Received: 14 February 2025 | Accepted: 14 May 2025

DOI: 10.1002/agg2.70151

Agrosystems, Geosciences & Environment OPEN ACCESS

ORIGINAL ARTICLE

Agrosystems

Genetic diversity and population structure analysis of *Pisum sativum* var. *abyssinicum* genotypes from Northern Ethiopia using ISSR markers

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Assigned to Associate Editor Hanh Pham.

Funding information
HU-MU-NMBU-NORAD

Abstract

Pisum sativum var. *abyssinicum* is a globally important legume crop, and its genetic diversity is vital for crop improvement, sustainable agriculture, and biodiversity conservation. This study investigated the genetic variability and population structure of *P. sativum* var. *abyssinicum* genotypes from Northern Ethiopia using inter simple sequence repeat (ISSR) markers. A total of 120 genotypes from five geographically based populations were analyzed using 12 ISSR primers. Scorable bands ranged from 443 to 1298, with an average of 815.75 per primer. Primer UBC-880 (where UBC is University of British Columbia) produced the most bands (1298), while UBC-841 generated the highest number of polymorphic bands (421), indicating its effectiveness in detecting genetic diversity. Genetic analysis showed that 84% of variation occurred within populations, with only 16% between them, suggesting greater diversity among individuals of the same population. Factors like gene flow, mutation, and natural selection may contribute to this internal variability. The highest Nei's genetic distance (0.468) was observed between populations SET and R, suggesting strong differentiation, while the lowest (0.06) was between NW and SET, indicating close

Abbreviations: EBI, Ethiopian Biodiversity Institute; FST, Fixation Index; Gst, genetic differentiation statistics by locus; Ne, effective number of alleles; Nm, estimate of the number of migrants (gene flow); PIC, polymorphic information content; R1, Raya 1; R2, Raya 2; SET, South Eastern Tigray; SS, some of squares; UBC, University of British Columbia.

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genetic similarity. Principal coordinate analysis (PCoA) revealed that the ST population was distributed across all three clusters, reflecting genetic similarities with other populations. Four genetically distinct clusters were identified using UPGMA clustering. South Tigray showed the greatest genetic variability. Despite its unique traits and historical importance, the genetic variability of *P. sativum* var. *abyssinicum* remains underexplored. This study provides essential insights for breeding and conservation, supporting the use of ISSR markers to guide genetic improvement and preservation strategies.

Plain Language Summary

Molecular genetic analysis in laboratories helps reproduceability of your work by other researchers easily. Principal component analysis (PCA) is a technique used in data analysis to reduce dimensionality of data. It is used to convert a large set of potentially correlated variables in to smaller set of uncorrelated variables known as principal components. Inter simple sequence repeat (ISSR) molecular marker is used by researchers to study genetic diversity of other crops. The purpose of this study was to investigate the genetic diversity of Dekoko (*Pisum sativum* var. *abyssinicum*) crop collections from the local potential areas of Northern Ethiopia. The result was as hypothesized by the authors. The molecular markers used were polymorphic in the PCA analysis used in the study. Researchers may benefit from this research study as there are no similar studies conducted for this specific orphan crop.

1 | INTRODUCTION

Genetic diversity is a cornerstone of crop improvement, playing a vital role in enhancing yield stability, stress tolerance, and adaptability to changing environmental conditions. In the face of global challenges such as climate change and food insecurity, the conservation and utilization of diverse genetic resources have become increasingly important for sustainable agriculture. Legume crops, including *Pisum sativum* (pea), are essential components of food systems due to their nutritional value and role in soil fertility. Among them, *Pisum sativum* var. *abyssinicum* is a little-studied and regionally adapted variety believed to possess unique genetic characteristics. Its exploration can provide valuable insights for breeding programs aimed at broadening the genetic base of cultivated pea and enhancing resilience to biotic and abiotic stresses. This variety is considered a valuable genetic resource due to its adaptation to diverse agro-ecological conditions and potential traits for disease resistance, drought tolerance, and enhanced yield performance. Ethiopia, recognized as a center of diversity for many crop species, harbors significant genetic variation in *P. sativum* var. *abyssinicum*. Despite its potential for crop improvement, limited research has been conducted on its genetic variability and population structure, particularly in its native environments.

Dekoko (*Pisum sativum* var. *abyssinicum*) is a major crop in mixed farming and is produced alongside the main cereal crops in Tigray (Gebreegziabher & Tsegay, 2016). *Pisum sativum var. abyssinicum* is native to Ethiopia and is well-adapted to medium- to high-altitude and cooler temperatures of the Tigray highlands. Besides, Dekoko is an important agricultural crop used to make an Ethiopian traditional dish called "Dekoko wot." In northern Ethiopia, Dekoko is also used as a source of protein and essential nutrients in the local diet, contributing to food security and nutrition in the region. In developing countries where it is difficult to obtain and consume animal products, field peas are an important source of protein. In the developed world, pulse crops replace vital protein and fat content while also serving as the primary source of feed (Teshome et al., 2015). Moreover, Ethiopia is a site of origin for cultivated flora, resulting in a concentrated distribution of *P. sativum* subsp. *abyssinicum* (Gebreslassie & Abraha, 2016).

Pisum sativum is beneficial to crop rotations because they disrupt disease and insect cycles, provide nitrogen, increase soil microbial diversity and activity, promote soil aggregation, save soil water, and provide economic diversification. Additionally, Dekoko produces antifungal proteins that aid the plant in combating phytopathogenic fungi, thereby protecting the plant from devastating fungal infections and preventing

massive economic losses (Gebreslassie & Abraha, 2016). Furthermore, *P. sativum* also improves soil structure and reduces the need for synthetic fertilizers, ultimately leading to a more sustainable and environmentally friendly agricultural system. Legumes are also grown as green manures and cover crops because they grow quickly and add nitrogen to the soil. Overall, incorporating legumes like *P. sativum* into crop rotations can lead to improved soil health, increased crop yields, and reduced environmental impact.

Pisum sativum var. *abyssinicum* plants produce a large number of basal branches that are at least as tall as the main stem once the seeds ripen and the main stem begins to wilt (Kosterin, 2017). This feature of *P. sativum* var. abyssinicum promotes enhanced seed production and overall plant health, making it an important crop for sustainable farming techniques. Furthermore, this variety's deep root system improves soil structure and water retention, which increases its environmental benefits. Moreover, *P. sativum* var. *abyssinicum*, often known as the Ethiopian pea, is valued for its drought tolerance and capacity to grow in difficult conditions (Smykal et al., 2015; Yang et al., 2022). *Pisum sativum* is especially significant for food security in areas with unpredictable rainfall and harsh weather. Its flexibility makes it an essential crop for sustainable agriculture in difficult conditions.

Understanding genetic diversity and population structure of genotype is essential for developing effective conservation and breeding programs (Erbano et al., 2015). Besides genetic diversity is critical for enhancing plant species' overall resistance to numerous stresses, including disease and climate change (Tian et al., 2018). For genetic diversity investigation, inter simple sequence repeats (ISSRs) are one of the most widely used polymerase chain reaction (PCR)-based marker systems, in which they are an effective tool for analyzing genetic diversity and population structure of many legume crops (Fang & Roose, 1997). To assess this diversity, molecular markers are often employed, with ISSR markers being one of the most effective tools. ISSRs offer several advantages, including high reproducibility, the ability to detect a wide range of polymorphisms across the genome, and their cost-effectiveness, especially in species with limited genomic resources. Unlike other markers such as simple sequence repeats (SSRs) or single nucleotide polymorphisms (SNPs), ISSRs do not require prior genomic sequencing, making them ideal for studying species like *P. sativum* var. *abyssinicum*, which has limited genetic data available. Therefore, ISSRs were selected for this study to evaluate the genetic diversity of *P. sativum* var. *abyssinicum* and explore its potential contributions to breeding and conservation efforts. Furthermore, studying this species' genetic diversity can assist in identifying important traits that can be used to improve crop yield, disease resistance, and nutritional values. However, the genetic diversity study of *P. sativum* var abyssinicum using ISSR markers in Ethiopia is limited. Therefore, this study is aimed at investigating information that is crucial for developing breeding programs that can help farmers in the region improve their *P. sativum* crop. Despite its unique adaptation to the Ethiopian highlands and its ecological role in improving soil fertility, *P. sativum* var. *abyssinicum* remains poorly studied in terms of genetic diversity. This lack of data limits its inclusion in breeding programs and conservation strategies. As climate change intensifies and agricultural systems face growing pressure to produce more with fewer resources, identifying and utilizing genetically diverse and resilient crop varieties is critical. Assessing the genetic variability of *P. sativum* var. *abyssinicum* is therefore essential not only for preserving this underutilized germplasm but also for exploring its potential contributions to improving the genetic base, adaptability, and sustainability of pea breeding programs.

