

# Evaluating Genetic Diversity in Kenyan Pineapple (*Ananas comosus*) Germplasm Using Sequence-Related Amplified Polymorphism (SRAP) Markers

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## Abstract

Pineapple (*Ananas comosus*), a widely cultivated tropical fruit, is known for its global economic and nutritional significance. The study aimed to investigate the genetic diversity of 80 pineapple accessions from Kenya using Sequence-Related Amplified Polymorphism (SRAP) markers. Out of the 48 SRAP primers, six polymorphic primers were identified. Genetic diversity was examined using DARWin, GenAlEx, and PopGene software. DNA (deoxyribonucleic acid) extraction followed a modified CTAB (Cetyltrimethylammonium bromide) method, ensuring quality DNA for analysis. Polymerase Chain Reaction (PCR) was conducted with selected primers to amplify DNA segments, revealing polymorphisms. The findings revealed significant genetic differences among (22%) and within (78%) populations, with a PhiPT value of 0.221, Nei's gene diversity (H) of 0.1761, and Shannon's Information index (I) of 0.2699, indicating moderate population differentiation. Principal Coordinates Analysis (PCoA) explained 53.47% of the overall genetic variation, revealing a relatively homogenous genetic composition within accessions. Gene flow (Nm) was 1.3623, suggesting moderate gene flow among populations. The dendrogram grouped samples into two clusters, corroborating the homogenous genetic composition. No differentiation among populations was observed using Nei's genetic distance matrix. The overall genetic similarity suggests that most populations share a common genetic background. Improving the genetic diversity of Kenyan pineapples is essential for enhancing resilience and sustainability. This can be achieved through collecting local varie-

ties, cross-breeding, importing new cultivars, conducting genomic studies, and establishing gene banks. These efforts will help make Kenyan pineapples more adaptable, productive, and resistant to climate change and other challenges.

## Keywords

Pineapple, Genetic Diversity, Genetic Analysis, SRAP, Polymorphism

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## 1. Introduction

Pineapple (*Ananas comosus*) thrives in tropical climates and is known to have a rich content of bioactive compounds, dietary fiber, minerals, and nutrients. The fruit has potential health advantages, such as anti-inflammatory and antioxidant properties [1]. Pineapple is a significant source of vital minerals and vitamins, including calcium, potassium, fiber, and vitamin C [2]. The distinct aroma and flavor of the pineapple fruits are attributed to specific volatile organic compounds (VOCs) [3]. Varieties such as the MD2 are preferred for sensory attributes, including juice volume and overall preference [4].

The cultivation of pineapples in Kenya holds significant importance, playing a vital role in domestic consumption and regional trade. Kenya had an export quantity of 30567.83 tonnes (t) of pineapples in 2022, increasing from 22527.56 t in 2021 [5]. Pineapple's economic significance is emphasized by its cultivation in diverse regions, providing benefits to both small-scale farmers and larger commercial growers [6].

The advantages of pineapple over other fruits include its hardiness and adaptability to diverse climates [7], making it suitable for cultivation in various regions of Kenya. The fruit has an extended shelf life, and its suitability for processing contributes to its economic viability [8]. Moreover, previous research shows that pineapple cultivation significantly increases the income of growers. [9] reported a 146.72 thousand average income increase per annum for pineapple cultivators from the Madhupur upazila in the Tangail District. [10] suggested that small-scale pineapple farmers can enhance their production efficiency through better use of available resources. However, the pineapple market in Kenya is oligopsonistic, with limited integration between urban and rural markets [11].

The findings of this study revealed moderate genetic diversity among the 80 pineapple accessions analyzed. These results are consistent with a study conducted by [12], which demonstrated low genetic diversity among the cultivars grown in Tanzania. Similar results were observed in a 2020 study by Ismail *et al.* in Malaysia, which also revealed moderate genetic diversity among 65 accessions. The lack of characterization of Kenyan pineapple accessions significantly hampers efficient cultivation practices and agricultural management, which is further compounded by the misidentification of varieties, such as "Smooth Cayenne" and MD2. Fur-

ther, some farmers claim to have local landraces, which have not been verified. Addressing this issue is pivotal for enhancing the precision of pineapple cultivation, promoting informed decision-making among farmers, and fostering sustainable agricultural practices in Kenya.

Genetic diversity is a fundamental aspect of plant breeding and conservation, playing a pivotal role in the creation of new cultivars, conservation of germplasm, and adaptation to environmental challenges [13] [14]. *Ananas comosus* holds both economic and cultural significance in Kenya, and the assessment of genetic variation within the germplasm is of paramount importance. Assessment of genetic variation not only aids in understanding the genetic structure of the local gene pool but also contributes to improving crop management and breeding programs.

Genetic diversity is crucial for the adaptability, productivity, and resilience of crops [15], including genetic variation present within a species, which can be exploited to enhance desirable traits and address vulnerabilities. Traditionally, plant genetic diversity assessment relied on morphological characteristics and agronomic traits [16]-[18]. However, these methods often have limitations in terms of accuracy and sensitivity. The dawn of molecular markers has dramatically transformed genetic diversity research by offering precise, high-throughput, and reproducible means to evaluate genetic variation [19].

Molecular markers are specific sequences of DNA that are utilized to identify genetic differences at a molecular level. They have played an instrumental role in quantifying genetic diversity, identifying the relationships among plant populations, and assisting in germplasm characterization [20]. Numerous molecular markers have been developed, such as Restriction Fragment Length Polymorphisms (RFLPs), Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSRs), and Amplified Fragment Length Polymorphisms (AFLPs) [21]. Each marker comes with its benefits and drawbacks, influencing the choice of marker for a specific genetic diversity study [22] [23].

