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Correlation between molecular diversity and biochemical traits of edible aerial parts of *Basella alba* L. from different geographical locations of Sri Lanka

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Abstract

Background *Basella alba* L. a widely consumed green leafy vegetable, exhibits considerable nutritional and therapeutic potential attributed to its bioactive constituents. Prior investigations revealed significant variation in phytochemical and antioxidant activity across agro-climatic zones in Sri Lanka, suggesting potential genetic influence. This study is designed to explore underlying genetic variation using RAPD markers to investigate the correlations and contributions of genotype on previously reported bioactivity variation.

Results From a screening of 15 RAPD primers, four primers (OPA 9, OPA 10, OPA 16, and OPB 10) produced, polymorphic, consistent and clearly scorable banding profiles (under optimized PCR conditions) in *B. alba* L. collected from 15 Sri Lankan locations. These primers collectively yielded 36 bands, 35 of which were polymorphic, resulting in a high polymorphism rate of 97.2%, confirming the informativeness of the selected primers for genetic diversity analysis. Genetic similarity was assessed using Jaccard's coefficient in NTSYSpc.v2·10e, revealing values ranging from 0.44 to 0.97, with the highest similarity from the samples from Ratnapura and Kandy and the lowest similarity in Ratnapura and Kalutara. A dendrogram constructed via UPGMA grouped the samples into two major clusters and five sub-clusters, demonstrating substantial genetic differentiation influenced by geographic origin. Cluster I included Ratnapura and Kandy, while the remaining samples formed Cluster II and its subgroups, each representing different ecological zones. When compared to the phytochemical and antioxidant clustering data of the previous study, partial correspondence was observed. A Mantel test comparing genetic diversity and biochemical/antioxidant potential revealed a weak negative correlation which was not significantly different. Some of the locations within similar genetic cluster shared similar biochemical traits, while others diverged significantly, indicating that environmental conditions also influence bioactive compound synthesis. Notably, Cluster I (Ratnapura and Kandy) showed both genetic similarity and lower antioxidant traits. Samples from Ella and Polonnaruwa showed similar bioactive traits even though they were grouped into different genetic clusters.

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Conclusion These findings suggest that both genetic makeup and environmental adaptation contribute to observed biochemical diversity in *B. alba* L. with a clear geographical correlation. This study highlights the value of integrating molecular and biochemical analyses to develop regionally adapted *B. alba* L. cultivars with enhanced nutritional traits, supporting sustainable agriculture in Sri Lanka and beyond.

Keywords Antioxidants, *Basella alba*L., Genetic variation, Genotype-environment interaction, Phytochemicals, RAPD markers

Background

Green leafy vegetables (GLVs) are vital components of the human diet, renowned not only for their rich nutritional content but also for their significant phytochemical and antioxidant properties [1]. Among them, *Basella alba* L. (commonly known as Malabar spinach) holds particular importance in tropical and subtropical areas due to its widespread culinary and medicinal uses [2]. The nutritional and therapeutic potential of *B. alba* L. is triggered by its accumulation of bioactive compounds such as chlorophylls, carotenoids, phenolics, and flavonoids, which also confer strong antioxidant capacity [3]. However, these biochemical traits can be influenced by external environmental factors, including soil characteristics, climate, and elevation, which can vary with geographical location [4].

In previous study, we evaluated the proximate composition, phytochemical content, and antioxidant activity of *B. alba* L. collected from 15 locations across diverse agro-climatic zones in Sri Lanka [4]. The results revealed significant variability among samples in both nutritional and biochemical traits. Furthermore, certain locations showed elevated levels of phytochemicals and antioxidant potential. Multivariate analyses such as principal component and cluster analysis, demonstrated a strong association between these traits and geographical location, underscoring the influence of environmental conditions on the biochemical profile of *B. alba* L. While these findings offer important guidance for location-specific cultivation strategies aimed at enhancing nutritional quality and sustainable agriculture, they also raised critical questions about the underlying genetic factors contributing to the observed variability.

To address this, the present study investigates the genetic variation among the same *B. alba* L. samples using Random Amplified Polymorphic DNA (RAPD) markers. By exploring the genetic variation and clustering patterns in relation to previously assessed phytochemical and antioxidant properties, study aim to investigate the correlations and contributions of genotype and environmental factors with the bioactivity variation. These investigations can offer valuable insights into genotype-environment interactions and their role in shaping the nutritional and therapeutic potential of GLVs. Understanding such dynamics is critical for the strategic conservation of genotypes with high performance, for the development of targeted breeding programs, and for the promotion of sustainable agricultural practices in specific environments.

Material and methods

Materials

The large vine-type *B. alba* L. variety with green leaves was collected from 15 locations in Sri Lanka (Table 1). Each sample was acquired from home gardens, grown with organic farming practices [4]. Fresh edible aerial parts (leaves and immature stems) of *B. alba* L. were randomly collected from each site. Samples were immediately placed in clean polyethylene bags and brought to the laboratory within 12 hours of collection for further analysis. Chemical reagents, cetyltrimethylammonium bromide (CTAB), NaCl, EDTA, Tris-HCl, polyvinylpolypyrolidone (PVPP), and β mercaptoethanol, were obtained by BrecklandScientificTM, UK. Chloroform, isopropyl alcohol, and ethanol were obtained by Sigma-Aldrich, USA. GoTaq® Green PCR buffer, $MgCl_2$, dNTP, DNA ladder (100 bp), Taq polymerase, and Primers were obtained by Promega, USA. SYBR SAFE™ gel stain was obtained by Thermo Fischer Scientific, USA.

Sample preparation

The collected samples were taxonomically identified according to the online herbarium, the Atlas of Florida Plants from the Institute for Systematic Botany, the University of South Florida, USA as described in [4]. The samples were cleaned well with tap water followed by distilled water to remove dust, mud, and other possible impurities [4]. The shoot parts of the samples were air dried at room temperature under shade, to remove excess water. Then they were stored under −20 °C until DNA extraction.

Dna extraction

Total genomic DNA was extracted from the young leaves of *B. alba* L. following the method described by [5] with slight modifications [5]. First, 100 mg of leaf shoot was crushed into a fine paste and homogenized with 500 µL 2× CTAB buffer [2% (w/v) CTAB, 1.4 M NaCl, 20 mM EDTA (pH 8.0), 0.1 M Tris-HCl (pH 8.0), 1% (w/v) PVPP, 1.0% (w/v) β mercaptoethanol] in an Eppendorf tube. Then the tube was incubated in a heating block (MaXtable H10, Daihan Scientific, South Korea) at 60 °C

Table 1 Sampling locations of *B. alba* L. made from different parts of Sri Lanka

Agro-climatic Zone	Sample location	Geographical coordinate	
		Latitude	Longitude
Low-country dry zone (LD)	Jaffna	9.738817	80.01751
	Anuradhapura	8.310921	80.38111
	Polonnaruwa	7.772025	81.211141
	Hambanthota	6.145451	81.144426
	Kalpitiya	8.039823	79.714747
Mid-country intermediate zone (MI)	Badulla	6.907357	81.215564
	Monaragala	6.855969	81.342407
Upcountry intermediate zone (UI)	Ella	6.861539	81.02505
	Welimada	6.869918	80.943397
Low-country wet zone (LW)	Kalutara	6.546935	80.045276
	Hikka	6.130542	80.10267
	Gampaha	7.071295	79.972292
	Rathnapura	6.710008	80.389119
	Kegalle	7.217894	80.2478
Up-country wet zone (UW)	Kandy	7.30824	80.720608