Core Ideas

- All the inter simple sequence repeat (ISSR) markers tested were polymorphic and highly informative. The genotypes collected from southern Tigray exhibited the highest values across multiple genetic diversity parameters, including the number of observed alleles, estimated allele frequency, effective allele count, Nei's gene diversity, Shannon's information index, and the percentage of polymorphic loci.
- This research study's findings will be highly valuable for agronomists seeking to further explore this specific orphan crop.
- Dekoko, a native crop of Ethiopia, is a staple in local cuisine, often served in restaurants as food and stew. With approximately 200 fasting days observed annually in Ethiopia, the study of this nutrient-rich crop holds significant potential to attract global researchers interested in its dietary, cultural, and agricultural importance.

2 | MATERIALS AND METHODOLOGY

2.1 | Plant materials collection and genomic DNA extraction

In this research, a total of 120 genotypes were studied: 83 genotypes from Southern Tigray (ST), 20 genotypes from North Wello (NW) (Amhara regional state), six genotypes from the Ethiopian Biodiversity Institute (EBI), nine genotypes from South-eastern Tigray (SET), and two additional commercial varieties called Raya 1 and Raya 2 randomly (Table S1; Figure 1). For genotyping, high molecular weight

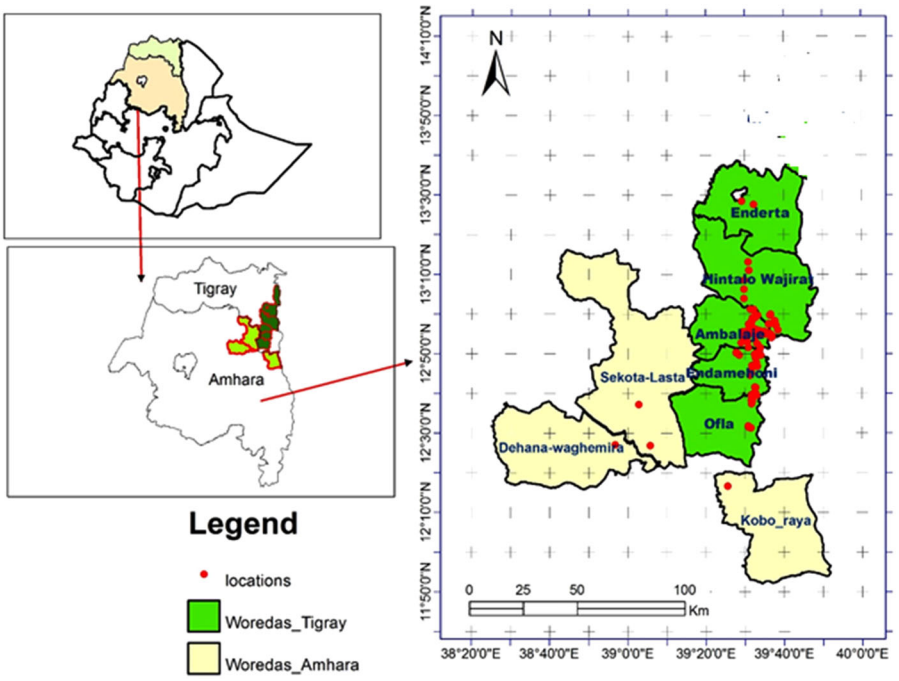


FIGURE 1 Map of the plant materials sampling areas.

genomic DNA (gDNA) was extracted from 2-week-old silica gel-dried leaf samples using a modified cetyltrimethylammonium bromide (CTAB) method (Atsbeha et al., 2023; Mekonnen et al., 2020; Abd El-Fatah & Nafea, 2020; Ogbonnaya et al., 2001). The quality and quantity of isolated DNA were checked using 1% agarose gel and NanoDrop spectrophotometry, respectively.

This study utilizes ISSR markers to analyze the genetic diversity and population structure of *P. sativum* var. *abyssinicum* genotypes from Northern Ethiopia. ISSR markers were chosen due to their cost-effectiveness, making them a budget-friendly alternative to SSR and SNP markers. No need for prior sequence information—unlike SSRs and SNPs, which require species-specific primers or sequencing data. While SSRs and SNPs are superior in terms of precision and repeatability, ISSRs provide a balanced approach when resources are limited, species-specific markers are unavailable, or a preliminary genetic diversity assessment is needed before investing in more refined techniques (Jones et al., 2007).

2.2 | ISSR genotyping and data scoring

Twenty-eight ISSR primers, ranging in length from 16 to 28 bp and representing di-, tri-, and tetramer repeats, were examined for polymorphism and repeatability. Out of the 28 examined primers, 12 reproducible primers with different banding patterns were chosen and used (Figure 2). These primers were dissolved in sterilized, double-distilled water at a concentration of 10 pmol/L. Several quantities of template DNA, dNTPs, and Taq polymerase were tested for optimal amplification. The amplification procedure included experimenting with different quantities of template DNA, dNTPs, and Taq polymerase to find the best combination for optimal amplification efficiency. Subsequently, PCR was performed in a total volume of 20 μL containing 2 μL template DNA, 2 μL $MgCl_2$, 0.5 μL dNTP mix, 0.25 μL primer, 0.5 μL Taq DNA, and 11.7-μL double-distilled water. Besides, the PCR condition (annealing temperature) was optimized using a Biometra 2003 T3 thermocycler. An initial denaturation at 94˚C for 5 min followed by 40 cycles of denaturation at 94˚C for 1 min was performed. The optimization of the PCR condition was carried out to determine the most suitable annealing temperature for primer binding. In optimum primer annealing conditions (45–52˚C for 1 min, primer extension at 72˚C for 2 min, and a final extension step of 10 min at 72˚C were used.

Amplification products were fractionated by loading 10 μL of the amplification product of each sample with 2 μL of 6X loading dye on 1.67% agarose gel electrophoresis supplemented with ethidium bromide in 1 × TBE buffer at 120 V for 2 h (this percentage agar gel electrophoresis was used after optimization). The gel was photographed using a BIO-RAD Gel Doc TM EZ Imaging System after being visualized under UV light. Then, the amplification size was estimated using a 100-bp DNA ladder. Since ISSR markers are dominant markers, for scoring, each locus was deemed a bi-allelic locus with one amplifiable and one null allele. Consequently, each primer was carefully scored based on presence (1), absence (0), or ambiguity (?). The band scoring produced a “0” and “1” data