SRAP is a relatively recent molecular marker technique that offers several advantages for genetic diversity studies over other markers, which include: high polymorphism detecting a wide range of genetic variation, which will be useful for assessing genetic diversity within pineapple populations; efficient because they use relatively simple primers to amplify specific regions of the genome, producing multiple bands in a single reaction, which helps in screening large numbers of individuals; prior sequence information not required. Unlike other markers like SSRs [simple sequence repeats] or SNPs (single nucleotide polymorphisms), they are cost-effective, highly reproducible and have good genome coverage giving a comprehensive understanding of the genetic makeup of pineapple plant [24], thus making them a valuable tool.

They were developed in 2001 by Li and Quiros and are based on polymerase chain reaction (PCR), utilizing primers that target coding regions in the genome. SRAP markers have been successfully applied to investigate genetic diversity in

various plant species [25] and are particularly suited to species with complex genomes like pineapple.

Previous genetic diversity studies in pineapple have employed various molecular markers, including RAPD, AFLP, and SSRs, with varying degrees of success [26]. These studies have uncovered genetic variation within cultivated pineapple germplasm and provided insights into the origin and diversity of pineapple varieties. However, there remains a need for comprehensive assessments, especially considering the emerging importance of pineapple as a cash crop in Kenya.

The assessment of genetic variation in Kenyan pineapple germplasm using SRAP markers represents a promising avenue for enhancing our understanding of the genetic structure of commercial and local pineapple accessions. Given the importance of pineapple in Kenya, such studies can facilitate the development of improved cultivars, conservation strategies, and more sustainable cultivation practices.

## 2. Materials and Methods

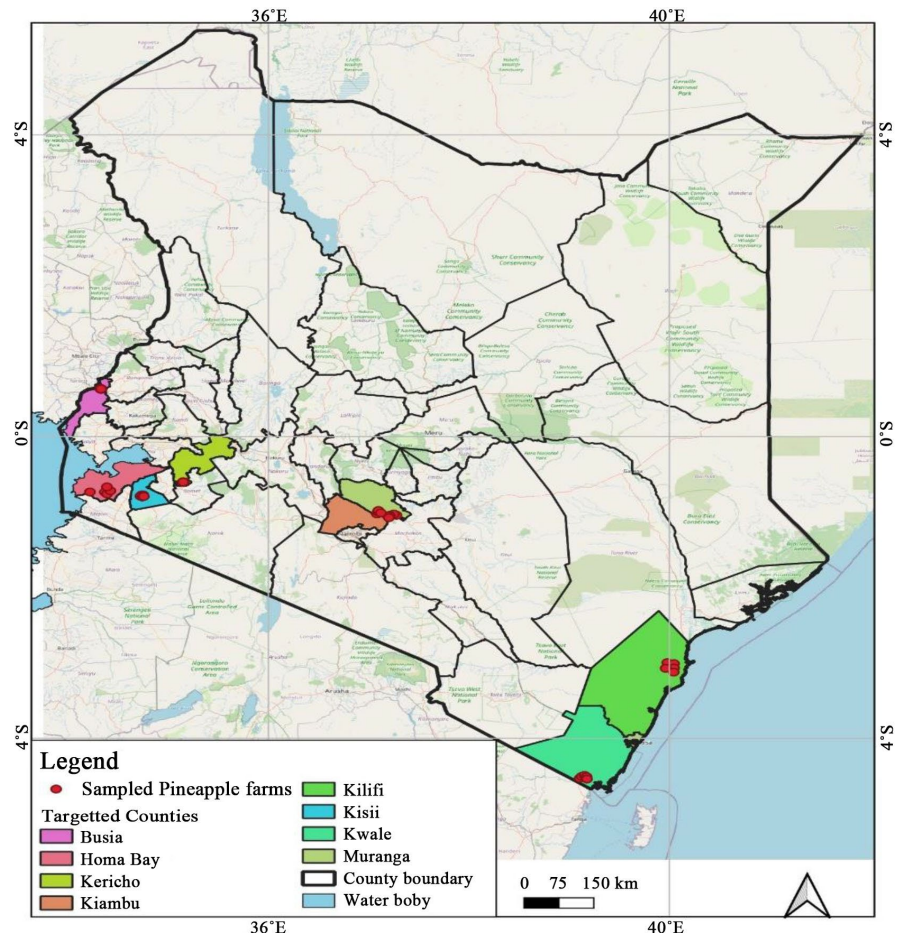
### 2.1. Sample Collection and Preparation

Eighty (80) pineapple (*Ananas comosus*) suckers were collected from eight counties in Kenya: Kisii, Kwale, Busia, Kericho, Kilifi, Murang'a, Kiambu, and Homa Bay (Figure 1). The selected sites represented the major pineapple-growing areas in Kenya. Farmers from each region were chosen randomly, and purposeful sampling was employed, as suckers were collected only from healthy plants on the farms [27]. Ten accessions were selected from each region, resulting in a sample size of 80, which is consistent with previous studies on genetic diversity in similar crops and aligns with plant genetic research. This sample size allows for analysis of genetic differentiation both within and among populations while remaining manageable in terms of resource conservation [28] [29].

The pineapple suckers were collected from farmers in the diverse regions of Kenya and later established in a screen house at the Kenya Agricultural and Livestock Research Organization (KALRO)-Biotechnology Centre, Kabete, until ready for DNA extraction.