Table 2 Primers and the optimized annealing temperature for the PCR amplification

Primer code	Sequence (5'to 3' end)	Optimized annealing temperature - T_a(°C)	References
OPA 1	CAG GCC CTT C	30	[6]
OPA 9	GGG TAA CGC C	37	[6]
OPA 10	GTG ATC GCA T	30	[5, 6]
OPA 16	AGC CAG CGA A	30	[6]
OPB 3	CAT CCC CCT G	30	[6]
OPB 4	CGA CTG GAG T	30	[5, 7]
OPB 6	TGC TCT GCC C	30	[6]
OPB 8	GTC CAC ACG G	30	[6, 7]
OPB 10	CTG CTG GGA C	37	[5, 6]
OPB 11	GTA GAC CCG T	30	[5, 7]
OPB 12	GTG ATC GCA G	30	[8]
OPB 17	AGG GAA CGA G	30	[6]
OPB 18	CCA CAG CAG T	30	[9]
OPB 19	ACC CCC GAA G	30	[9]
OPC 5	GAT GAC CGC C	37	[9]

for 15 min. 500 µL of chloroform was added and mixed well, followed by centrifugation at 13,000 rpm for 10 min. The aqueous phase was transferred into a new sterile microcentrifuge tube. The solution was re-extracted with an equal volume of chloroform followed by centrifugation at 13,000 rpm for 10 min. Then, 600 µL of 100% ice-cold isopropyl alcohol was added to the aqueous phase in a new microcentrifuge tube And mixed carefully and thoroughly for DNA to precipitate. Then centrifuged at 13,000 rpm for 5 min at 4 °C. The DNA pellet was washed with 70% (v/v) ethanol and re-centrifuged at 13,000 rpm for 2 min. The DNA pellet was air-dried and suspended in 100 µL of sterile distilled water. DNA quality was assessed using 1% (w/v) agarose gel electrophoresis, while its purity and concentration were determined by Nabi UV-Vis Nano spectrophotometer (MicroDigital, South Korea) before PCR amplification.

Random amplified polymorphic DNA (RAPD) assay

RAPD analysis was conducted with random decamer primers, and a master mix was prepared using GoTaq® Green PCR buffer according to the protocols outlined by [5] with modifications [5]. The master mix for PCR amplification reaction with a final volume of 20 µL, contained 4 µL of 10× Green PCR buffer, 3 µL of 4 mM $MgCl_2$, 2 µL of 1.5 mM dNTPs (dATP, dGTP, dCTP, and dTTP), 2 µL template DNA, 2 µL of 100 mM primers, 0.25 units of Taq DNA polymerase, and 6.75 µL PCR grade water. The respective PCR amplifications were conducted using BIOER LifeECO Thermocycler (Hangzhou Bioer Technology Corporation Limited, China) by using the following amplification profile: initial denaturation at 94 °C for 3 min, followed by 35 cycles consisted of denaturation at 94 °C for 1 min, annealing at T_a (Table 2) for 30 sec., extension at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. After completion of PCR cycles, DNA amplicons were stored at – 20 °C until further analysis.

After completion of PCR cycles, DNA amplicons were stored at 4 °C. For the initial PCR screening of primers, 15 random decamer primers (Integrated DNA Technologies, Inc. - USA) (Table 2) were used.

Agarose gel electrophoresis

The PCR products were electrophoresed in 1.5% agarose gel stained with SYBR SAFE™ (Thermo Fischer Scientific - USA) (1 µL/100 mL). Gel electrophoresis was carried out in 1× Tris Acetic Acid EDTA buffer (TAE, pH 8.0) and run at 60 V for 60 min. 5 µL from each PCR was loaded into the agarose gel for electrophoresis. The PCR amplicons were visualized using a gel documentation apparatus (Gel DocTM-EZ imager, USA). The band size of the amplicons was determined with reference to a 100 bp DNA ladder (Promega – USA).

Statistical analysis

A binary matrix was created using the DNA fingerprint pattern by coding 1 and 0 for the presence [1] or absence (0) of bands of various molecular weight sizes. Only polymorphic, consistent, and clearly scorable (under optimized PCR conditions)fragments were considered for the analysis. The total number of fragments (TF), the number of polymorphic fragments (PF), and the percentage of polymorphic fragments (PPF) were determined for each primer used. The binary matrix was used as the

input file in the NTSYSpc.v2.10e. software to generate a dendrogram based on the unweighted pair group method analysis (UPGMA) clustering method. The similarity coefficients between varieties were computed using the Jaccard Similarity Coefficient (J) algorithm followed by cluster analysis based on UPGMA which was employed to generate a dendrogram as a graphical representation of the RADP analysis and to interpret the genetic variation within the species [10]. Furthermore, a Mantel test was performed between the pairwise RAPD-based genetic distance matrix (1 – Jaccard similarity) and the biochemical/antioxidant data as a Euclidean distance matrix to evaluate the relationship between genetic variation and biochemical/antioxidant diversity in *B. alba*. The Euclidean distance matric was computed using PAST 4.03 software based on the biochemical and antioxidant diversity data reported for *B. alba* by [4]. The PAST 4.03 software was used with 9,999 permutations to assess the statistical significance of the correlation. The Mantel statistic (R) was calculated as a Pearson correlation coefficient between the two distance matrices, and significance was determined using a one-tailed test. To examine the association of genetic variation with phytochemical and antioxidant traits, *B. alba* L. accessions were grouped according to the genetic clusters obtained from RAPD marker analysis. Then the phytochemical and antioxidant results of our former study [4] were compared among resulting genetic clusters using one-way analysis of variance ANOVA test followed by Tukey's post hoc test at a 95% confidence level ($p < 0.05$). The analysis was performed using IBM SPSS Statistics data editor.

Results

The screened primers gave different band patterns. However, from the 15 primers screened, 4 primers gave consistent and clearly scorable banding profiles (under optimized PCR conditions) and were used for the determination of genetic variation among the samples collected from different locations. An additional file shows the gel images of the RAPD marker analysis of *B. alba* L. from different locations for the primers OPA 9, OPA 10, OPA 16, and OPB 10 (Additional file 1).

Table 3 RAPD marker summary statistics

Primer code	TF	PF	PPF (%)
OPA 9	11	11	100.0
OPA 10	6	6	100.0
OPA 16	8	8	100.0
OPB 10	11	10	90.9

TF – total no of fragments, PF – no of polymorphic bands, PP – percentage of polymorphic fragments

In the RAPD analysis of *B. alba* L. from the 15 locations, the four primers evaluated produced a total of 36 fragments with 35 (97.2%) bands being polymorphic. The TF, PF, and PPF % values are presented in Table 3. The number of bands produced by the RAPD primers ranged from 6 (OPA10) to 11 (OPA 9 and OPB 10). On an average, each primer generated 9 total fragments and 8.75 polymorphic fragments. The percentage of polymorphism ranged from 90.9% (OPB 10) to 100% (OPA 9, OPA 10, and OPA 16), with an overall average polymorphism frequency of 97.2%.