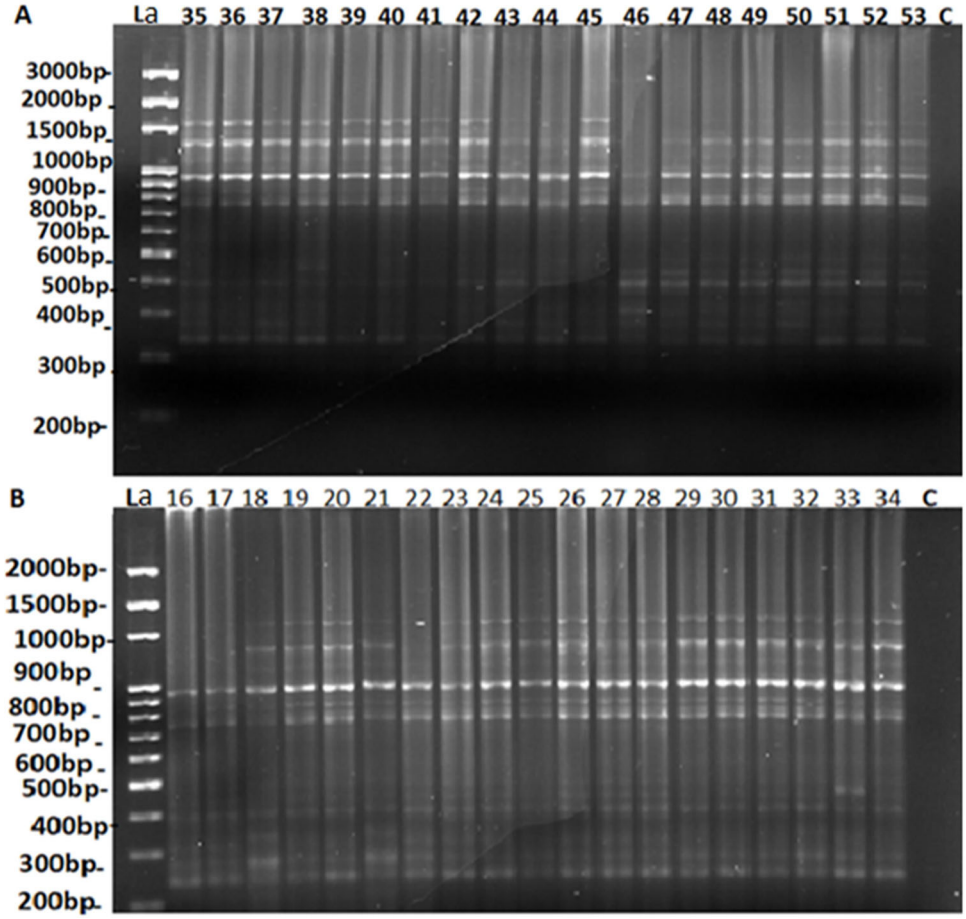


FIGURE 2 Banding pattern of ISSR primer UBC-841 (where UBC is University of British Columbia) in 19 genotypes: (A) 19 genotypes and (B) additional genotypes. La = DNA ladder, C = negative control.

matrix that was analyzed and allowed for the determination of genetic diversity and population structure of *P. sativum var. abyssinicum* genotypes.

2.3 | Data analysis

Several statistical software packages were used to investigate the genetic diversity and population structure of the genotypes. POPGENE version 1.31 software was used to compute locus-based diversity parameters such as the number of observed alleles (Na), effective number of alleles (Ne), Shannon's information statistic (*I*), Nei's genetic diversity, and genetic differentiation statistics by locus (Gst), and gene flow (Nm). Additionally, GenAlEx ver. 6.501 software was used to calculate population genetic diversity parameters such as number of observed alleles (Na), effective number of alleles (Ne), Shannon's Information index (*I*), Nei's genetic diversity (h), and percent of polymorphic loci (PPL) across loci (Peakall & Smouse, 2012). Moreover, the same software was used for investigation analysis of molecular variance (AMOVA) and variance component estimation. In addition, polymorphic information content (PIC) was calculated using the formula $PIC = 2f(1-f)$ (Roldan-Ruiz et al., 2000), where f is the frequency at which the polymorphic bands emerge. Besides, clustering was performed using molecular evolutionary genetics analysis software and the unweighted pair group method with arithmetic mean (UPGMA). For population structure analysis, STRUCTURE software, version 2.3.4, was used to perform Bayesian model-based clustering with postulated K populations on the tested 120 genotypes. The parameters of the experiment were 80,000 burn-in iterations followed by 80,000 Monte Carlo Markov Chain iterations (Pritchard et al., 2000).

3 | RESULT

3.1 | ISSR markers performance

Twelve primers were chosen for their capacity to generate unambiguous and repeatable banding patterns. The primers generated a total of 9789 bands, yielding bands ranging in size from 200 to 1500 bps. The scorable bands ranged between 443 and 1298, with an average number of bands of 815.75 per primer. Primer UBC-880 (where UBC is University of

TABLE 1 Summary of inter simple sequence repeat (ISSR) primers performance.

ISSR primer	Total number of bands	Number of polymorphic bands	Polymorphism percentage
UBC-811	959	194	20.22%
UBC-812	625	204	32.64%
UBC-818	647	194	29.98%
UBC-834	569	222	39.02%
UBC-835	908	353	38.88%
UBC-841	1039	421	40.52%
UBC-848	443	325	73.34%
UBC-857	730	300	41.10%
UBC-873	1158	351	30.31%
UBC-880	1298	285	21.96%
UBC-881	855	406	47.48%
UBC-895	558	265	47.49%
Average	815.75	293.33	–
Total	9789	3520	–

Abbreviation: UBC, University of British Columbia.

British Columbia) generated higher number of bands (1298), followed by primer UBC-873 (1158). The highest number of polymorphic bands (421) was recorded for the ISSR primer UBC-841, followed by UBC-881 with value of 406 (Table 1).

3.2 | Genetic diversity indices

The analysis revealed that the ISSR markers used were highly polymorphic and informative with a mean number of alleles of 1.94, an effective allele count of 1.55, a Shannon information index of 0.51, Nei's gene diversity of 0.33, and gene flow of 5.94 (Table 3). The highest number of alleles (Na = 2.00), effective allele count (Ne = 1.86), gene diversity (H = 0.46), and Shannon information index (I = 0.46) were obtained for primer UBC-812. Primer UBC-848 and UBC-881 resulted in an estimate of the number of migrants (Nm [gene flow]) = 16.82 and PIC = 0.43, respectively. On the other hand, primer UBC-835 produced the lowest observed number of alleles = 1.82 and Shannon information index = 0.41. Accordingly, 100% of the ISSR markers showed considerable PIC (Table 1). Moreover, 50% of the primers (5) demonstrated PIC values greater than the average value (0.38) (Table 2).

3.3 | Population structure analysis

3.3.1 | Analysis of molecular variance

Using the 182 ISSR bands generated by the 12 primers, an AMOVA was conducted to investigate variation between and within populations. AMOVA revealed that within-population genetic variability accounted for the majority (84%) of total genetic variation, leaving only 16% for between-population genetic variation. FST (Fixation Index) compares genetic variation within subpopulations to the total genetic variation across all populations. The populations showed statistically significant low genetic differentiation (FST value of = 0.081; $p < 0.001$ among the five *P. sativum* populations (Table 3).

3.3.2 | Structure

The genetic structure of the 120 *P. sativum* individuals was assessed using a Bayesian clustering approach in STRUCTURE v2.3.4. The analysis was performed for K values ranging from 1 to 10, and the optimal number of genetic clusters was determined to be $K = 3$ using CLUMPAK (Cluster Markov Packager across K). The resulting STRUCTURE bar plot (Figure 3B) revealed that individuals were assigned to three major genetic clusters, but with varying degrees of admixture. The clusters did not correspond strictly to the geographic origin of the populations, suggesting that gene flow and historical mixing have occurred among regions. Many individuals exhibited membership in more than one cluster, further supporting the presence of genetic intermixing. This observation aligns with the results of UPGMA and principal coordinate analysis (PcoA) analyses, which also indicated a lack of clear geographic separation among the populations.

3.3.3 | Principal coordinate analysis

PCoA is a commonly applied multivariate technique used to visualize genetic relationships among individuals and quantify the genetic variation explained by different axes (Atsbeha et al., 2023; Mekonnen et al., 2020). In this study, the first three principal axes accounted for a cumulative 34.78% of the total genetic variation, with axis 1 explaining 13.89%, axis 2

TABLE 2 Summary statistics of informativeness and genetic diversity indices.