Briefly, 10 suckers per farmer field were collected by carefully slicing suckers from mature pineapple plants. The samples were collected during the peak harvest season of pineapples to ensure the genetic variability was well-represented. Each sample was labelled with essential information, including the region of origin, the number of contributing farmers, GPS, photos, and the farm.

The pineapple suckers, upon collection, were carefully packed in 2 kg khaki bags to ensure their freshness and vitality during transportation to KALRO-Biotechnology Centre, Kabete. Upon arrival, the suckers were acclimatized and established in a screen house. Subsequently, the samples were further processed for DNA extraction, and genetic analysis was conducted using Sequence-Related Amplified Polymorphism (SRAP) markers.



**Figure 1.** Map displaying the geographic coordinates of pineapple sample collection sites in Kenya. Each point represents a sample location.

## 2.2. DNA Extraction

Genomic DNA was extracted following a modified Cetyltrimethylammonium bromide (CTAB) method. The CTAB buffer contained 100 mM Tris-HCl pH 8.0, 2% CTAB, 20 mM EDTA pH 8.0, 1.4 M NaCl, and 2%  $\beta$ -mercaptoethanol. Before use, the buffer was pre-heated to 65°C. Approximately 100 mg of leaves were collected, 750  $\mu$ L of preheated CTAB extraction buffer was added, and the mixture was ground using a pestle and mortar into a homogenous sample. The mixture was transferred into 2-mL microcentrifuge tubes.

The mixture was incubated at 65°C for 30 minutes, with intermittent mixing by gentle inversion after every 10 minutes. After incubation, the mixture was left to cool to RT, and chloroform: isoamyl alcohol (24:1) 600  $\mu$ L was added, and the mixture was gently inverted 10 times. The subsequent step involved centrifugation at 13,000 rpm for 10 minutes at RT. This process was repeated twice.

The upper aqueous phase was carefully transferred to a new tube, and DNA was precipitated by adding an equal volume (~600  $\mu$ L) of ice-cold isopropanol. The tubes were incubated at -20°C for 60 minutes to facilitate DNA precipitation, followed by centrifugation at 13,000 rpm for 10 minutes, and the supernatant was

discarded. The DNA was washed by adding 600  $\mu\text{L}$  of ice-cold 70% ethanol and spun at 13,000 rpm for 5 minutes. The supernatant was discarded by carefully decanting. The process was repeated twice. Thereafter, DNA was air-dried for 30 minutes at RT to remove residual ethanol and re-suspended in 100  $\mu\text{L}$  of DNase-free water.

### 2.3. Quality and Integrity Assessment of Extracted DNA

The quality of the DNA was checked using a 0.8% (w/v) agarose gel prepared in 1X TAE buffer. Six (6)  $\mu\text{L}$  of DNA samples were loaded into the wells of the 0.8% (w/v) agarose gel, which was pre-stained with 0.5  $\mu\text{g}/\text{ml}$  ethidium bromide [30]. Gel electrophoresis was carried out at 70 volts for 1 hour. DNA was visualized using UV illumination (Uvitec Gel Documentation | Thermo Fisher Scientific - AU, USA). Photographs (digital images) were taken to document the DNA bands. The extracted DNA was evaluated based on the presence of intact, high-molecular-weight DNA bands. Additionally, the quantity and purity of the DNA were assessed using a spectrophotometer (Thermo Scientific NanoDrop 2000c, USA). DNA concentrations were normalized to 20 ng/ $\mu\text{L}$  across all samples based on the NanoDrop readings. Subsequently, genetic analyses were performed using SRAP markers.

### 2.4. SRAP Marker Analysis

The SRAP protocol developed by [31] was employed. The sequences of the forward and reverse primers used in this study are shown in **Table 1**.

**Table 1.** List of forward and reverse SRAP primers used for genetic analysis of pineapple samples. Sequences are provided in the 5'-3' direction.

Forward primer (name: sequence 5'-3')	Reverse primer (name: sequence 5'-3')
	Em5: GACTGCGTACGAATTAAC
Me1: TGAGTCCAAACCGGATA	Em7: GACTGCGTACGAATTCAA
Me2: TGAGTCCAAACCGGAGA	Em9: GACTGCGTACGAATTCGA
Me5: TGAGTCCAAACCGGAAG	Em10: GACTGCGTACGAATTCCAG
Me8: TGAGTCCAAACCGGTGC	Em11: GACTGCGTACGAATTCCA
Me11: TGAGTCCAAACCGGTGT	Em12: GACTGCGTACGAATTATG
Me12: TGAGTCCAAACCGGCAT	Em15: GACTGCGTACGAATTTAG
	Em17: GACTGCGTACGAATTGTC

A total of 48 primer pairs were evaluated for their ability to generate detectable fragments. The primer pairs were derived from 6 forward primers and 8 reverse primers. From the 48, six were selected due to their levels of fragment polymorphism. **Table 2** shows the 48 primer combinations. The six polymorphic primer pairs are indicated in bold.

PCR was performed using a thermal cycler (T100 thermal cycler, USA) with a total reaction volume of 20  $\mu\text{L}$ . The PCR mixture contained 1X Green GoTaq<sup>®</sup> Reaction buffer (Promega Corporation, USA), 0.2 mM each of dNTPs (Promega

Corporation, USA), and both 0.5  $\mu\text{M}$  forward and reverse primers, 5U Taq Polymerase (Promega Corporation, USA), and 40 ng/ $\mu\text{L}$  of the template. The PCR conditions were as follows: initial denaturation at 94°C for 5 min, 5 cycles (1 minute at 94°C, 1 minute at 35°C for every primer pair, and 1 minute at 72°C). Subsequently, an additional 35 cycles (1 min at 94°C, 1 min at 50°C for every primer pair, and an extension of 1 min at 72°C) were performed. The final extension was set at 72°C for 7 min.