Figure 1 shows the similarity coefficient matrix generated using Jaccard's coefficient (J) algorithm by feeding the presence or absence of RAPD bands as binary data into NTSYSpc.v2.10e. software. The Jaccard's similarity coefficient among the *B. alba* L. samples ranged from 0.44 to 0.97, with the highest similarity in Ratnapura and Kandy (Fig. 1) and the lowest similarity in Ratnapura and Kalutara.

The dendrogram (Fig. 2), based on a similarity matrix, grouped the *B. alba* L. sample locations into two major

	Kalutara	Gampaha	Jaffna	Hikkaduwa	Monaraga	Ella	Welimada	BadullaRatnapura	Anuradha	Kandy	Kalpitiya	Polonnar	Hambanth	Kegalle
Kalutara	1.0000													
Gampaha	0.7222	1.0000												
Jaffna	0.6111	0.7222	1.0000											
Hikkaduwa	0.7500	0.7500	0.5833	1.0000										
Monaragala	0.5556	0.7222	0.7222	0.5833	1.0000									
Ella	0.4722	0.6944	0.6389	0.6667	0.7500	1.0000								
Welimada	0.5556	0.7222	0.6667	0.6944	0.7222	0.7500	1.0000							
Badulla	0.5556	0.7222	0.7222	0.5833	0.6111	0.5278	0.6667	1.0000						
Ratnapura	0.4444	0.7222	0.5556	0.5278	0.6667	0.6944	0.6111	0.5000	1.0000					
Anuradhapura	0.6944	0.8056	0.8611	0.6667	0.7500	0.6667	0.6944	0.8056	0.6389	1.0000				
Kandy	0.4722	0.7500	0.5833	0.5556	0.6944	0.7222	0.6389	0.5278	0.9722	0.6667	1.0000			
Kalpitiya	0.5833	0.7500	0.8611	0.6111	0.7500	0.7222	0.6944	0.6944	0.6389	0.8889	0.6667	1.0000		
Polonnaruwa	0.6111	0.7778	0.8889	0.6389	0.7778	0.7500	0.7222	0.7222	0.6111	0.9167	0.6389	0.9722	1.0000	
Hambanthota	0.6667	0.8333	0.8889	0.6389	0.7778	0.7500	0.7778	0.7222	0.6667	0.9167	0.6944	0.9167	0.9444	1.0000
Kegalle	0.6667	0.7222	0.7222	0.8611	0.6111	0.6944	0.6667	0.6667	0.5556	0.8056	0.5833	0.7500	0.7778	0.7778

Fig. 1 The Jaccard's similarity coefficients between the *B. alba* L. samples from different locations

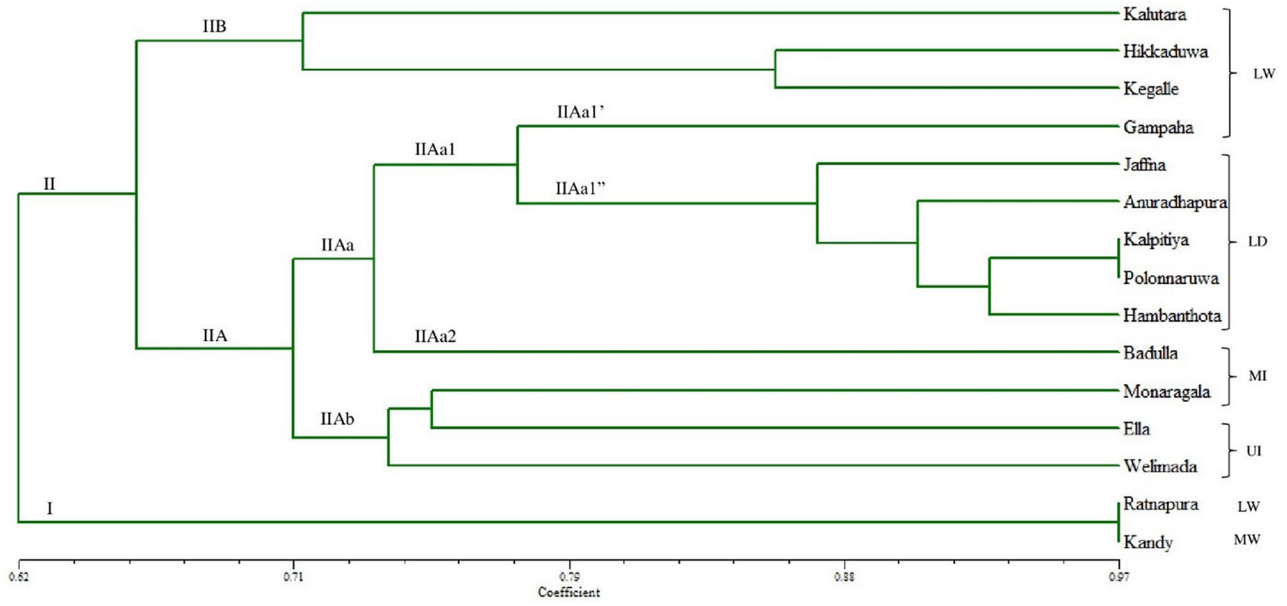

Fig. 2 The dendrogram generated by the NTSYSpc.V2.10e software based on the Jaccard's similarity coefficient

	Kalutara	Gampaha	Jaffna	Hikkaduwa	Monaragala	Ella	Welimada	Badulla	Rathnapura	Anuradhapura	Kandy	Kalpitiya	Polonnaruwa	Hambanthota	Kegalle
Kalutara	0														
Gampaha	2.332498	0													
Jaffna	15.7642	15.54959	0												
Hikkaduwa	14.38249	13.10241	10.28399	0											
Monaragala	6.241323	7.718751	12.84893	15.61186	0										
Ella	29.4129	29.53533	14.28464	22.91412	25.13835	0									
Welimada	10.2921	10.81536	7.818796	13.01303	6.108278	19.80494	0								
Badulla	13.46835	13.36941	4.510136	10.26198	10.67129	16.8379	4.950181	0							
Rathnapura	7.278479	8.932094	18.19097	20.07838	5.713305	30.20014	11.48004	16.11194	0						
Anuradhapura	16.35547	17.22513	10.30926	18.84351	10.67098	16.75123	6.955515	9.044533	14.85341	0					
Kandy	6.132057	7.090061	11.89686	13.75503	3.562622	24.5374	4.887798	8.772416	8.112854	10.81557	0				
Kalpitiya	8.715218	10.23016	17.68105	19.45536	6.965126	29.39794	12.11877	16.20736	5.214633	15.44061	9.225237	0			
Polonnaruwa	27.28734	27.12438	12.31381	20.276	23.54573	6.986621	18.20184	15.06125	28.63963	16.20246	22.97205	28.69254	0		
Hambanthota	5.456764	6.187146	12.20894	13.08244	3.682133	25.43129	6.7026	10.08938	7.601935	12.42186	4.056093	7.855319	23.54004	0	
Kegalle	6.806492	8.448849	15.8514	18.38762	3.322069	27.75679	8.815482	13.54752	2.800181	12.33917	5.719723	6.058986	26.18758	5.87822	0

Fig. 3 The Euclidean distance coefficients for biochemical and antioxidant diversity between the *B. alba* L. samples from different locations

clusters: cluster I and cluster II. Cluster I has two locations; Ratnapura and Kandy, while cluster II has two subclusters, cluster IIA and IIB. The sub cluster IIB has three locations (Kalutara, Hikkaduwa, and Kegalle). The subgroup IIA is further divided into two, with one (IIAb) having Monaragala, Ella, and Welimada together and the other one (IIAa) having two more subgroups, in which Badulla is isolated from six other locations. IIAa1 subclusters into two, isolating Gampaha from Jaffna, Anuradhapura, Kalpitiya, Polonnaruwa, and Hambanthota. The cluster pattern shows that Ratnapura and Kandy are the most diverting locations from Kalurata.

