leaf shape varied from obovate in *L. sativa* accessions to runcinate and lyrate in *L. taraxacifolia* accessions. Leaf margin types included dendate, irregularly dendate, and coarsely serrate. The depth of incision ranged from entire to pinnatiset, with *L. taraxacifolia* generally showing deeper incisions. Leaf texture varied from smooth in most *L. sativa* accessions to rough and hairy in *L. taraxacifolia* accessions. These morphological differences clearly distinguished the two species and showed intraspecific variation within *L. taraxacifolia*.

3.3. Marker Polymorphism and Genetic Diversity

All four markers (LSSA14, LSSA43, LSSB28, and UBC816) successfully amplified across both *L. sativa* and *L. taraxacifolia* accessions, demonstrating the transferability of SSR markers from *L. sativa* to *L. taraxacifolia*. The SSR markers generated 2 alleles each, while the ISSR marker UBC816 generated 7 alleles, of which 4 were polymorphic (Table 3). All markers showed 100% polymorphism, indicating high genetic diversity among the accessions. The polymorphism information content (PIC) values ranged from 0.1948 (LSSB28) to 0.7916 (UBC816), with the ISSR marker showing the highest discriminatory power. Expected heterozygosity values ranged from 0.2188 to 0.8166, with UBC816 again showing the highest value.

Table 3 Polymorphism and diversity parameters of SSR and ISSR markers

Parameter	LSSA14	LSSB28	LSSA43	UBC816
No. of alleles	2	2	2	7
No. of monomorphic allele	0	0	0	0
No. of polymorphic allele	2	2	2	4
Percentage polymorphism (%)	100	100	100	100
PIC	0.3318	0.1948	0.2149	0.7916

Expected Heterozygosity	0.42	0.2188	0.2449	0.8166

3.4. Cluster Analysis and Genetic Relationships

Cluster analysis based on combined morphological and molecular data revealed clear groupings among the accessions. The UPGMA dendrogram separated the accessions into two main clusters corresponding to the two species (Figure 2). *L. sativa* accessions formed a distinct cluster with high genetic similarity (Jaccard coefficient > 0.75), while *L. taraxacifolia* accessions showed more diversity, forming subclusters based on geographical origin. Accessions from the same location tended to cluster together, suggesting local adaptation and limited gene flow between populations.

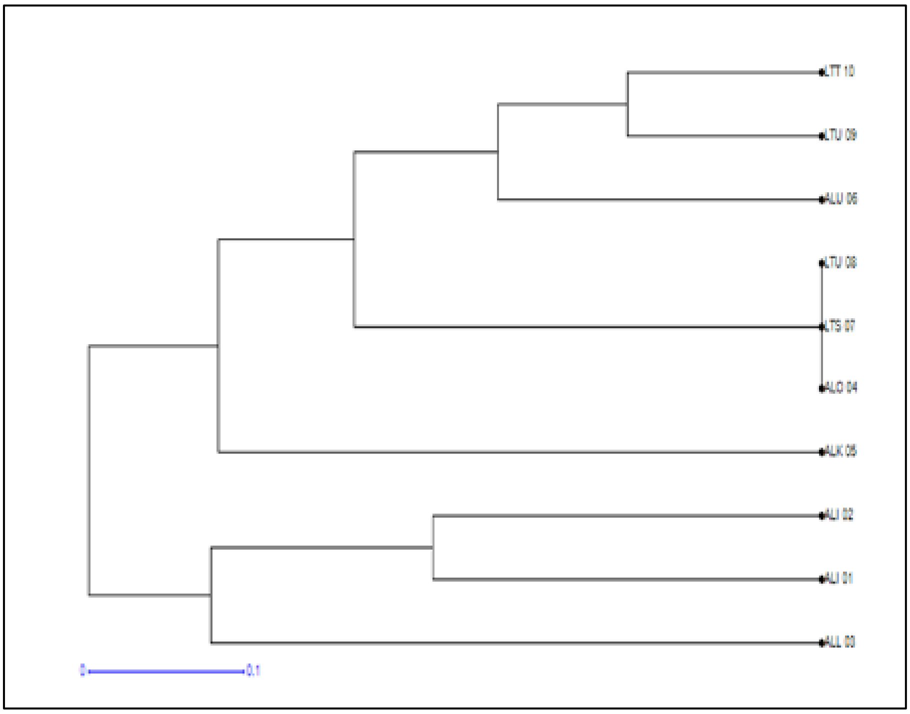

Figure 2 UPGMA dendrogram showing genetic relationships among Lactuca accessions based on combined morphological and molecular data

Principal coordinate analysis (PCoA) further supported the clustering pattern, with the first two principal coordinates explaining 68.3% of the total variation. The PCoA plot clearly separated *L. sativa* and *L. taraxacifolia* into distinct groups, with *L. taraxacifolia* showing greater dispersion, indicating higher intraspecific diversity.