Primer	Na	Ne	*I*	*H*	Ht	Hs	Gst	Nm	PIC
UBC-811	1.93	1.64	0.51	0.35	0.35	0.27	0.19	5.26	0.29
UBC-812	2.00	1.86	0.65	0.46	0.37	0.25	0.32	1.81	0.40
UBC-818	2.00	1.63	0.54	0.36	0.30	0.23	0.17	4.90	0.34
UBC-834	1.92	1.50	0.54	0.36	0.31	0.25	0.15	4.53	0.44
UBC-835	1.82	1.49	0.46	0.31	0.35	0.22	0.33	3.49	0.34
UBC-841	1.94	1.62	0.52	0.35	0.24	0.24	0.33	1.56	0.42
UBC-848	1.92	1.42	0.41	0.23	0.27	0.23	0.11	16.82	0.34
UBC-857	1.93	1.45	0.49	0.31	0.35	0.24	0.27	7.73	0.36
UBC-873	1.95	1.42	0.42	0.27	0.41	0.21	0.42	1.80	0.42
UBC-880	2.00	1.68	0.62	0.43	0.41	0.31	0.25	3.79	0.42
UBC-881	2.00	1.46	0.46	0.27	0.33	0.28	0.12	15.81	0.43
UBC-895	1.9	1.48	0.45	0.29	0.16	0.18	0.38	3.75	0.37
Mean	1.94	1.55	0.51	0.33	0.32	0.24	0.25	5.94	0.38
SEM	0.02	0.04	0.02	0.02	0.02	0.01	0.03	1.49	0.01
SD	0.05	0.13	0.07	0.07	0.07	0.03	0.11	5.15	0.05
Minimum	1.82	1.42	0.41	0.23	0.16	0.18	0.11	1.56	0.29
Maximum	2.00	1.86	0.65	0.46	0.41	0.31	0.42	16.82	0.44

Note: Na = observed number of alleles, Ne = effective number of alleles, I = Shannon's Information index, H = Nei's gene diversity, Ht = total genetic diversity Hs = genetic differentiation, Gst = genetic differentiation statistics by locus, Nm = estimate of the number of migrants (gene flow) from Gst where Nm = 0.5 (1 − Gst)/Gst, PIC = polymorphic information content, and UBC = UBC, University of British Columbia.

TABLE 3 Analysis of molecular variation among and within populations.

Source	*df*	SS	MS	EV	PV	FST	*p*-value
Among Pops	4	384.345	96.086	4.310	16%	0.05	0.001
Within Pops	115	3832.671	33.328	33.328	84%	–	0.001
Total	119	4217.017	–	37.637	100%	–	–

Abbreviations: EV, estimated variation; *df*, degree of freedom; FST, Fixation Index; MS, mean square; Pops, populations; PV, percent of variation; SS, some of squares.

accounting for 11.83%, and axis 3 contributing 9.09%. The PCoA scatter plot revealed the presence of three main genetic clusters among the 120 *P. sativum* genotypes (Figure 4). Populations EBI, ST, SET, and R largely grouped together in Cluster 3 (C3), whereas individuals from the NW and ST populations were more dispersed between Cluster 1 (C1) and Cluster 2 (C2). Importantly, no cluster was composed exclusively of genotypes from a single geographic population, indicating a high degree of genetic admixture. These findings are broadly consistent with the STRUCTURE results, which also identified three genetic clusters with considerable intermixing. Together, the PCoA and STRUCTURE analyses suggest that gene flow and shared ancestry are likely contributing to the observed lack of strict population differentiation.

3.3.4 | Phylogenetic relationships (UPGMA)

The unweighted pair group method with arithmetic mean (UPGMA) based clustering method identified four core clusters (C1, C2, C3, and C4). In C4, 36 (30%) genotypes were included, while C3 had 31 (26%). Furthermore, clusters C2 and C1 had fewer genotypes, with 25 (21%) and 28 (23%), respectively (Figure 5). The clustering observed in the UPGMA tree reveals a pattern of genetic differentiation and possible historical relationships among the populations. These clusters suggest the existence of distinct genetic groups within *P. sativum*, although some overlap is evident, indicating genetic exchange among the populations. The clustering does not strictly correlate with geographic origin, which is consistent with the findings from STRUCTURE and PCoA analyses, further highlighting the complexity of the genetic structure and gene flow dynamics.

The average estimated allele frequency (p and q) was $p = 0.39$ (range 0.29–0.51) and $q = 0.61$ (range 0.49–0.72). Likewise, the observed alleles were 1.52 (range 1.25–2.00), and effective alleles were 1.43 (range 1.16–1.61). Moreover, the Shannon information index was 0.37 (range 0.14–0.52) and Nei's gene diversity was 0.25 (range 0.09–0.35), respectively. The ST population showed the highest observed

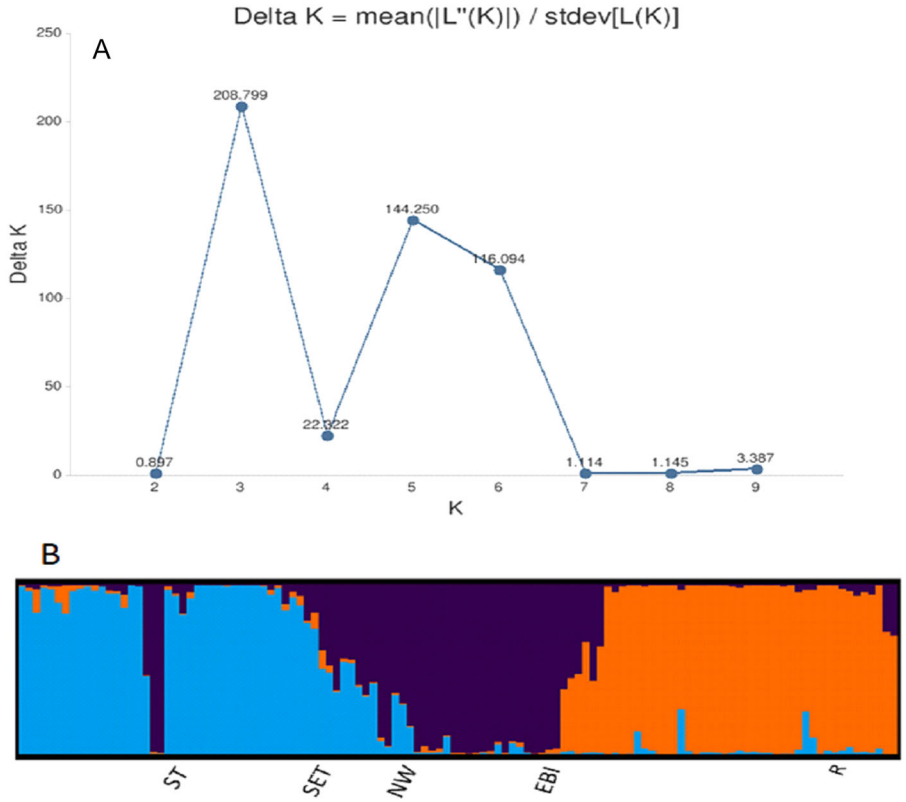


FIGURE 3 Population structure of 120 *P. sativum* var. *abyssinicum* genotypes grouped in to five populations. (A) Best estimated delta *K* value for $K = 3$ and (B) estimated population structure; the blue, purple, and orange colors represent genetic groups designated by Structure Harvester analytic software. Population abbreviations are NW = North Wello, SET = South East Tigray, ST = South Tigray, EBI = Ethiopian Biodiversity Institute, and R = Raya 1 and Raya 2.

TABLE 4 Summary of diversity indices across 5 Dekoko populations as revealed by 12 inter simple sequence repeat (ISSR) markers.