Furthermore, the Mantel test revealed no significant correlation between RAPD-based genetic distance and biochemical/antioxidant distance (Fig. 3) in *B. alba* ($R = -0.1135$, $p = 0.7974$).

The results of the ANOVA test revealed significant differences ($p < 0.05$) in phytochemical and antioxidant traits; total phenolic content (TPC), total flavonoid content (TFC), total chlorophyll, total carotene, DPPH radical scavenging activity and ferric ion reducing power (FRAP) among the genetic clusters identified by RAPD analysis (Table 4).

Discussion

RAPD marker analysis

The RAPD analysis revealed a high level of polymorphism (> 90%) among *B. alba* accessions, confirming the

Table 4 Significant difference in phytochemicals and antioxidant activity of *B. alba* L. from different genetic clusters

Genetic cluster				Location	Chlorophyll (μg/g)	Carotene (μg/g)	TFC (mg QE/g)	TPC (mg GAE/g)	DPPH (%)	FRAP (mg AAE/g)	Reference
I				Ratnapura (LW)	14.54 ± 1.47^{de}	2.87 ± 0.35^{e}	5.65 ± 0.54^{bcd}	3.02 ± 0.20^{cd}	11.40 ± 2.12^{a}	1.23 ± 0.08^{a}	[4]
				Kandy (MW)	14.08 ± 0.35^{cd}	1.90 ± 0.28^{abc}	4.58 ± 0.58^{abc}	0.46 ± 0.12^{a}	18.94 ± 0.64^{bcd}	1.53 ± 0.11^{ab}	
II	IIA	IIAb		Monaragala (MI)	15.17 ± 0.37^{def}	2.70 ± 0.36^{cde}	5.70 ± 0.42^{bcd}	2.40 ± 0.12^{bcd}	16.84 ± 1.79^{abc}	2.72 ± 0.19^{f}	
				Ella (UI)	27.53 ± 0.94^{i}	1.54 ± 0.29^{ab}	10.51 ± 0.35^{f}	4.50 ± 0.92^{ef}	38.03 ± 2.04^{g}	3.76 ± 0.40^{g}	
				Welimada (UI)	17.12 ± 0.66^{g}	2.07 ± 0.56^{bcde}	4.99 ± 0.32^{abcd}	1.86 ± 0.38^{bc}	22.46 ± 0.38^{cde}	1.86 ± 0.36^{abcd}	
		IIAa	IIAa2	Badulla (MI)	$16.27 \pm 1.0e^{fg}$	1.85 ± 0.25^{ab}	5.07 ± 0.54^{abcd}	1.75 ± 0.40^{b}	27.32 ± 0.78^{ef}	1.59 ± 0.18^{abc}	
			IIAa1	Gampaha (LW)	8.04 ± 0.15^{ab}	1.15 ± 0.28^{a}	3.94 ± 0.42^{a}	3.47 ± 0.24^{de}	17.01 ± 0.34^{abcd}	1.27 ± 0.07^{a}	
			IIAa1′	Jaffna (LD)	16.82 ± 0.65^{fg}	1.85 ± 0.21^{ab}	7.44 ± 0.53^{e}	4.92 ± 0.28^{f}	29.17 ± 2.71^{f}	2.57 ± 0.17^{ef}	
			IIAa1″	Anuradhapura (LD)	23.98 ± 0.24^{h}	2.87 ± 0.18^{e}	5.72 ± 0.17^{bcd}	1.80 ± 0.32^{b}	22.77 ± 2.44^{de}	1.96 ± 0.21^{bcde}	
				Kalpitiya (LD)	13.66 ± 0.49^{cd}	3.80 ± 0.10^{f}	10.54 ± 0.40^{f}	2.27 ± 0.34^{bc}	12.43 ± 2.01^{a}	1.28 ± 0.08^{a}	
				Polonnaruwa (LD)	24.92 ± 0.11^{h}	2.31 ± 0.04^{bcde}	5.41 ± 0.71^{bcd}	8.33 ± 0.09^{g}	37.37 ± 3.00^{g}	4.33 ± 0.31^{g}	
				Hambanthota (LD)	12.45 ± 0.18^{c}	4.56 ± 0.03^{f}	5.79 ± 0.24^{cd}	2.66 ± 0.21^{bcd}	18.45 ± 1.41^{bcd}	2.15 ± 0.12^{bcdef}	
		IIB		Kalutara (LW)	9.33 ± 0.11^{b}	1.20 ± 0.07^{a}	4.44 ± 0.22^{ab}	2.53 ± 0.48^{bcd}	15.87 ± 1.33^{ab}	2.42 ± 0.12^{def}	
				Hikkaduwa (LW)	6.69 ± 0.41^{a}	1.99 ± 0.47^{abcd}	6.01 ± 0.49^{d}	4.24 ± 0.16^{ef}	29.79 ± 2.74^{f}	2.26 ± 0.13^{cdef}	
				Kegalle (LW)	15.52 ± 0.52^{defg}	2.82 ± 0.17^{de}	5.05 ± 0.47^{abcd}	2.35 ± 0.68^{bcd}	13.84 ± 2.14^{ab}	1.50 ± 0.30^{ab}	

Rows followed by the same letters in a column are not significantly different at $p < 0.05$. AAE; Ascorbic acid equivalents, GAE; Gallic acid equivalents, QE; Quecertin equivalents. LW – low country wet zone, MW – mid-country wet zone, MI – mid-country intermediate zone, UI – up-country intermediate, LD – low-country dry zone

effectiveness of the selected primers in detecting intra-specific diversity [11].