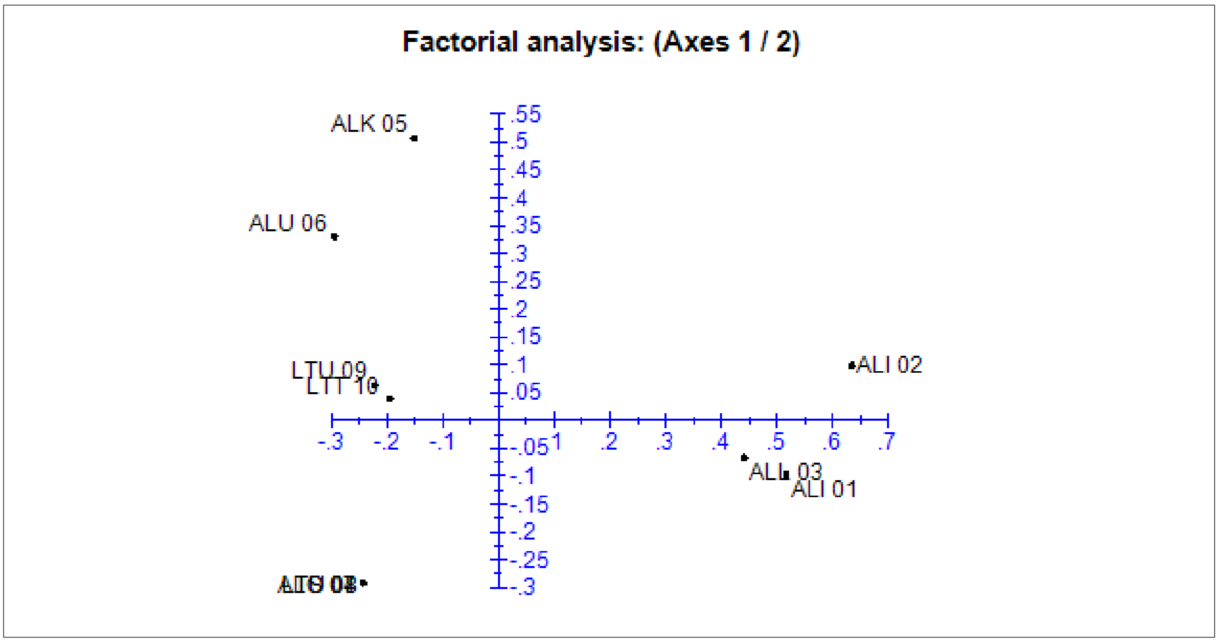

Figure 3 Principal coordinate analysis (PCoA) plot showing genetic relationships among Lactuca accessions

3.5. Gel Electrophoresis Results

SDS-PAGE analysis of PCR products showed clear banding patterns for all markers across the accessions (Figure 4). The SSR markers produced 1-2 bands per accession, consistent with their co-dominant nature, while the ISSR marker produced multiple bands ranging from 3-7 per accession. Band sizes ranged from approximately 150 bp to 900 bp for SSR markers and 200 bp to 1500 bp for ISSR markers. The amplification success rate was 100% for all markers across all accessions, confirming the robustness of the marker systems and the quality of the extracted DNA.

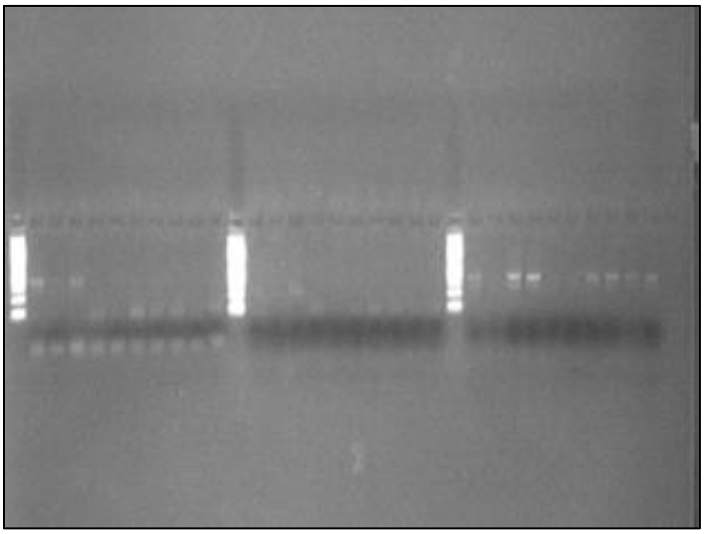

Figure 4 Representative of Agarose gel Electrophoresis showing PCR amplification products of SSR and ISSR markers across Lactuca accessions