Population	*N*	Band frequency	*p*	*q*	Na	Ne	*I*	*H*	uHe	PPL (%)
NW	20	0.45	0.29	0.72	1.90	1.58	0.50	0.33	0.34	94.51%
SET	9	0.54	0.41	0.59	1.54	1.46	0.39	0.26	0.28	69.78%
ST	83	0.48	0.30	0.70	2.00	1.61	0.52	0.35	0.35	100.0%
EBI	6	0.57	0.47	0.53	1.25	1.36	0.28	0.20	0.21	48.35%
R	2	0.00	0.51	0.49	0.89	1.16	0.14	0.09	0.12	22.53%
Mean	24	0.41	0.39	0.61	1.52	1.43	0.37	0.25	0.26	67.03%

Note: *N* = sample size, Na = number of observed alleles, *p* and *q* = frequency of estimated alleles, Ne = effective number of alleles, *H* = Nei's gene diversity, *I* = Shannon's information index, uHe = unbiased and expected heterozygosity, and PPL = percentage of polymorphic loci. Na = number of different alleles, Ne = number of effective alleles = $1/(p^2 + q^2)$, *I* = Shannon's information index = $-1 \times [p \times \text{Ln}(p) + q \times \text{Ln}(q)]$, He = expected heterozygosity = $2 \times p \times q$, uHe = unbiased expected heterozygosity = $[2N/(2N-1)] \times$ He where for diploid binary data and assuming Hardy–Weinberg equilibrium $q = (1 - \text{Band Freq.})^{0.5}$, and $p = 1 - q$.
Abbreviations: EBI, Ethiopian Biodiversity Institute; NW, North Wello; R, Raya 1 and Raya 2; SET, South East Tigray; ST, South Tigray.

number of alleles, effective number of alleles, Nei's gene diversity, Shannon's information index, and PPL. Over 50% of the populations under consideration had genetic diversity above the mean ($h = 0.25$), and the PPL per population ranged from 22.53% (R) to 100% (ST), with an overall mean PPL of 67.03%. The estimates of genetic diversity across loci and populations are summarized in Table 4.

The highest mean heterogeneity (0.35) was observed by ST, followed by NW (0.33) and SET (0.26) (Table 5). Additionally, private bands used for distinguishing populations from each other were available in the SET and EBI populations (Figure 6).

3.4 | Populations' Genetic Relationships

Estimates using genetic distance (below diagonal) and gene flow (above diagonal) showed the highest genetic distances

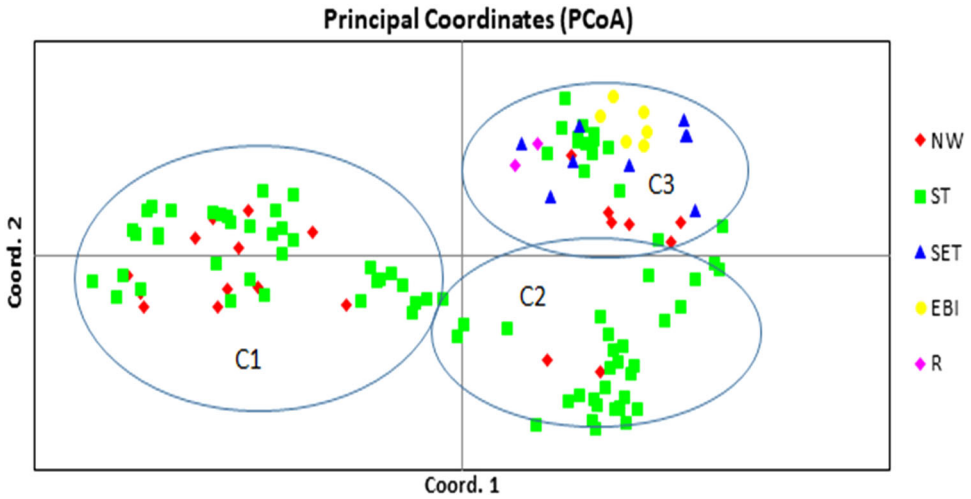


FIGURE 4 Principal coordinate analysis (PCoA) of 120 *P. sativum* var. *abyssinicum* genotypes as revealed by 12 molecular markers. Samples coded with same color and shape belong to the same population. EBI, Ethiopian Biodiversity Institute; NW, North Wello; R, Raya 1 and Raya 2; SET, South East Tigray; ST, South Tigray.

TABLE 5 Pairwise matrix of Nei's genetic distance of the population.

NW	SET	ST	EBI	R	
0.000					NW
0.060	0.000				SET
0.075	0.161	0.000			ST
0.226	0.313	0.119	0.000		EBI
0.384	0.468	0.220	0.167	0.000	R

Abbreviations: EBI, Ethiopian Biodiversity Institute; NW, North Wello; R, Raya 1 and Raya 2; SET, South East Tigray; ST, South Tigray.

of 0.468, 0.384, and 0.313 between SET and R, NE and R, and SET and EBI, respectively. The lowest genetic distance (0.06) was observed between NW and SET populations (Table 5).

4 | DISCUSSION

There has been very little research done on *P. sativum* var. *abyssinicum* so far. However, initial studies have shown promising results in terms of its yield and nutrition potential (Yemane & Skjelvåg, 2003). As a result, researcher attention to the crop is increasing. In this study, the genetic diversity and population structure of 120 *P. sativum* var. *abyssinicum* populations were analyzed to facilitate the selection of parent plants with desirable traits for future breeding programs and to ensure the sustainable utilization of this valuable genetic resource. Moreover, marker-based genetic diversity analysis in this study has revealed a wide range of genetic variability within *P. sativum* var. *abyssinicum* populations.

4.1 | ISSR markers performance

The selection of 12 primers for genetic analysis showcases their efficiency in producing clear and repeatable banding patterns. A total of 9789 bands were generated, highlighting a remarkable level of genetic variation and marker resolution. The band sizes, ranging from 200 to 1500 bps, align well with the expected range for ISSR markers, confirming their suitability for diverse genetic studies. With a scorable band range of 443–1298 and an average of 815.75 bands per primer, the primers demonstrated consistent amplification efficiency. However, notable differences were observed among them. Primer UBC-880 stood out by generating the highest number of bands (1298), followed closely by UBC-873 (1158), underscoring their superior amplification capabilities.

Polymorphism plays a pivotal role in evaluating genetic diversity, and among the ISSR primers tested, UBC-841 exhibited the highest number of polymorphic bands (421), followed closely by UBC-881 (406). These findings align with previous studies that have demonstrated the effectiveness of ISSR markers in detecting genetic variation across various plant and animal species (Bornet & Branchard, 2001; Reddy et al., 2002). The high polymorphism levels observed suggest substantial genetic variability within the analyzed samples, a factor that has been widely reported in studies on genetic fingerprinting and population genetics (Kumar et al., 2020). Such genetic diversity is particularly advantageous for applications such as breeding programs, conservation genetics, and evolutionary research. Similar results have been observed in studies on plant germplasm characterization, where ISSR markers, including UBC-841 and UBC-881, have been effective in differentiating closely related genotypes. Overall, the results validate the reliability of the selected primers for