The four primers to detect the genetic variation of *B. alba* L., were selected based on their ability to generate polymorphic, consistent, and clearly scorable banding profiles (under optimized PCR conditions) According to the results, DNA amplified with OPA 9 primer resulted in PCR products of approximately 1500–300 bp length while DNA amplified with OPA 10 primer resulted in PCR products of approximately 1000–300 bp length. Amplification of the DNA with OPA 16 and OPB 10 primers yielded PCR products within the range of 900–200 bp and 1500–400 bp length respectively. Each primer produced a distinct set of amplification products, reflecting random regions across the *B. alba* L. genome. The banding profiles varied in number and intensity among the locations, indicating a considerable degree of polymorphism. The presence of unique bands in certain samples, as well as shared bands among others, was used to construct a binary matrix for genetic similarity analysis. The resulting dendrogram highlighted both genetically similar clusters and distinct lineages, demonstrating the effectiveness of these primers in revealing intra-specific genetic diversity. OPA-9 and OPB-10, which have a high GC content, yielded multiple sharp and well-resolved bands, suggesting a strong annealing affinity to conserved genomic regions (Fig. 1). OPA-10 and OPA-16 also contributed significantly to the overall polymorphism detected, with several bands unique to specific regions, implying possible localized genetic adaptations [12].

RAPD is a widely used PCR-based technique for assessing genetic variation in plants, particularly when genomic information is limited [13]. In the present study, RAPD markers were used to analyze the genetic diversity of *B. alba* L. collected from various locations in Sri Lanka due to its simplicity, cost effectiveness, and ability to detect polymorphisms across the genome without requiring prior sequence data [13]. One of the key advantages of RAPD is that it generates multiple loci simultaneously using short arbitrary primers, allowing for the rapid screening of genetic diversity [14, 15]. It is particularly useful for non-model organisms like *B. alba* L., where genomic resources are scarce [15]. Additionally, RAPD is useful for detecting inter- and intra-population variability, and its high-throughput capability makes it suitable for screening large sample sizes [13, 16]. Nevertheless, RAPD has certain limitations [17] which are discussed at the end of the discussion section. However, by careful standardization and replication we can obtain reliable and reproducible results [18].

Jaccard's similarity coefficient

In this study, the presence or absence of RAPD bands was scored as binary data and fed into NTSYSpc.v2.10e.

software to generate a similarity coefficient matrix, using Jaccard's coefficient (J) algorithm. Jaccard's coefficient is simpler and is most widely used with the RAPD marker [19–21]. Here, the algorithm has derived a similarity coefficient matrix (Fig. 1) from all possible pairwise combinations of individuals (locations), considering the genetic compatibility of one individual (location) to every other individual (location). The dendrogram (Fig. 2) graphically represents the above similarity coefficients, revealing the genetic relationships among samples from different locations. According to Jaccard's similarity coefficient, the highest similarity was observed in Ratnapura and Kandy (Fig. 1), Zwhile the lowest similarity was in Ratnapura and Kalutara. The similarity coefficients showed a wide range of genetic diversity among locations [22].

The genetic variation study using RAPD markers revealed distinct clusters (Fig. 2) in *B. alba* L. sample locations, implying environmental and geographical factors may influence genetic differentiation. Overall, RAPD remains a valuable tool for preliminary genetic diversity assessment and can be particularly informative when combined with geographical, morphological, or biochemical data [23–25].

Association of genetic variation with the phytochemical composition and antioxidant potential of *B. alba* L.

Understanding how genetic variation influences phytochemical composition is essential for selecting and conserving nutritionally and medicinally valuable plant germplasm [26]. According to Dahanayka et al. (2025), geographical variation showed a significant impact on the phytochemical and antioxidant activity of *B. alba* L. [4]. In the present study, the *B. alba* L. collected from diverse agro-climatic zones across Sri Lanka were grouped into several distinct genetic clusters based on RAPD marker analysis. To evaluate whether these genetically distinct clusters exhibit significant differences in their phytochemical profiles, we incorporate the results of our former study [4] which was conducted to investigate the key traits including TPC, TFC, total chlorophyll, total carotene, and antioxidant activities (DPPH and FRAP) of *B. alba* L. By applying the statistical test ANOVA to compare these traits among genetic clusters, the cluster grouping based on the phytochemical study can be used to compare and match the dendrogram generated by molecular markers [27].

The Table 4 demonstrates clear and statistically significant variation ($p < 0.05$) in total chlorophyll, carotene, TFC, TPC, DPPH radical scavenging activity, and FRAP among the genetic clusters. Genetic cluster I include samples from Ratnapura and Kandy, representing the same phytochemical and antioxidant activity cluster (Dahanayaka el al., 2025), which displayed some of the lowest values for antioxidant activity (Dahanyaka et al., 2025) [4]. According to [4] Ratnapura recorded the lowest DPPH and FRAP activities, suggesting a weak antioxidant potential [4]. Additionally, this location showed relatively lower chlorophyll and TPC levels [4]. Kandy also showed a low TPC value and moderate-to-low antioxidant activity [4]. These results suggest that Cluster I harbors genotypes with inferior antioxidant properties, potentially due to the reduced biosynthesis of phenolics and pigments. However, regarding the phytochemical content, genetic cluster I is closely related to the location Kalpitiya of cluster IIAa1". In the cluster analysis based on phytochemical and antioxidant activity [4], Kalpitiya, Rathnapura, and Kandy exist in the same cluster (II) contrasting the results of the genetic clusters [4]. Also, they exhibit closer TPC results to Monaragala, Anuradhapura, and Polonnaruwa according to the results of [4] which belong to different genetic clusters.

Cluster II being genetically more diverse, includes subclusters with considerable variations. In the sub-cluster IIA are the locations, Ella (with higher chlorophyll, TFC, and TPC) Welimada and Badulla (intermediate values across most traits), Gampaha (lower TFC and FRAP values), Jaffna, Anuradhapura, and Polonnaruwa (higher antioxidant activity and chlorophyll content) [4]. However, according to the results of [4] in phytochemical and antioxidant activity-based clustering, these locations are dispersed into several clusters. Cluster IIB (Kalutara, Hikkaduwa, and Kegalle) also showed significant intracluster variation. According to [4] Kalutara showed high chlorophyll and TFC levels, while Hikkaduwa and Kegalle had moderate to lower values across traits [4]. However, in genetic clustering Kalutara, Kegalle, Rathnapura, and Kandy are divergent even though they belong to the same phytochemical and antioxidant-based cluster [4].

The divergence between genetic clustering and phytochemical/antioxidant activity clustering in *B. alba* L. suggests that genetic similarity is not always similar to biochemical similarity [28]. Furthermore, the weak negative correlation ($R = -0.1135$, $p = 0.7974$) resulted by the Mantel test, indicates that there is no significant association between genetic variation and biochemical/antioxidant diversity among the *B. alba* accessions. This suggests that variation in biochemical traits of *B. alba* L. is independent of the genetic diversity captured by RAPD markers. Similar findings have been reported in the study of Khan et al. (2008), where RAPD-based genetic diversity showed a low and non-significant correlation ($r = 0.027$) with biochemical traits, highlighting the need for complementary approaches in assessing genetic diversity related to specific traits [23]. These observations show the complexity of genotype-phenotype relationships and the influence of evolutionary and environmental factors on plant traits. However, these results suggest that genetic clustering based on RAPD markers shows

a partial correspondence with phytochemical and antioxidant diversity. The observed variations can be attributed to both genetic and environmental factors affecting metabolite biosynthesis pathways [29]. Importantly, some locations within the same genetic cluster still showed significant differences in phytochemical traits, indicating that environmental conditions and local adaptations also play critical roles (ex: Rathnapura and Kandy, Table 4).