4. Discussion

A comprehensive evaluation of a species for the extent of genetic diversity present among the populations is highly beneficial for sustainable utilization of its available resources and for their management. The present study was an effort towards characterizing and correlating various morphological, molecular and phytochemical traits in *L. taraxacifolia* and *L. sativa*. As morphological traits can be observed directly and easy to score, presence of phenotypic variation among the studied accessions could be useful for their preliminary identification and classification.

The phytochemical analysis revealed that both *L. sativa* and *L. taraxacifolia* possess high levels of phenols and saponins, with *L. taraxacifolia* showing slightly higher concentrations. This finding corroborates previous studies by Arawande et al. (2013) and Adinortey et al. (2012) who reported high phenolic content in *L. taraxacifolia* leaves. Phenolic compounds are known for their antioxidant properties and play important roles in plant defense mechanisms and human health (Ololade et al., 2017). The presence of saponins contributes to the medicinal properties of these plants, including anti-inflammatory and cholesterol-lowering activities (Bello et al., 2018). The higher phytochemical content in *L. taraxacifolia* may explain its widespread use in traditional medicine across West Africa.

Morphological assessment revealed clear differences between *L. sativa* and *L. taraxacifolia*, particularly in leaf shape, margin type, and texture. These differences are consistent with taxonomic descriptions of the two species (Lebeda et al., 2004; Sakpere and Aremu, 2008). The variation observed within *L. taraxacifolia* accessions suggests adaptation to local environmental conditions and potential for selection of superior genotypes for cultivation. This intraspecific variation is valuable for breeding programs aimed at developing improved varieties with desirable agronomic and nutritional traits.

The molecular characterization using SSR and ISSR markers revealed high levels of genetic diversity among the Lactuca accessions. All markers showed 100% polymorphism, indicating their effectiveness for diversity studies in Lactuca. The successful transferability of SSR markers from *L. sativa* to *L. taraxacifo*lia is particularly significant, as it demonstrates the conservation of microsatellite loci across these species. This finding is consistent with studies by El-Esawi (2015) who reported successful cross-species amplification of SSR markers within the genus Lactuca. The transferability of markers between species suggests a close evolutionary relationship and provides a cost-effective approach for genetic studies in related species where marker development may be limited.

The PIC values obtained in this study ranged from 0.1948 to 0.7916, with the ISSR marker UBC816 showing the highest value. According to the classification by Botstein et al. (1980), PIC values greater than 0.5 indicate highly informative markers, while values between 0.25 and 0.5 indicate moderately informative markers. The high PIC value of UBC816 (0.7916) makes it particularly useful for genetic diversity studies and fingerprinting applications in Lactuca. The SSR markers showed moderate informativeness, which is still valuable for genetic studies, especially given their co-dominant nature and reproducibility.

Cluster analysis revealed distinct groupings of *L. sativa* and *L. taraxacifolia*, confirming their taxonomic separation. The high genetic similarity among L. sativa accessions (Jaccard coefficient > 0.75) suggests limited genetic diversity in the cultivated species, which is typical of domesticated crops that have undergone selection and breeding (de Vries, 1997). In contrast, the greater diversity observed within *L. taraxacifolia* indicates that this wild species maintains higher genetic variation, which is important for its adaptation to diverse environments and for its long-term evolutionary potential. This diversity represents a valuable genetic resource that could be utilized in lettuce breeding programs to introduce novel traits such as disease resistance, stress tolerance, and enhanced nutritional quality.

The geographical clustering observed within *L. taraxacifolia* accessions suggests that genetic differentiation may be occurring among populations from different locations. This pattern could result from limited gene flow between populations, local adaptation to environmental conditions, or genetic drift in small populations. Similar findings have been reported in other wild plant species where geographical isolation leads to population differentiation (Alansi et al., 2016). Understanding these genetic relationships is important for conservation strategies, as it highlights the need to preserve genetic diversity across multiple populations rather than focusing on a single location.