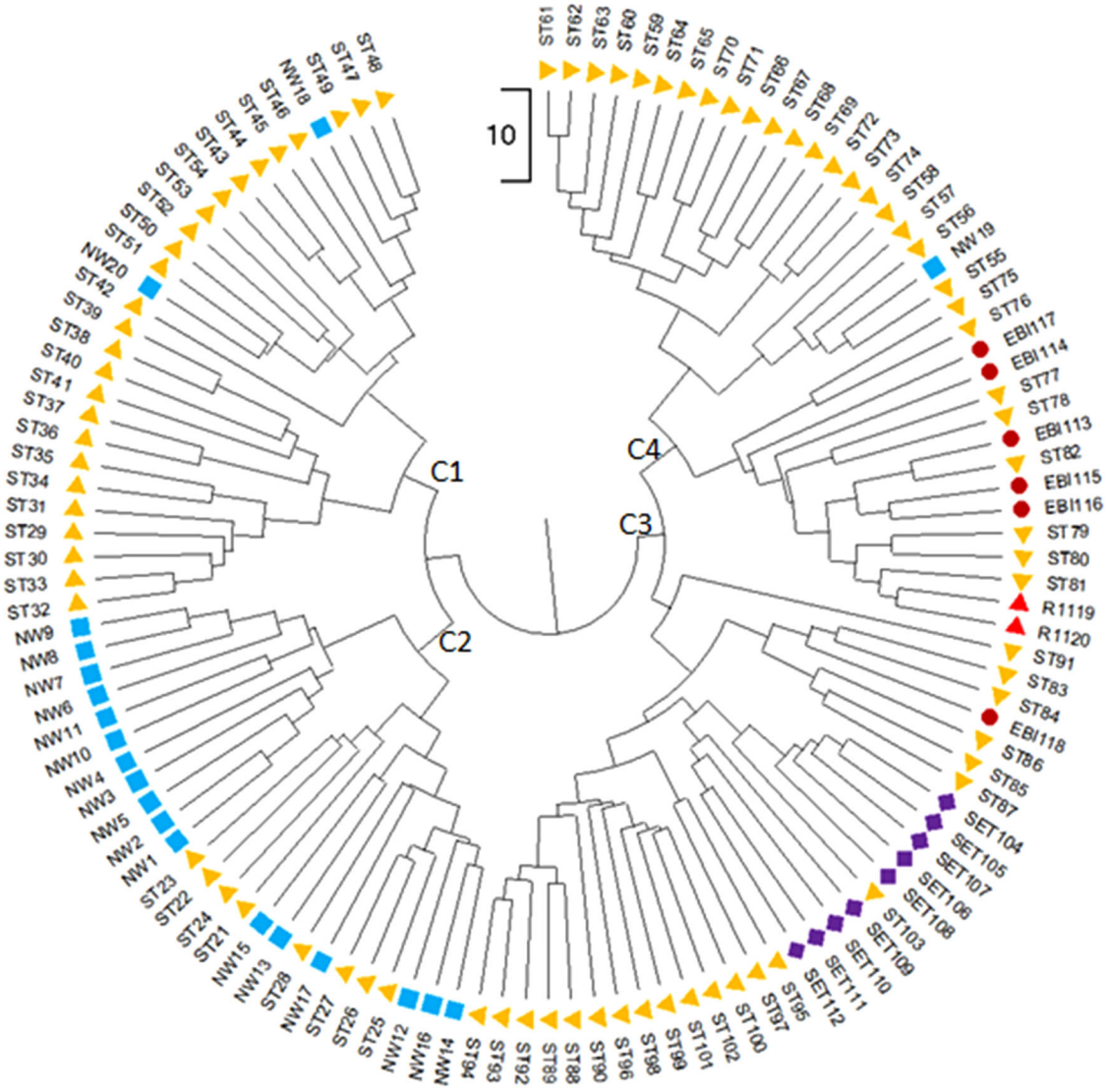


FIGURE 5 The neighbor joining-based clustering of 120 *P. sativum* genotypes from five populations. Samples coded with the same symbol and color belong to the same population. Population abbreviations are NW = North Wello, SET = South East Tigray, ST = South Tigray, EBI = Ethiopian Biodiversity Institute, R1 = Raya 1 and R2 = Raya 2.

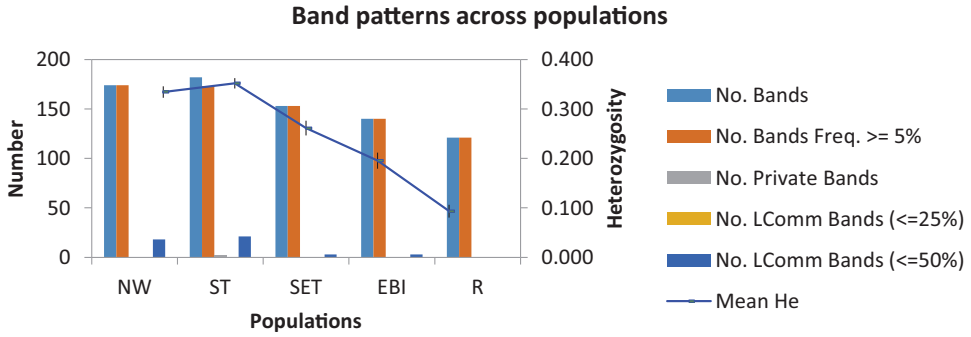


FIGURE 6 Pattern of bands formed between populations of genotypes. EBI, Ethiopian Biodiversity Institute; NW, North Wello; R, Raya 1 and Raya 2; SET, South East Tigray; ST, South Tigray.

genetic fingerprinting, with UBC-841 and UBC-881 demonstrating particular efficacy in polymorphism detection. Future research should explore their applicability across diverse populations to assess their reproducibility and efficiency in broader genetic contexts.

4.2 | Genetic Diversity Indices

Genetic diversity indicators: Na (number of alleles per locus): The mean value of Na = 1.94, ranging from 1.82 to 2.00, suggests that the primers used generally amplify two alleles per locus, indicating moderate genetic diversity within the studied population. Ne (effective number of alleles): The average Ne = 1.55, with a range of 1.42–1.86, shows that although multiple alleles exist, their contributions to genetic diversity are not equal, likely due to allele frequency variations. Informative measures of diversity: *I* (Shannon's information index): The mean *I* = 0.51 suggests moderate genetic variability, with UBC-812 showing the highest diversity (0.65), while UBC-848 has the lowest (0.41). *H* (Nei's gene diversity): The mean *H* = 0.33 indicates an intermediate level of genetic variation. The highest *H* value (0.46 for UBC-812) implies a higher degree of polymorphism for that primer, whereas the lowest (0.23 for UBC-848) suggests lower diversity.

Genetic structure and differentiation: Ht (total gene diversity) and Hs (within-population diversity): The mean values Ht = 0.32 and Hs = 0.24 indicate that genetic diversity is more structured within populations rather than across populations. Gst (genetic differentiation coefficient): The mean Gst = 0.25 suggests that about 25% of genetic variation is due to differences among populations, while 75% is within populations. This moderate genetic differentiation indicates possible gene flow or shared ancestry among studied populations. Nm (gene flow estimate): The mean Nm = 5.94 suggests high gene flow, which helps maintain genetic homogeneity among populations. High Nm values like 16.82 for UBC-848, indicate extensive gene flow, reducing genetic differentiation.

PIC: The mean PIC = 0.38, ranging from 0.29 to 0.44, indicates moderate informativeness of the primers. PIC values above 0.40 (for the ISSR primers, UBC-812, UBC-841, UBC-873, UBC-880, and UBC-881) suggest these primers are highly informative for genetic studies, whereas lower PIC values indicate less polymorphic loci.

In this study, the mean *H* value of 0.33 is comparable to the 0.34 observed in *Aframomum corrorima* (Chombe & Bekele, 2018) suggesting a moderate level of genetic variation. The mean *I* value of 0.51 in of the study aligns with the 0.51 found in *Aframomum corrorima* indicating a similar or greater degree of genetic diversity. The mean Nm value of 5.94 in our data suggests higher gene flow compared to the Nm of 1.08 observed in the other studies, indicating more substantial genetic exchange among populations in this current study. These comparisons suggest that the genetic diversity in this study is moderate and aligns with findings from recent research on similar plant species. The relatively higher gene flow in our populations may contribute to the moderate genetic differentiation observed.

4.3 | Analysis of Molecular Variance

Only 16% of the total genetic variation is attributed to differences among populations. The FST value (0.05) suggests a low level of genetic differentiation among populations, indicating substantial gene flow or a shared ancestry. The *p*-value (0.001) confirms that this differentiation is statistically significant.

A large proportion (84%) of genetic diversity is found within populations, meaning that individual plants within the same population are genetically diverse. This suggests that factors such as recombination, mutation, and possibly local adaptation contribute to within-population genetic variation. This confirms the total variation analyzed and shows that most diversity is retained at the within-population level.

Recent studies on *Pisum* spp. have found similar patterns of low differentiation among landraces, which may be due to historical gene flow, shared ancestry, or similar selective pressures across different environments. The presence of significant within-population diversity suggests that on-farm conservation, breeding, and environmental adaptation play a key role in maintaining genetic variability.

Since diversity is largely within populations, in-situ conservation strategies should focus on maintaining diverse accessions rather than relying solely on distinct population collections. The low genetic differentiation implies that cross-breeding between populations may not introduce significantly novel alleles, which should be considered in breeding programs. Recent studies on pea (*Pisum sativum* L.) genetic diversity have employed various molecular markers and analytical methods to assess genetic variation and population structure (Sharma et al., 2022). Research on common bean (*Phaseolus vulgaris* L.) in Chongqing, China, assessed genetic diversity to support the development of superior germplasm and genetic resources (Chen et al., 2021).