Influence of geographical locations on genetic variation, phytochemical composition, and antioxidant potential

The grouping of *B. alba* L. samples from different locations into distinct genetic clusters (Fig. 2) appears to reflect the influence of their respective agro-climatic zones. Genetic Cluster IIAa1", which includes locations, Jaffna, Anuradhapura, Kalpitiya, Polonnaruwa, and Hambanthota, is composed entirely of samples from the LD zone [4]. This suggests a degree of genetic adaptation to the dry, high-temperature environments and possibly shared ancestral origins or gene flow within this zone [30]. Similarly, Cluster IIB comprises Kalutara, Hikkaduwa, and Kegalle—all located in the LW zone [4], characterised by high rainfall and humidity. Meanwhile, IIAb, which includes Ella and Welimada, represents populations from the UI zone, known for its cooler temperatures and higher altitudes. These geographical patterns in genetic clustering highlight the potential role of environmental selection pressures in shaping genetic variation [31]. Local adaptation to factors such as temperature, moisture availability, altitude, and soil conditions may drive the divergence observed among clusters [32]. Furthermore, the phytochemical composition and antioxidant potential of these populations also appear to be influenced by geography.

According to [4], samples from Ella (from IIAb genetic cluster) exhibit different phytochemical accumulation profiles compared to the locations from LD, LW, MW, and MI zones, potentially due to altitude-related stress responses that stimulate phenolic and flavonoid biosynthesis pathways [33]. However, Monaragala and Welimada are in a similar genetic cluster with Ella, though they are significantly different in phytochemical clustering. The present study confirms that geographical structuring supports the concept that both genetic markers (RAPD) and functional traits (phytochemicals/antioxidants) can be influenced by geographical location where local environmental conditions varies [4, 34–36]. These findings highlight the importance of considering the agro-ecological context when selecting germplasm for conservation, breeding, or nutraceutical development [37]. Identifying and preserving location-specific genotypes with desirable biochemical traits can contribute to enhancing the resilience and nutritional quality of future *B. alba* L. cultivars. Furthermore, building on the insights of this study, we can extend the thorough investigations on other plant species with higher nutraceutical or therapeutic value.

However, this study has certain limitations that should be acknowledged. Firstly, the number of sampling sites per accession was restricted to one, while the number of accessions from different agro-climatic zones differed, due to natural variation and the limited availability of *B. alba* L. samples cultivated under organic farming practices. This imbalance may influence clustering outcomes by over-representing some regions while under-representing others. A larger sample size with multiple sites per accession would have provided greater statistical power to capture micro-environmental variability. Moreover, as samples were collected only from home gardens under organic practices, the results may not fully reflect the properties of *B. alba* cultivated under conventional farming systems or occurring in the wild. Another limitation was that sampling was conducted within a single season, without accounting for seasonal and inter-annual influences on phytochemical and nutrient composition. Additionally, environmental variables such as soil pH, mineral content, and microclimatic conditions were not directly incorporated, although these factors are known to affect plant biochemical traits. Furthermore, the molecular marker system employed (RAPD) has several limitations. Being a dominant marker system, RAPD cannot distinguish between homozygous and heterozygous loci [17], and its reproducibility can be affected by minor PCR condition variations [13]. These factors restrict its capacity to capture fine-scale allelic richness and may limit the power of correlation analyses between genetic diversity and biochemical traits [38]. Moreover, independent repeat PCR assays were conducted in the present RAPD analysis, but inter-laboratory validation were not performed. Therefore, while banding patterns were clear and consistent under optimized conditions, inter laboratory reproducibility cannot be fully confirmed at this stage.

To overcome these limitations, future research should expand the sampling strategy by including multiple sites per location, incorporating more locations across Sri Lanka, and considering both organic and conventional farming systems. Longitudinal sampling across seasons and years would also help capture temporal variation in phytochemical and antioxidant profiles. Incorporating environmental data such as soil composition, pH, mineral content, and microclimatic conditions would allow more precise attribution of observed variations to ecological drivers. On the molecular level, the use of co-dominant, highly reproducible marker systems such as microsatellites/Simple Sequence Repeats (SSRs) or single nucleotide polymorphisms (SNPs) would provide higher discriminatory power by differentiating heterozygotes

from homozygotes, providing accurate estimates of heterozygosity, gene flow, and population structure [25]. Future studies could increase number of primers to better capture biochemical-associated variation. Additionally, the polymorphic loci detected here can be sequenced and developed into SCAR markers in future studies to improve marker reliability for use in genetic resource management and breeding applications [39]. In future research, morphological parameters can be systematically quantified with standardized descriptors, for better interpret cases such as Ella and Polonnaruwa, where biochemical similarity does not follow molecular clustering.

Despite these limitations, the findings provide novel insights into the genetic diversity of *B. alba* L. across Sri Lanka and its relationship to phytochemical and antioxidant properties. Future work employing more comprehensive sampling and advanced molecular marker systems will further refine the understanding of the genetic and biochemical basis of diversity in *B. alba* L., ultimately supporting its effective conservation and utilization.

Conclusion

In this study, we explored the genetic variability of *B. alba* L. across 15 geographically distinct locations in Sri Lanka using RAPD markers. The Jaccard similarity coefficients revealed genetic similarities ranging from 0.44 to 0.97, indicating substantial genetic variation among samples. Clustering analysis grouped the accessions into two main genetic clusters and five sub-clusters, reflecting both genetic divergence and potential adaptation to different agro-climatic zones by clustering *B. alba* L. samples from similar agro-climatic zones into similar genetic clusters. Importantly, we also explored the association between genetic variation and phytochemical composition and antioxidant activity based on our previous research [4]. Among the studied locations, Ella (UI) and Polonnaruwa (LD) showed both high genetic diversity and superior phytochemical and antioxidant profiles, while the locations, Kalutara (LW) and Kegalle (LW) demonstrated moderate yet consistent performance across traits. Furthermore, region-specific accessions, such as Polonnaruwa of LD zone, Ella of UI zone, and Hikkaduwa of LW zone, exhibited particularly strong antioxidant potential. Interestingly, our findings reveal that while RAPD analysis reflects distinct genetic clustering associated with agro-ecological zones, these genetic patterns do not entirely align with the biochemical profiles. This divergence suggests a complex interplay between genetic makeup and environmental influence, offering valuable insights into genotype-environment interactions and their role in shaping the nutritional and therapeutic potential of GLVs. This study shows the value of integrating molecular marker analysis with biochemical trait evaluation to understand and develop regionally adapted *B. alba* L. cultivars with superior nutritional and therapeutic qualities, supporting sustainable agriculture in Sri Lanka and beyond.