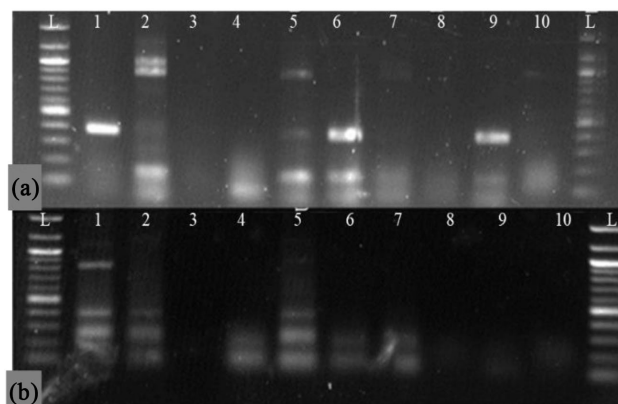
To screen for polymorphism, the PCR products were separated using 1 $\times$  TAE buffer and electrophoresed in 1.5% agarose gels. Six (6)  $\mu\text{L}$  of products and a DNA ladder of known size were run at 70 V for 120 mins. The DNA bands were visualized using a UV gel imaging system (Uvitec Gel Documentation | Thermo Fisher Scientific - AU, USA).

**Table 2.** Forty-eight (48) primer pairs used in SRAP marker analysis, showing the combinations of forward (ME) and reverse (EM) primers. Polymorphic primer pairs are indicated with an asterisk.

F/R Primer	ME1	ME2	ME5	ME8	ME11	ME12
EM5	ME1xEM5	ME2xEM5	ME5xEM5	ME8xEM5	ME11xEM5	<b>*ME12xEM5</b>
EM7	ME1xEM7	<b>*ME2xEM7</b>	ME5xEM7	ME8xEM7	ME11xEM7	ME12xEM7
EM9	ME1xEM9	ME2xEM9	ME5xEM9	ME8xEM9	ME11xEM9	ME12xEM9
EM10	ME1xEM10	ME2xEM10	ME5xEM10	ME8xEM10	ME11xEM10	ME12xEM10
EM11	ME1xEM11	ME2xEM11	ME5xEM11	ME8xEM11	ME11xEM11	ME12xEM11
EM12	ME1xEM12	ME2xEM12	ME5xEM12	ME8xEM12	ME11xEM12	ME12xEM12
EM15	ME1xEM15	<b>*ME2xEM15</b>	ME5xEM15	ME8xEM15	ME11xEM15	ME12xEM15
EM17	<b>*ME1xEM17</b>	ME2xEM17	ME5xEM17	ME8xEM17	<b>*ME11xEM17</b>	<b>*ME12xEM17</b>

\* indicates polymorphic primer pairs used in the genetic diversity analysis.

The six selected primer pairs were utilized to analyze the genetic variation of 80 pineapple accessions. The PCR and electrophoresis were done, and a representative agarose gel image is shown in **Figure 2**.



**Figure 2.** Representative agarose gel images showing polymorphic SRAP primers used in pineapple genotyping. Lanes: (a) ME11/EM17 and (b) ME12/EM17. Numbers 1 to 10 represent samples collected from different regions. 'L' indicates the ladder (molecular weight marker).

The DNA banding patterns were recorded as either “1” (for presence) or “0” (for absence) within an Excel workbook. The binary data matrix was employed for the upstream data analysis.

## 2.5. Data Analyses

Genetic diversity analysis and analysis of molecular variance (AMOVA), used to assess diversity among and within the population, were conducted using GenAlEx [32]. PopGen32 [33] was employed to perform a population-based analysis using F statistics, gene diversity over loci, proportion of polymorphic loci, Shannon index, and gene frequency [34] [35]. Additionally, PIC was calculated for each primer pair. PIC assessed marker informativeness, particularly in their capacity to differentiate genotypes within the pineapple accessions. To determine the relationships between 80 pineapple accessions, principal coordinate analysis and cluster analysis using an unweighted pair group method with arithmetic mean (UPGMA) were performed using Darwin software [36]. Branch support in the consensus tree was determined using bootstrap at 2000.

## 3. Results and Discussion

The result of screening for amplification and polymorphism is shown in **Table 3**. Based on our findings, the primers were categorized into: no amplification, poor quality, monomorphic, and polymorphic.

**Table 3.** Summary of amplification and polymorphism analysis for 48 SRAP primer pairs in pineapple samples. The table categorizes primer pairs based on their amplification results (no amplification, poor quality, monomorphic, and polymorphic).