The AMOVA showed that most of the genetic variation occurred within populations rather than among populations, suggesting a high degree of genetic diversity within individual populations. This high within-population variation indicates that there is significant genetic structuring at the local level, potentially due to factors such as local adaptation, environmental influences, or gene flow within populations. Furthermore, the AMOVA results align with the F_ST values, which revealed low to moderate genetic differentiation among populations. This consistency supports the idea that while there is some genetic distinction between populations,

the majority of the variation resides within populations. These findings highlight the complexity of population structure in *P. sativum* and suggest that genetic diversity is largely shaped by internal factors within populations, with gene flow and adaptation playing key roles.

4.4 | Structure

Best estimated delta *K* value for *K* (3) suggests that the most likely number of genetic clusters (*K*) in the population is three, based on an analysis using Structure Harvester. Delta *K* is a statistical measure used to determine the optimal number of genetic groups in population structure analysis. This part presents the genetic composition of each individual genotype, usually displayed as a bar plot. The different colors (blue, green, purple, and orange) represent distinct genetic groups identified through Structure Harvester analysis. Each individual genotype is depicted as a vertical bar, where different colors within a bar indicate genetic admixture from multiple populations.

The five populations include NW (North Wello), SET (South East Tigray), ST (South Tigray), EBI, and R (Raya 1 and Raya 2). These groups likely represent different geographic origins or breeding sources of the genotypes. Overall, the figure illustrates the genetic structure and diversity within these populations, providing insights into their relatedness, genetic differentiation, and potential gene flow between them. The graph shows the Evanno method results used to determine the optimal number of genetic clusters (*K*).

Each vertical bar represents an individual genotype, with different colors indicating genetic group membership. The colors (blue, purple, and orange) correspond to the three genetic clusters identified in (A). Populations ST (South Tigray) and SET (South East Tigray) show a dominant blue genetic background. NW (North Wello) has a mix of blue and purple, suggesting admixture. EBI appears predominantly purple. R (Raya) is mainly orange, showing a distinct genetic structure from the other populations.

The structure analysis supports the existence of three major genetic groups in *Pisum sativum* var. *abyssinicum*. The varying levels of admixture indicate gene flow between populations, with some groups being more genetically distinct (e.g., R). The clustering reflects possible geographic or historical selection pressures shaping genetic diversity.

4.5 | Principal Coordinate Analysis

The principal coordinate analysis (PCoA) plot presents a visual representation of the relationships among different groups based on their similarities or dissimilarities. The *x*-axis (Coord. 1) and *y*-axis (Coord. 2) represent the principal coordinate dimensions, which account for variation among the data points.

The points are grouped into three main clusters (C1, C2, and C3) as indicated by the ellipses. Clustering suggests that samples within each cluster share more similarity with each other than with samples in other clusters.

Different symbols/colors represent distinct groups (NW, ST, SET, EBI, and R). Green squares (ST) are widely distributed across all clusters but appear concentrated in C1 and C2. Red diamonds (NW) are interspersed in C1, C2, and C3, indicating dispersion. Blue triangles (SET) are more dominant in cluster C3. Yellow circles (EBI) are also in C3, showing an overlap with SET. Purple diamonds (R) are fewer and mainly positioned near C3. C1 is mostly composed of ST and NW samples. C2 is dominated by ST samples with some NW.C3 contains a mixture of SET, EBI, and some ST and NW samples.

There is clear clustering, suggesting structured variation among the groups. ST (green squares) appears to be the most widespread. SET and EBI (blue triangles and yellow circles) tend to group together in C3. NW (red diamonds) is scattered throughout, showing no strong affiliation with a specific cluster. The PCoA indicates distinct clustering patterns among the groups, suggesting differentiation based on the analyzed variables. The presence of overlapping groups suggests some shared characteristics, while separate clusters indicate divergence.

4.6 | Phylogenetic Relationships (UPGMA)

This image represents a phylogenetic tree constructed using the UPGMA (unweighted pair group method with arithmetic mean) clustering method.

The tree is structured into distinct clusters labeled C1, C2, C3, and C4. These clusters indicate groups of closely related sequences that share common ancestors. The branch lengths represent evolutionary distances between the populations. A scale bar of "10" is present, indicating the genetic divergence measure.

Different populations are marked with distinct symbols and colors, such as yellow triangles, blue squares, red circles and purple diamonds. This likely represents different groups, such as species, strains, or lineages. Closely related genotypes are grouped together, implying shared evolutionary histories. Some branches show deeper divergence, suggesting distinct evolutionary paths. The clustering may indicate evolutionary relationships, host adaptation, or geographic distribution. Identifying shared clusters can provide insights into genetic similarities or evolutionary pressure.

Populations within the same cluster may be closely related species or strains. The presence of well-defined clusters suggests possible subspecies differentiation. Closer branches

indicate recent divergence events. Larger distances suggest long-term separation and evolutionary divergence.

5 | CONCLUSION

In the current study, genetic diversity and population structure of 120 *P. sativum var. abyssinicum* genotypes were analyzed using highly informative ISSR markers. This provides valuable understandings into the genetic diversity and population structure of studied *P. sativum var. abyssinicum* genotypes. The research demonstrated that the markers utilized were completely informative, making them excellent tools for describing genetic diversity and population structure of *P. sativum* var. *abyssinicum* genotypes. Among the five *P. sativum var.abyssinicum* populations, the ST population had the highest observed number of alleles, effective number of alleles, Nei's gene diversity, Shannon's information index, and PPL. The populations were adequately grouped using UPGMA-based clustering, PCoA, and STRUCTURE analysis, confirming significant genetic intermixing and high gene flow between populations. In conclusion, the *P. sativum* var.abyssinicumgenotypes from the ST population exhibit high genetic diversity and could be valuable resources for breeding programs aimed at improving *P. sativum var.abyssinicum*. The genetic research results show that there is considerable gene flow and genetic intermixing among *P. sativum* populations, implying a complex population structure within the species. The genetic diversity identified in this study has significant implications for breeding programs aimed at improving the genetic resilience of *P. sativum var. abyssinicum*, particularly in the face of environmental stresses like drought. Populations exhibiting higher levels of genetic variation, especially those with greater diversity within populations, should be prioritized as sources of genetic material for breeding. These populations likely harbor alleles that contribute to traits such as drought tolerance and stress resistance. By incorporating these genetically diverse lines into breeding programs, it is possible to enhance the genetic pool of *P. sativum* and develop varieties better adapted to changing environmental conditions. This approach would increase the crop's resilience and improve its sustainability in regions prone to drought stress.

AUTHOR CONTRIBUTIONS

Gebru Equar Gebremichael: Conceptualization; data curation; formal analysis; investigation; methodology; project administration; software; visualization; writing—original draft; writing—review and editing. **Yemane Tsehaye Bayratsion**: Conceptualization; supervision; validation; writing review and editing. **Fetien Abay Abera**: Conceptualization; supervision. **Yemane G. Egziabher**: Conceptualization; supervision. **Desta Berhe Sbhatu**: Conceptualization; supervision. **Kassahun Tesfaye**: Conceptualization; funding acquisition; methodology; resources; supervision. **Tilahun Mekonnen**: Conceptualization; methodology; resources; supervision; validation. **Genet Atsbeha**: Conceptualization; formal analysis; methodology; software; supervision; writing—review and editing.