Abbreviations

A	Adenine
ANOVA	Analysis of variance
Bp	Base pairs
C	Cytosine
CTAB	Cetyltrimethylammonium bromide
dATP	Deoxyadenosine triphosphate
dCTP	Deoxycytidine triphosphate
dGTP	Deoxyguanosine triphosphate
DNA	Deoxyribonucleic acid
dNTP	Deoxyribonucleoside triphosphate
DPPH	2,2-Diphenyl-1-picrylhydrazyl
dTTP	Deoxythymidine triphosphate
EDTA	Ethylenediaminetetraacetic acid
FRAP	Ferric reducing antioxidant power
G	Guanine
GAE	Gallic acid equivalents
LD	Low-country dry zone
LI	Low-country Intermediate zone
LW	Low-country wet zone
$MgCl_2$	Magnesium chloride
MI	Mid-country intermediate zone
mM	Milimolar
MW	Mid-country wet zone
NaCl	Sodium chloride
PCR	Polymerase chain reaction
PF	Polymorphic fragments
PPF	Percentage of polymorphic fragments
PVPP	Polyvinylpolypyrolidone
QE	Quecertin equivalents
RAPD	Random amplified polymorphic DNA
SD	Standard deviation
SNP	Single nucleotide polymorphisms
SSR	Simple Sequence Repeats
T	Thymine
TAE	Tris Acetic Acid EDTA
TF	Total number of fragments
TFC	Total flavonoid content
TPC	Total phenolic content
Tris-HCl	Tris-Hydrochloride
UI	Upcountry intermediate zone
UK	United Kingdom
UPGMA	Unweighted pair group method analysis
USA	United States of America
UW	Up-country wet zone.

Supplementary information

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Supplementary Material 1

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Author contributions

LWD – methodology, data curation, formal analysis, writing ± original draft, writing ± review, and editing; MSTM – funding acquisition, project administration, conceptualization, supervision, formal analysis, writing ± original draft, writing ± review and editing; CCK – conceptualization, supervision, resources, writing ± review and editing; DU – conceptualization, supervision, writing ± review, and editing of the manuscript. All authors have read and approved the final manuscript.

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Data availability
The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

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Competing interests
The authors declare that they have no competing interests.

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References

1. Moyo SM, Serem JC, Bester MJ, Mavumengwana V, Kayitesi E. African Green leafy vegetables health benefits beyond nutrition. Food Rev Int [Internet]. 2021, Aug, 18;37(6):601–18. Available from: https://www.tandfonline.com/doi/full/10.1080/87559129.2020.1717519
2. Sagar V, Pragya B, Devi R, Singh, Sk J, Singh P, et al. The inheritance of betalain pigmentation in *Basella alba* L. South Afr J Botany [Internet]. 2022, Mar, 1;145:360–69. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0254629922000345
3. Kumar PC, Oberoi HS, Azeez S. *Basella* - an underutilized green leafy vegetable with a potential for functional food development. Food Rev Int [Internet]. 2022, Nov, 1;38(sup1):456–73. Available from: https://www.tandfonline.com/doi/full/10.1080/87559129.2021.1874410
4. Dahanayaka LW, Mmst M, Kadigamuwa CC, Udayanga D. Impact of geographical variation on nutritional and antioxidant properties of *basella alba* L. from Sri Lanka. BMC Plant Biol [Internet]. 2025, Jan, 27;25(1):118. Available from: https://bmcplantbiol.biomedcentral.com/articles/10.1186/s12870-025-06143-3
5. Venkatachalam P, Malar S, Thiyagarajan M, Indiraarulselvi P, Geetha N. Effect of phycochemical coated silver nanocomplexes as novel growth-stimulating compounds for plant regeneration of *alternanthera sessilis* L. J Appl Phycol [Internet]. 2017, Apr, 12;29(2):1095–106. Available from: http://link.springer.com/10.1007/s10811-016-0977-2
6. Sharma S, Kumar P, Gambhir G, Kumar R, Srivastava DK. Assessment of genetic diversity in lettuce (*Lactuca sativa* L.) germplasm using RAPD markers. 3 biotech [Internet]. 2018, Jan, 11;8(1):9. Available from: http://link.springer.com/10.1007/s13205-017-1039-4.
7. Sobowale OI, Akinyele BO, Odiyi AC, Okunlola AI, Ajayi EO, Aderonmu OL. Assessment of genetic diversity in Indian spinach (*Basella spp* l) accessions using RAPD markers. Asian J Biol [Internet]. 2020, Nov, 21;1–8. Available from: https://journalajob.com/index.php/AJOB/article/view/171.
8. Idehen EO, Oduwaye OA, Lateef LA, Ikeora CJ. Evaluation of genetic diversity in some *amaranthus spp.* using morphological and random amplified polymorphic DNA (RAPD) analysis. Niger J Biotechnol [Internet]. 2019, Mar, 4;35(2):167. Available from: https://www.ajol.info/index.php/njb/article/view/184124.
9. Singh AK, Singh M, Singh R, Kumar S, Kalloo G. Current science association genetic diversity within the genus *solanum* (solanaceae) as revealed by RAPD markers [Internet]. 2006 Mar. Available from: https://www.jstor.org/stable/24089120.
10. Daghighi S, Alizadeh Z, Habibi H. Determination of chromosome number and genetic diversity using SSR and RAPD markers in *ziziphus jujuba* mill. Iran J Sci Technol, Trans A: Sci [Internet]. 2021, Feb, 23;45(1):77–89. Available from: https://link.springer.com/10.1007/s40995-020-00982-5.
11. Alzahib RH, Migdadi HM, Al GA, Alwahibi MS, Afzal M, Elharty EH, et al. Exploring genetic variability among and within hail tomato landraces based on sequence-related amplified polymorphism markers. Diversity (basel) [Internet]. 2021, Mar, 22;13(3):135. Available from: https://www.mdpi.com/1424-2818/13/3/135.
12. Nybom H. Comparison of different nuclear dna markers for estimating intraspecific genetic diversity in plants. Mol Ecol [Internet]. 2004, May, 6;13(5):1143–55. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1365-294X.2004.02141.x.
13. Babu KN, Sheeja TE, Minoo D, Rajesh MK, Samsudeen K, Suraby EJ, et al. Random amplified polymorphic DNA (RAPD) and derived techniques. In. 2021;219–47. Available from: http://link.springer.com/10.1007/978-1-0716-0997-2_13.
14. Williams JGK, Kubelik AR, Livak KJ, Rafalski JA, Tingey SV. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucleic Acids Res [Internet]. 1990;18(22):6531–35. Available from: https://academic.oup.com/nar/article-lookup/doi/10.1093/nar/18.22.6531.
15. James KE, Schneider H, Ansell SW, Evers M, Robba L, Uszynski G, et al. Diversity arrays technology (DArT) for pan-genomic evolutionary studies of non-model organisms. PLoS One [Internet]. 2008, Feb, 27;3(2):e1682. Available from: https://doi.org/10.1371/journal.pone.0001682. Michalak P, editor..
16. Alavi-Siney SM, Saba J, Nasiri J. Genetic variability and population genetic structure in autotriploid saffron using allelic phenotypes of microsatellite markers. Scientia Hortic [Internet]. 2022, Jun, 1;299:111043. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0304423822001686.
17. Bhaskar R, Agnita Sharon E. Molecular genetic approaches in wildlife conservation. Genetic diversity - recent advances and applications [Internet]. 2023. Available from: https://www.intechopen.com/chapters/83797. IntechOpen..
18. Stefańska I, Kwiecień E, Górzyńska M, Sałamaszyńska-Guz A, Rzewuska M. RAPD-PCR-Based fingerprinting method as a tool for epidemiological analysis of *trueperella pyogenes* infections. Pathogens [Internet]. 2022, May, 10;11(5):562. Available from: https://www.mdpi.com/2076-0817/11/5/562.
19. Duarte JM, dos SJ, Melo LC. Comparison of similarity coefficients based on RAPD markers in the common bean. Genet Mol Biol [Internet]. 1999, Sep;22(3):427–32. Available from: http://www.scielo.br/scielo.php?script=sci_arttext%26pid=S1415-47571999000300024%26lng=en%26tlng=en.
20. Lakhani KG, Gamit AJ, Suthar KP, Suthar HG, Parekh VB, Kapadia C. Genetic relatedness analysis of Little Millet (*panicum sumatrense* roth. Ex Roem & Schult.) using RAPD markers. Appl Biol Res [Internet]. 2023, Sep, 1;25(3):311–18. Available from: https://acspublisher.com/journals/index.php/abr/article/view/11655.
21. Hromadová Z, Gálová Z, Mikolášová L, Balážová Ž, Vivodík M, Chňapek M. Efficiency of RAPD and SCoT markers in the genetic diversity assessment of the common bean. Plants [Internet]. 2023, Jul, 25;12(15):2763. Available from: https://www.mdpi.com/2223-7747/12/15/2763.
22. Taunk J, Yadav NR, Chaudhary RK, Singh C, Yadav RC, Kumar R. Genetic diversity among greengram [*Vigna radiata* (L.) Wilczek] genotypes varying in micronutrient (fe and Zn) content using RAPD markers [internet]. Article In Indian J Biotechnol. 2012. Available from: 11. https://www.researchgate.net/publication/235661278.
23. Khan MA, von Witzke-Ehbrecht S, Maass BL, Becker HC. Relationships among different geographical groups, agro-morphology, fatty acid composition and RAPD marker diversity in safflower (*Carthamus tinctorius*). Genetic Resour Crop Evol [Internet]. 2009, Feb, 10;56(1):19–30. Available from: http://link.springer.com/10.1007/s10722-008-9338-6.
24. Molin WT, Kronfol RR, Ray JD, Scheffler BE, Bryson CT. Genetic diversity among geographically separated *cyperus rotundus* accessions based on RAPD markers and morphological characteristics. Am J Plant Sci [Internet]. 2019;10(11):2034–46. Available from: https://www.scirp.org/journal/doi.aspx?doi=10.4236/ajps.2019.1011143.
25. Bidyananda N, Jamir I, Nowakowska K, Varte V, Vendrame WA, Devi RS, et al. Plant genetic diversity studies: insights from dna Marker analyses. Int J Multiling Plant Biol [Internet]. 2024, Jul, 8;15(3):607–40. Available from: https://www.mdpi.com/2037-0164/15/3/46.
26. Oladosu Y, Rafii MY, Arolu F, Chukwu SC, Salisu MA, Olaniyan BA, et al. Genetic diversity and utilization of cultivated eggplant germplasm in varietal