Primer Pairs	No amplification	Poor quality	Monomorphic	Polymorphic
ME5xEM10, ME5xEM11, ME8xEM7, ME11xEM7, and ME11xEM9	√			
ME5xEM12, ME5xEM15, and ME5xEM17		√		
ME1xEM5, ME1xEM9, ME1xEM11, ME1xEM12, ME1xEM15, ME2xEM5, ME2xEM9, ME2xEM10, ME2xEM11, ME2xEM12, ME2xEM17, ME5xEM5, ME5xEM7, ME5xEM9, ME8xEM5, ME8xEM9, ME8xEM10, ME8xEM11, ME8xEM12, ME8xEM15, ME8xEM17, ME11xEM5, ME11xEM10, ME11xEM11, ME11xEM12, ME11xEM15, ME12xEM7, ME12xEM9, ME12xEM10, ME12xEM11, and ME12xEM12			√	
ME1xEM17, ME2xEM7, ME2xEM15, ME11xEM17, ME12xEM5, and ME12xEM17				√

The informativeness of each selected primer pair is shown in **Table 4**. Obtained fragment sizes ranged from 100 to about 1200 base pairs. The number of polymorphic bands varied between 13 and 92, with an average of 53.2 bands for all the

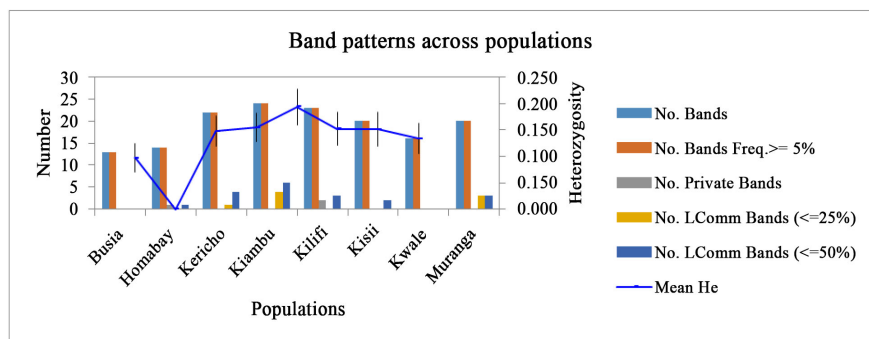
primers from 713 bands generated, representing 42.10% of the observed polymorphic loci.

ME12xEM5 accounted for the highest polymorphic fragments (56.4% of the total bands), while ME1xEM17 accounted for the lowest polymorphic pieces (12.3%). In terms of marker informativeness, ME12xEM17 and ME12xEM5 recorded the highest Polymorphism Information Content (PIC) values of 0.6 and 0.5, respectively. However, ME1xEM17 and ME11xEM17 recorded a low PIC value of 0.1. A binary data matrix generated after analysis of the 80 samples using the six SRAP markers was used upstream. The six SRAP primer pairs collectively generated 713 DNA fragments, of which 319 were polymorphic. The average polymorphism rate across all primer pairs was approximately 42.1%, with a mean PIC value of 0.3.

**Table 4.** Analysis of six polymorphic primer pairs used in SRAP marker analysis of pineapple samples. The table lists the total number of bands (DNA fragments), polymorphic fragments, percentage of polymorphic fragments, and Polymorphism Information Content (PIC) for each primer pair. Summary statistics (total and mean) are provided at the bottom.

Primer Combination	Total Bands (DNA Fragments)	Polymorphic Fragments	% Polymorphic Fragments	Polymorphism Information Content
ME2xEM7	83	35	42.2	0.3
ME1xEM17	106	13	12.3	0.1
ME12xEM5	163	92	56.4	0.5
ME12xEM17	147	81	55.1	0.6
ME2xEM15	86	26	30.2	0.2
ME11xEM17	128	72	56.3	0.1
TOTAL	713	319		
MEAN	118.8	53.2	42.1	0.3

The distribution of bands across the selected eight pineapple populations is shown (**Figure 3**). Notably, a variable number of bands was observed among populations, with Kericho and Kiambu standing out with 22 and 24 bands, respectively.



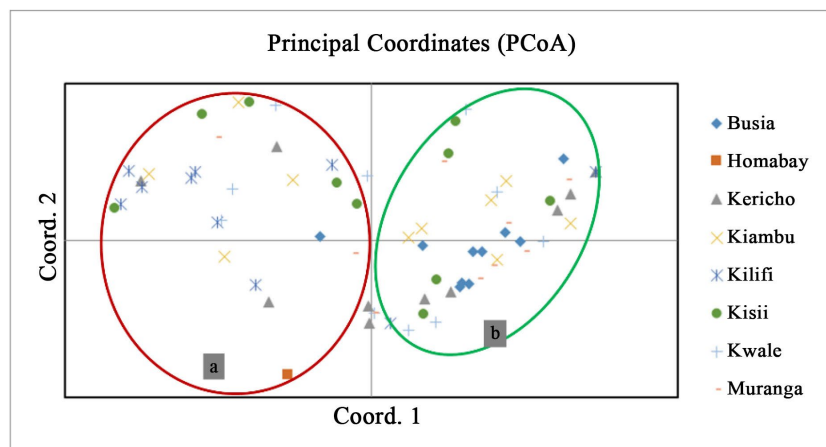
**Figure 3.** Genetic landscape of Kenyan pineapple populations revealed through band pattern analysis. The graph illustrates the number of bands (orange:  $\geq 5\%$  frequency; grey: private bands; yellow: locally common bands  $\leq 25\%$ ; blue: locally common bands  $\leq 50\%$ ) and the mean heterozygosity (blue line: mean He) across different populations: Busia, Homabay, Kericho, Kiambu, Kilifi, Kisii, Kwale, and Muranga.

At the same time, the frequency distribution of bands across populations unveiled a pattern where the majority of bands were present at frequencies equal to or exceeding 5%. Further exploration into the concept of locally common bands elucidated patterns of genetic sharing or distinctiveness. For instance, Kwale exhibited a locally common band that was present in 25% or fewer populations, shedding light on the regional specificity of certain genetic elements. Based on the assessment of expected heterozygosity ( $H_e$ ) and unbiased expected heterozygosity ( $uH_e$ ), accessions obtained from the Kiambu region emerged as particularly diverse, with the highest  $H_e$  and  $uH_e$  among the populations. The standard error values associated with  $H_e$  and  $uH_e$  provided insights into the precision of these diversity estimates, enhancing the reliability of the findings. The genetic distance, calculated using Nei's genetic diversity index, varied between 0.007 and 0.200, implying a moderate genetic differentiation (Table 5).