ACKNOWLEDGMENTS

HU-MU-NMBU-NORAD project is highly appreciated and acknowledged for the financial support. AAU, Institute of Biotechnology, Plant Genetics Laboratory is accordingly appreciated and acknowledged for the provision of lab space, chemicals, equipment.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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REFERENCES

Abd El-Fatah, B. E. S., & Nafea, D. S. (2020). Genetic relationships and diversity among pea (*Pisumsativum*L.) genotypes assessed using agro-morphological and molecular markers. *Journal of Agricultural Chemistry and Biotechnology*, *11*(12), 353–363. https://doi.org/10.21608/jacb.2020.137305

Atsbeha, G., Tesfaye, K., Mekonnen, T., Haileselassie, T., & Kebede, M. (2023). Genetic diversity and population structure analysis of bread wheat (*Triticumaestivum* L.) germplasms as revealed by inter simple sequence repeat (ISSR) markers. *Genetic Resources and Crop Evolution*, *71*(6), 2721–2735. https://doi.org/10.1007/s10722-023-01791-6

Bornet, B., & Branchard, M. (2001). Nonanchored inter simple sequence repeat (ISSR) markers: Reproducible and specific tools for genome fingerprinting. *Plant Molecular Biology Reporter*, *19*, 209–215. https://doi.org/10.1007/BF02772892

Chen, X., Zhou, G., Pang, J., & Srinives, P. (2021). Editorial: Domestication of agronomic traits in legume crops. *Frontiers in Genetics*, *12*, 707600. https://doi.org/10.3389/fgene.2021.707600

Chombe, D., & Bekele, E. (2018). Genetic diversity analysis of cultivated Korarima [*Aframomumcorrorima* (Braun) P.C.M. Jansen] populations from southwestern Ethiopia using inter simple sequence repeats (ISSR) marker. *Journal of Biological Research-Thessaloniki*, *25*(1), Article 1. https://doi.org/10.1186/s40709-017-0073-z

Erbano, M., Schuhli, G. S. E., & Santos, E. P. D. (2015). Genetic variability and population structure of Salvia lachnostachys: Implications for breeding and conservation programs. *International Journal of Molecular Sciences*, *16*, 7839–7850. https://doi.org/10.3390/ijms16047839

Fang, D., & Roose, M. (1997). Identification of closely related citrus cultivars with inter-simple sequence repeat markers. *Theoretical and Applied Genetics*, *95*, 408–417. https://doi.org/10.1007/s001220050577

Gebreslassie, B., & Abraha, B. (2016). Review: Distribution and productivity of dekoko (*Pisum sativum* var. *abyssinicum* A. Braun) in Ethiopia. *Global Journal of Science Frontier Research: C Biologi-*

cal Science, *16*(3), 45–57. https://www.researchgate.net/publication/3090642708

Jones, E. S., Sullivan, H., Bhattramakki, D., & Smith, J. S. (2007). A comparison of simple sequence repeat and single nucleotide polymorphism marker technologies for the genotypic analysis of maize (*Zea mays* L.). *Theoretical and Applied Genetics*, *115*(3), 361–371. https://doi.org/10.1007/s00122-007-0570-9

Kosterin, O. E. (2017). Abyssinian pea (*Lathyrus schaeferi* Kosterin pro *Pisum abyssinicum* A. Br.)—A problematic taxon. *Acta Biologica Sibirica*, *3*(3), 97–110. https://doi.org/10.14258/abs.v3i3.3621

Kumar, A., D'Souza, S. S., Uenishi, G., Park, M. A., Lee, J. H., & Slukvin, I. I. (2020). Generation of T cells from human and nonhuman primate pluripotent stem cells. *Bio-protocol Journal*, *10*(13), e3675. https://doi.org/10.21769/BioProtoc.3675

Mekonnen, T., Haile Selassie, T., Goodwin, S. B., & Tesfayea, K. (2020). Genetic diversity and population structure of *Zymoseptoriatritici* in Ethiopia as revealed by microsatellite markers. *Fungal Genetics and Biology*, *141*, 103413. https://doi.org/10.1016/j.fgb.2020.103413

Ogbonnaya, F. C., Subrahmanyam, N. C., Moullet, O., de Majnik, J., Eagles, H. A., Brown, J. S., Eastwood, R. F., Kollmorgen, J., Appels, R., & Lagudah, E. S. (2001). Diagnostic DNA markers for cereal cyst nematode resistance in bread wheat. *Australian Journal of Agricultural Research*, *52*(12), 1367–1374. https://doi.org/10.1071/AR01031

Peakall, R., & Smouse, P. E. (2012). Genetics and population analysis, GenAlEx 6.5: Genetic analysis in Excel. Population genetic software for teaching and research—An update. *Bioinformatics*, *28*(19), 2537–2539. https://doi.org/10.1093/bioinformatics/bts460

Pritchard, J. K., Stephens, M., & Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics*, *155*(2), 945–959. https://doi.org/10.1093/genetics/155.2.945

Reddy, M. P., Sarla, N., & Siddiq, E. (2002). Inter simple sequence repeat (ISSR) polymorphism and its application in plant breeding. *Euphytica*, *128*, 9–17. https://doi.org/10.1023/A:1020691618797

Roldan-Ruiz, I., Dendauw, J., Van Bockstaele, E., Depicker, A., & De Loose, M. (2000). AFLP markers reveal high polymorphic rates in ryegrasses (*Lolium spp.*). *Molecular Breeding*, *6*, 125–134. https://doi.org/10.1023/A:1009680614564

Sharma, A., Sharma, S., Kumar, N., Rana, R. S., Sharma, P., Kumar, P., & Rani, M. (2022). Morpho-molecular genetic diversity and population structure analysis in garden pea (*Pisumsativum* L.) genotypes using simple sequence repeat markers. *PLoS One*, *17*(9), e0273499. https://doi.org/10.1371/journal.pone.0273499

Smykal, P., Coyne, C. J., Ambrose, M. J., Maxted, N., Schaefer, H., Blair, M. W., Berger, J., Greene, S. L., Nelson, M. N., Besharat, N., Vymyslický, T., Toker, C., Saxena, R. K., Roorkiwal, M., Pandey, M. K., Hu, J., Li, Y. H., Wang, Y. X., Guo, Y., … Varshney, R. K. (2015). Legume crops phylogeny and genetic diversity for science and breeding. *Critical Reviews in Plant Sciences*, *34*, 43–104. https://doi.org/10.1080/07352689.2014.897904

Teshome, A., Bryngelsson, T., Dagne, K., & Geleta, M. (2015). Assessment of genetic diversity in Ethiopian field pea (*Pisum sativum* L.) accessions with newly developed EST-SSR markers. *BMC Genetics*, *16*, Article 102. https://bmcgenomdata.biomedcentral.com/articles/10.1186/s12863-015-0261-5

Tian, H. Z., Han, L. X., Zhang, J. L., Li, X. L., Kawahara, T., Lopez-Pujol, J., Kumar, P., Chung, M. G., & Chung, M. Y. (2018). Genetic diversity in the endangered terrestrial orchid *Cypripedium japonicum* in East Asia: Insights into population history and implications for conservation. *Scientific Reports*, *8*, Article 6467. https://doi.org/10.1038/s41598-018-24912-z

Yang, T., Liu, R., Luo, Y., Hu, S., Wang, D., Wang, C., Pandey, M. K., Ge, S., Xu, Q., Li, N., Li, G., Huang, Y., Saxena, R. K., Ji, Y., Li, M., Yan, X., He, Y., Liu, Y., Wang, X., … Zong, X. (2022). Improved pea reference genome and pan-genome highlight genomic features and evolutionary characteristics. *Nature Genetics*, *54*, 1553–1563. https://www.nature.com/articles/s41588-022-01172-2

Yemane, A., & Skjelvåg, A. O. (2003). Effects of fertilizer phosphorus on yield traits of Dekoko (*Pisum sativum* var. *abyssinicum*) under field conditions. *Journal of Agronomy and Crop Science*, *189*(1), 14–20. https://doi.org/10.1046/j.1439-037X.2003.00595.x

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How to cite this article: Gebremichael, G. E., Bayratsion, Y. T., Abera, F. A., Egziabher, Y. G., Sbhatu, D. B., Tesfaye, K., Mekonnen, T., & Atsbeha, G. (2025). Genetic diversity and population structure analysis of *Pisum sativum* var. *abyssinicum* genotypes from Northern Ethiopia using ISSR markers. *Agrosystems, Geosciences & Environment*, *8*, e70151. https://doi.org/10.1002/agg2.70151