The integration of morphological and molecular data provided a comprehensive assessment of genetic diversity in Lactuca. While morphological traits are influenced by environmental factors and can show plasticity, molecular markers provide a more direct assessment of genetic variation. The congruence between morphological and molecular-based clustering validates the use of both approaches and provides confidence in the observed patterns of diversity. This multi-faceted approach is recommended for comprehensive genetic diversity studies in crop species and their wild relatives.

The findings of this study have important implications for conservation and utilization of Lactuca genetic resources. The high diversity observed in *L. taraxacifolia* populations underscores the importance of in situ conservation of wild populations in their natural habitats. Additionally, ex situ conservation through seed banks and germplasm repositories should include samples from multiple geographical locations to capture the maximum genetic diversity. For *L. sativa*, efforts should be made to broaden the genetic base through introgression of genes from wild relatives, particularly *L. taraxacifolia*, which possesses valuable traits such as high phytochemical content and potential disease resistance.

5. Conclusion

This study successfully characterized the genetic diversity of *L. sativa* and *L. taraxacifolia* using morphological, phytochemical, and molecular approaches. The results demonstrate that:Both species possess high levels of phenolic compounds and saponins, with *L. taraxacifolia* showing higher concentrations, supporting its medicinal value.Morphological assessment revealed clear interspecific differences and intraspecific variation, particularly in *L. taraxacifolia*.SSR markers developed for *L. sativa* successfully transferred to *L. taraxacifolia*, demonstrating their utility for genetic studies across Lactuca species.High levels of genetic diversity exist within *L. taraxacifolia* populations, representing valuable genetic resources for crop improvement.Cluster analysis clearly separated the two species and revealed geographical structuring within *L. taraxacifolia*.These findings provide a foundation for conservation strategies, germplasm management, and breeding programs aimed at developing improved lettuce varieties with enhanced nutritional and medicinal properties. Future studies should focus on expanding the number of molecular markers used, including genome-wide markers such as SNPs, to gain a more comprehensive understanding of genetic diversity and to identify genes associated with important agronomic and nutritional traits.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Adebisi, A.A. (2000). Population of Neglected Indigenous Leafy Vegetables among the Yoruba tribe of South West Nigeria. CERNARD Development Series 06 CERNARD, Ibadan, Nigeria. pp. 86.

[2] Adebisi, A.A. (2004). Launaea Taraxacifolia (Willd) Amin ex C Jeffrey. In: PROTA (Plant Resources of Tropical Africa/Ressourcesvegetales de l'Afriquetropicale), Grubben, G.J.H. and O.A. Denton (Eds.). Wageningen, Netherlands.

[3] Adebooye, O.C., Ogbe, F.M.D., and Bamidele, J.F. (2003). Ethno Botany of Indigenous Leaf Vegetables of South West Nigeria. Delpinoa. 45: 295-299.

[4] Adedeji, O., and Jewoola, O.A. (2008). Importance of leaf epidermal characters in the Asteraceae family. NotulaeBotanicae Horti Agrobotanici Cluj-Napoca. 36(2): 7-16.

[5] Adegbite, A.E. (1987). Biosystematics studies of some species of the Tribe Cichorieae (Asteraceae) in Nigeria. M.Sc. Thesis, O.A.U Ile Ife Nigeria.

[6] Adewale, A., Ayoade, A.A., and Alani, E.A. (2013). Determination of vitamins in five selected West African green leafy vegetables. Journal of American Science. 9: 40-43.

[7] Adinortey, M.B., Sarfo, J.K., Kwarteng, J., Adinortey, C.A., Ekloh, W., Kuatsienu, L.E., and Nyarko, A.K. (2018). The Ethnopharmacological and Nutraceutical Relevance of Launaeataraxacifolia (Willd.) Amin ex C. Jeffrey. Evidence-Based Complementary and Alternative Medicine. 2018 (Article ID 7259146): 1-13.

[8] Adinortey, M.B., Sarfo, J.K., Quayson, E.Y., Weremfo, A., Adinortey, C.A., Ekloh, W., and Ocran, J. (2012). Phytochemical screening, proximate and mineral composition of Launaeataraxacifolia leaves. Research Journal of Medicinal Plant. 6(2): 171-179.