**Table 5.** Nei genetic distance matrix for pineapple populations. The diagonal represents populations from each region, while the off-diagonal elements show the genetic distances between pairs of populations. Values marked with an asterisk indicate the most (0.200) and least (0.007) related genetic distances based on Nei's genetic diversity.

Busia	Homabay	Kericho	Kiambu	Kilifi	Kisii	Kwale	Muranga	
0								Busia
0.191	0							Homabay
0.02	0.182	0						Kericho
0.017	0.182	0.021	0					Kiambu
0.046	0.150	0.032	0.030	0				Kilifi
0.026	*0.200	0.014	0.019	0.020	0			Kisii
0.011	0.185	0.02	0.023	0.033	0.017	0		Kwale
*0.007	0.196	0.019	0.021	0.043	0.031	0.012	0	Muranga

\* indicates the most and least related genetic distance based on Nei's genetic diversity.



**Figure 4.** Principal Coordinates Analysis (PCoA) plot showing the genetic relationship of 80 pineapple accessions. The PCoA revealed two main groups: (a) and (b). Different symbols and colors represent various regions, highlighting the genetic diversity and clustering within the populations.

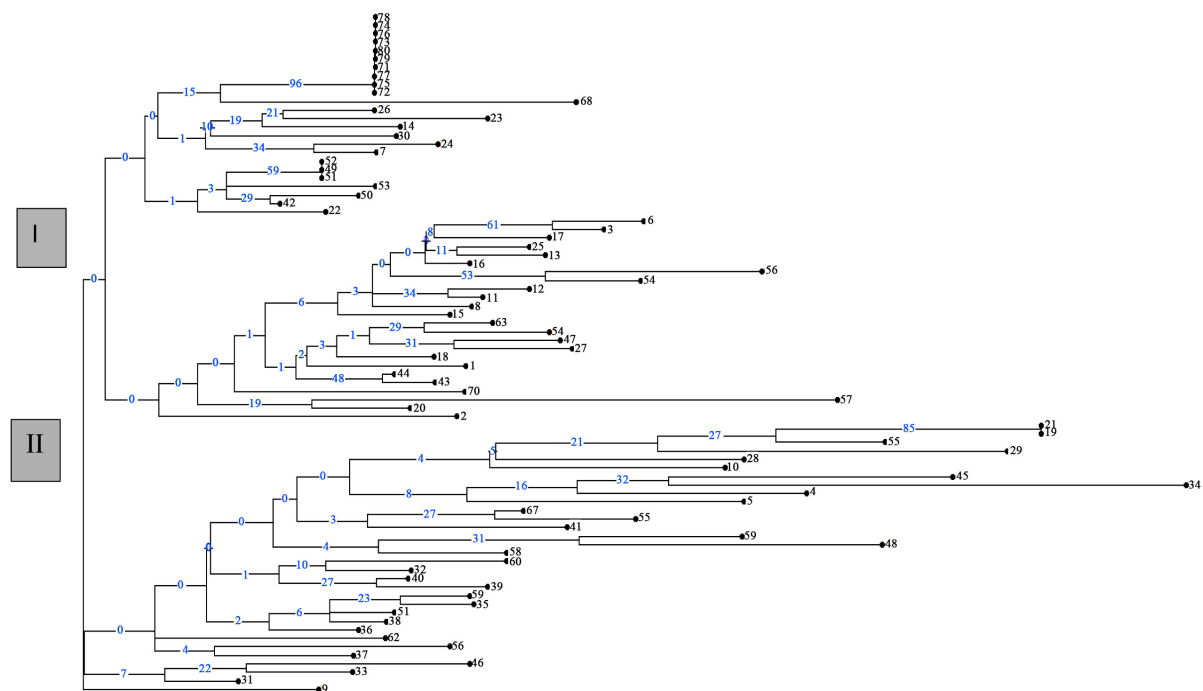
Principal Coordinates Analysis (PCoA) revealed moderate variation distrib-

uted across the first three principal axes (Figure 4). Axis 1 explained 25.23% of the overall genetic variation among the population. On the other hand, Axis 2 accounted for 19.29% of the overall variation. The cumulative effect of Axis 1 and 2 was at 44.52%. However, Axis 3 explained a smaller proportion (8.95%) of the genetic variation. Axis 1, 2, and 3 cumulatively accounted for 53.47% of the total genetic diversity within the pineapple populations.

AMOVA results revealed highly significant variance components ( $P < 0.001$ ), indicating greater variation within (78%) populations compared to among populations (22%) (Table 6). A moderate level of genetic differentiation was observed with a PhiPT value of 0.221.

**Table 6.** Summary of Analysis of Molecular Variance (AMOVA) results for Kenyan pineapple accessions. The significance of the molecular variance is represented by the PhiPT statistic ( $P(\text{rand} \geq \text{data}) = 0.001$ ).