improvement. Plants [Internet]. 2021, Aug, 20;10(8):1714. Available from: https://www.mdpi.com/2223-7747/10/8/1714.
27. 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 [Internet]. 2020, Jun, 1;174:112330. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0031942220301175.
28. Laurentin H, Ratzinger A, Karlovsky P. Relationship between metabolic and genomic diversity in sesame (*sesamum indicum* L.). BMC Genomics [Internet]. 2008, Dec, 29;9(1):250. Available from: https://bmcgenomics.biomedcentral.com/articles/10.1186/1471-2164-9-250.
29. Kliebenstein DJ. Secondary metabolites and plant/environment interactions: a view through *Arabidopsis thaliana* tinged glasses. Plant, Cell & Environ [Internet]. 2004, Jun, 8;27(6):675–84. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1365-3040.2004.01180.x.
30. Kremer A, Ronce O, Robledo-Arnuncio JJ, Guillaume F, Bohrer G, Nathan R, et al. Long-distance gene flow and adaptation of forest trees to rapid climate change. Ecol Lett [Internet]. 2012, Apr, 28;15(4):378–92. Available from: https://onlinelibrary.wiley.com/doi/10.1111/j.1461-0248.2012.01746.x..
31. Booker TR. The structure of the environment influences the patterns and genetics of local adaptation. Evol Lett [Internet]. 2024, Dec, 2;8(6):787–98. Available from: https://academic.oup.com/evlett/article/8/6/787/7735308..
32. Simin T, Davie-Martin CL, Petersen J, Høye TT, Rinnan R. Impacts of elevation on plant traits and volatile organic compound emissions in deciduous tundra shrubs. Sci of the Total Environ [Internet]. 2022, Sep, 1;837:155783. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0048969722028807.
33. Agati G, Biricolti S, Guidi L, Ferrini F, Fini A, Tattini M. The biosynthesis of flavonoids is enhanced similarly by UV radiation and root zone salinity in *L. vulgare* leaves. J Plant Physiol [Internet]. 2011, Feb, 15;168(3):204–12. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0176161710003755.
34. Sudhir SP, Kumarappan A, Malakar J, Verma HN. Genetic diversity of *nigella sativa* from different geographies using RAPD markers. Am J Life Sci [Internet]. 2016;4(6):175. Available from: http://www.sciencepublishinggroup.com/journal/paperinfo?journalid=118%26doi=10.11648/j.ajls.20160406.15.
35. Zhao N, Gao Y, Wang J, Ren A, Xu H. RAPD diversity of *Stipa grandis* populations and its relationship with some ecological factors. Acta Ecologica Sin [Internet]. 2006, May;26(5):1312–18. Available from: https://linkinghub.elsevier.com/retrieve/pii/S1872203206600231.
36. Wang J, Ali MM, Wu Y, Zhang G, Zhang X, Xie L, et al. Genetic diversity and comprehensive evaluation of physicochemical traits in *Phyllanthus emblica* L. for breeding and resource utilization. BMC Plant Biol [Internet]. 2025, Sep, 2;25(1):1187. Available from: https://bmcplantbiol.biomedcentral.com/articles/10.1186/s12870-025-07161-x.
37. Joshi DC, Zhang K, Wang C, Chandora R, Khurshid M, Li J, et al. Strategic enhancement of genetic gain for nutraceutical development in buckwheat: a genomics-driven perspective. Biotechnol Adv [Internet]. 2020, Mar, 1;39:107479. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0734975019301790X.
38. Casiva PV, Saidman BO, Vilardi JC, Cialdella AM. First comparative phenetic studies of Argentinean species of *Acacia* (Fabaceae), using morphometric, isozymal, and RAPD approaches. Am J Botany [Internet]. 2002, May;89(5):843–53. Available from: https://bsapubs.onlinelibrary.wiley.com/doi/10.3732/ajb.89.5.843.
39. Yang L, Fu S, Khan MA, Zeng W, Fu J. Molecular cloning and development of RAPD-SCAR markers for *Dimocarpus longan* variety authentication. Springerplus [Internet]. 2013, Dec, 3;2(1):501. Available from: https://springerplus.springeropen.com/articles/10.1186/2193-1801-2-501.

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