[9] Alansi, S., Tarroum, M., Al-Qurainy, F., Khan, S., and Nadeem, M. (2016). Use of ISSR markers to assess the genetic diversity in wild medicinal Ziziphus spina-christi (L.) Willd. collected from different regions of Saudi Arabia. Biotechnology and Biotechnological Equipment. 30(5): 942-947.

[10] Arawande, J.O., Amoo, I.A., and Lajide, L. (2013). Chemical and phytochemical composition of wild lettuce Launaeataraxacifolia. Journal of Applied Phytotechnology in Environmental Sanitation. 2(1): 25-30.

[11] Ayodele, A.E. (2005). The medicinally important leafy vegetables of South West Nigeria. Ethnobotanical Leaflets. 1(16): 1-6.

[12] Basu, A., Ghosh, M., Meyer, R., Powell, W., Basak, S., and Sen, S. (2004). Analysis of genetic diversity in cultivated jute determined by means of SSR markers and AFLP profiling. Crop Science. 44: 678-685.

[13] Bello, O.M., Ogbesejana, A.B., and Uduma, A.U. (2018). Launaeataraxacifolia: a Neglected Vegetable from Nigeria, its Antiinflammatory and Antioxidant Activities. ChemSearch Journal. 9(1): 9-12.

[14] Botstein, D., White, R.L., Skolnick, M., and Davis, R.W. (1980). Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 32(3): 314-331.

[15] Christopoulou, M., McHale, L.K., Kozik, A., Wo, S.B., Wroblewski, T., and Michelmore, R.W. (2015). Dissection of two complex clusters of resistance genes in lettuce (Lactuca sativa). Molecular Genetics and Genomics. 28(7): 751-765.

[16] Dario, S., Scott, W., Winkler, S. and Jones, R.B. (2012). Lettuce (Lactuca sativa L.) growth and quality response to applied nitrogen under hydroponic conditions. Acta Horticulturae. 927: 353-360.

[17] de Vries, I.M. (1997). Origin and domestication of Lactuca sativa L. Genetic Resources and Crop Evolution. 44(2): 165-174.

[18] Dziechciarkova, M., Lebeda, A., Dolezalova, I., and Astley, D. (2004). Characterization of Lactuca spp. germplasm by protein and molecular markers - A review. Plant Soil and Environment. 50(2): 47-58.

[19] El-Esawi, M., Germaine, K., and Malone, R. (2012). Assessing the genetic diversity and relationships in Irish Brassica oleracea species based on microsatellites markers. Proceedings of the Fifth Saudi Science Conference, Umm Al-Qura University, Saudi Arabia.

[20] El-Esawi, M.A. (2012). Assessing the genetic diversity, phylogenetic relationships, and disease resistance genes in Irish Brassica oleracea species. PhD Thesis, Dublin Institute of Technology, Ireland.

[21] El-Esawi, M.A. (2015). Molecular genetic markers for assessing the genetic variation and relationships in Lactuca germplasm. Annual Research and Review in Biology. 8(5): 1-13.

[22] El-Esawi, M.A. and Sammour, R. (2014). Karyological and phylogenetic studies in the genus Lactuca L. (Asteraceae). Cytologia. 79(2): 269-275.

[23] Keseli, R., Paran, I., and Michelmore, R.W. (1991). Analysis of a detailed genetic linkage map of Lactuca sativa (lettuce) constructed from RFLP and RAPD markers. Genetics. 136: 1435-1446.

[24] Lebeda, A., Dolezalova, I., Ferakova, V., and Astley, D. (2004). Geographical distribution of wild Lactuca species (Asteraceae, Lactuceae). Botanical Review. 70(3): 328-356.

[25] Ololade, Z.S., Fakoya, S., Adelusi, S.A., and Udi, O.U. (2017). Chemical composition and antioxidant activity of Launaeataraxacifolia methanol leaf extract. International Journal of Biological Chemistry. 11(1): 13-20.

[26] Park, K.C., Kwak, J.H., Kim, N.S., and Lee, J. (2009). Introgression of chromosome segments from wild species into cultivated rice. International Journal of Genomics. Article ID 829865.

[27] Rauscher, G. and Simko, I. (2013). Development of genomic SSR markers for fingerprinting lettuce (Lactuca sativa L.) cultivars and mapping genes. BMC Plant Biology. 13: 11.

[28] Sakpere, A.M.A. and Aremu, M.T. (2008). Seed morphology and germination studies in three species of Launaea Cass. (Compositae). Notulae Scientia Biologicae. 1: 94-98.