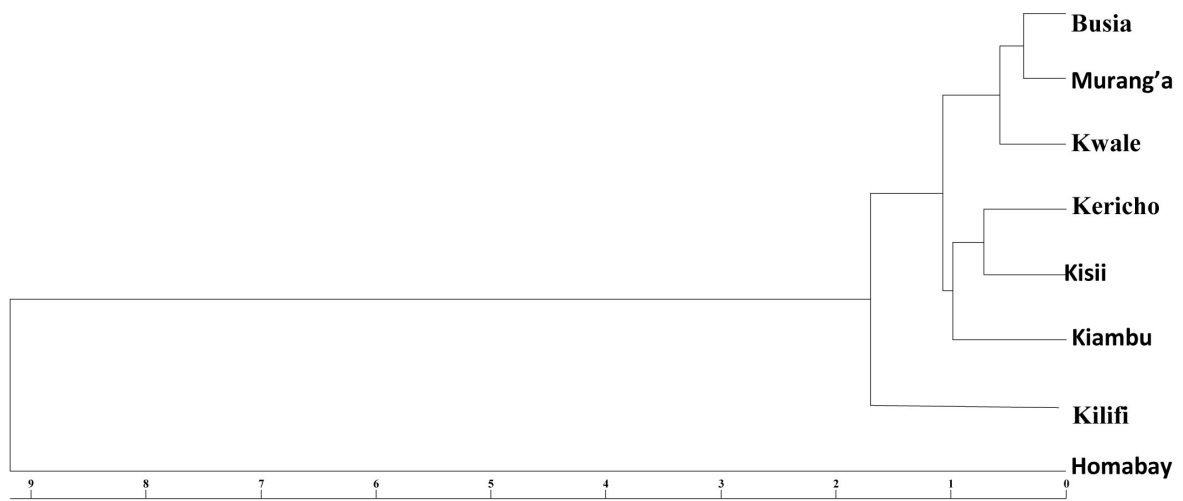
Source	Df	SS	M	Est. Var.	%
Among Pops	7	86.863	12.409	0.917	22%
Within Pops	72	233.100	3.238	3.238	78%
Total	79	319.963		4.155	100%
Stat	Value	P(rand $\geq$ data)			
PhiPT	0.221	0.001			
PhiPT max	0.846				
Phi'PT	0.261				



**Figure 5.** Hierarchical clustering of Kenyan pineapple accessions based on six polymorphic SRAP marker primer combinations. The dendrogram reveals two main groups: I and II. Each branch represents a different pineapple accession (list of samples as per the tree 9, 31, 33, 46, 37, 56, 62, 36, 38, 61, 35, 59, 39, 40, 32, 60, 58, 48, 69, 41, 65, 67, 5, 4, 34, 45, 10, 28, 29, 55, 19, 21, 2, 20, 57, 70, 43, 44, 1, 18, 27, 47, 54, 63, 15, 8, 11, 12, 64, 66, 16, 13, 25, 17, 3, 5, 22, 42, 50, 53, 51, 49, 52, 7, 24, 30, 14, 23, 26, 68, 72, 75, 77, 71, 79, 80, 73, 76, 74, 78).

Hierarchical clustering using the UPGMA method discerned two distinct clusters (**Figure 5**), suggesting limited genetic diversity within the sampled pineapple accessions. The clusters (I and II) did not correspond to the geographical locations, as previously supported by the PCoA above.

The dendrogram based on Nei's (1978) genetic distance utilizing the UPGMA method to visually elucidate the genetic associations among eight distinct pineapple populations revealed genetic homogeneity across regions (**Figure 6**). Populations from Busia and Murang'a had a relatively short branch length of 0.02437. Similarly, Kwale and Kericho exhibit a close genetic relationship with a branch length of 0.16544. Conversely, Homabay had the longest branch length at 8.95526, signifying significant genetic divergence from other populations. The genetic distance between accessions obtained from Kwale and Kericho is detailed with a branch length of 0.24411. Furthermore, populations of Kilifi and Kiambu demonstrated notable genetic differentiation, reflected in a branch length of 0.50912.



**Figure 6.** Dendrogram based on Nei's (1978) genetic distance using the UPGMA method. The dendrogram illustrates the genetic relationships among pineapple accessions from different regions. Branch lengths reflect genetic distance, with shorter branches indicating greater genetic similarity. This analysis shows how pineapple populations vary across regions.

SRAP markers were successfully used to assess the genetic diversity of pineapple accessions in Kenya. The varying levels of polymorphism observed in this study have practical implications for pineapple breeding programs. The primer combinations ME12\_EM5 and ME12\_EM17, with their high levels of polymorphism and significant PIC values, could serve as valuable tools for marker-assisted selection and breeding efforts.

This is the first report on the genetic diversity of Kenyan pineapple accessions. A narrow genetic base was observed among the studied pineapple accessions. These results align with previous studies in other countries on pineapple genetic diversity using other marker systems, which have also reported limited genetic diversity in certain pineapple germplasms. For example, Ismail 2020 found moderate levels of polymorphism in Malaysia pineapple germplasm using SSR mark-

ers. However, our result contrasts with the findings of [37], who reported higher diversity among pineapple accessions from various countries using ISSR and SSR markers. This could be attributed to the fact that the pineapple varieties used in their study were sourced from different countries or regions, including Australia, Brazil, Costa Rica, Guangdong of China, Hainan of China, Indonesia, Japan, Mauritius, Taiwan of China, and Thailand.

Several factors could contribute to the low polymorphism observed in this study. Pineapples are often propagated clonally, which can reduce genetic diversity within germplasm collections. Pineapple is not an indigenous crop in Kenya, and only two varieties (MD2 and smooth cayenne) contributing to the narrow genetic diversity. The genetic background and breeding history of the accessions used may also play a role [38]. Furthermore, pineapple seed systems are not well defined apart from the multinationals like Delmonte. The commercial pineapple industry in Kenya tends to rely on a few elite MD2 and Smooth Cayenne varieties, which has led to genetic uniformity, a trend also observed in the Ghanaian market [32]. The MD2 variety is preferred for its sweetness, appealing aroma, and uniform fruit size, making it well-suited for the export market (Valleser, 2019). Furthermore, the biomass of the MD2 pineapple has been identified as a potential feedstock for biofuel production, with characteristics suitable for this application [17]. The Smooth Cayenne pineapple, on the other hand, is preferred for its versatility, robustness, and adaptability to different growing conditions [2].

Contrary, local farmers in Kenya do not know which varieties they grow. For farmers to be competitive, there is a need to know the type of varieties they are dealing with. In addition, the introduction of new, known, diverse pineapple varieties, as well as breeding to enhance the genetic diversity of the available material is essential.

When considering the overall genetic diversity assessment, it is evident that the average percentage of polymorphic fragments across all primer combinations is 42.1%. This figure suggests that, on average, approximately 42% of the bands generated by SRAP markers were polymorphic, signifying moderate genetic diversity within the pineapple germplasms sampled in this study. This observation is consistent with the idea that pineapples are predominantly vegetatively propagated, leading to lower genetic diversity compared to sexually reproducing crops.

#### 4. Conclusions

The study identified moderate genetic variability present among the studied pineapple germplasm.

Future research in pineapple genetic diversity should consider exploring additional molecular marker systems, such as SSRs or SNP markers, to complement the findings of this study. Introducing germplasms from different regions and with superior traits would ensure a wide genetic base.

To improve genetic diversity within Kenyan pineapple germplasm, a combination of broadening breeding sources, enhancing conservation efforts, and using

modern biotechnology tools like marker assisted selection would be essential.

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### Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the article.

### Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

### References

- [1] Mohd Ali, M., Hashim, N., Abd Aziz, S. and Lasekan, O. (2020) Pineapple (*Ananas comosus*): A Comprehensive Review of Nutritional Values, Volatile Compounds, Health Benefits, and Potential Food Products. *Food Research International*, **137**, Article 109675. <https://doi.org/10.1016/j.foodres.2020.109675>
- [2] de Araújo, H.S., do Carmo, S.A., dos Santos, N.C.B., Freitas, P.G.N. and Purquerio, L.F.V. (2021) Effect of Shading Screens on the Production and Quality of 'smooth Cayenne' Pineapple. *Pesquisa Agropecuária Tropical*, **51**, e69594. <https://doi.org/10.1590/1983-40632021v5169594>
- [3] Chaudhary, V., Kumar, V., Singh, K., Kumar, R. and Kumar, V. (2019) Pineapple (*Ananas cosmosus*) Product Processing: A Review. *Journal of Pharmacognosy and Phytochemistry*, **8**, 4642-4652.
- [4] Cleveland, M.A. and Deeb, N. (2012) Selecting Markers and Evaluating Coverage. In: Rifkin, S., Ed., *Methods in Molecular Biology*, Vol. 871, Humana Press, 55-71. [https://doi.org/10.1007/978-1-61779-785-9\\_5](https://doi.org/10.1007/978-1-61779-785-9_5)
- [5] FAOSTAT (2022) Crops and Livestock Products. <https://www.fao.org/faostat/en/#data/TCL>
- [6] George, J., Nguyen, T., Williams, D., Hardner, C., Sanewski, G. and Smyth, H.E. (2023) Review of the Aroma Chemistry of Pineapple (*Ananas comosus*). *Journal of Agricultural and Food Chemistry*, **71**, 4069-4082. <https://doi.org/10.1021/acs.jafc.2c08546>
- [7] Hasan, S., Ali, M. and Khalil, M. (1970) Impact of Pineapple Cultivation on the Increased Income of Pineapple Growers. *The Agriculturists*, **8**, 50-56. <https://doi.org/10.3329/agric.v8i2.7577>
- [8] Farid Hossain, M., Akhtar, S. and Anwar, M. (2015) Nutritional Value and Medicinal Benefits of Pineapple. *International Journal of Nutrition and Food Sciences*, **4**, 84-88. <https://doi.org/10.11648/j.ijnfs.20150401.22>
- [9] Hossain, F. (2016) World Pineapple Production: An Overview. *African Journal of Food, Agriculture, Nutrition and Development*, **16**, 11443-11456. <https://doi.org/10.18697/ajfand.76.15620>
- [10] Islam, M.A. and Ashilenje, D.S. (2018) Diversified Forage Cropping Systems and Their Implications on Resilience and Productivity. *Sustainability*, **10**, Article 3920. <https://doi.org/10.3390/su10113920>

- [11] Khan, F. (2015) Molecular Markers: An Excellent Tool for Genetic Analysis. *Journal of Molecular Biomarkers & Diagnosis*, **6**, Article 233. <https://doi.org/10.4172/2155-9929.1000233>
- [12] Koech, W., Ithinji, G.K. and Kibet, L.K. (2013) Evaluating Technical Efficiency of Small-Scale Pineapple (*Ananas comosus*) Production in Bureti District, Kenya. *Current Research Journal of Social Sciences*, **5**, 192-196. <https://doi.org/10.19026/crjss.5.5555>
- [13] Kordrostami, M. and Rahimi, M. (2015) Molecular Markers in Plants: Concepts and Applications. *Genetics in the Third Millennium*, **13**, 4024-4031.
- [14] Kronstad, W.E. (2015) Genetic Diversity and Plant Improvement. Agronomy Society of New Zealand, 16-20.
- [15] Li, G. and Quiros, C.F. (2001) Sequence-Related Amplified Polymorphism (SRAP), a New Marker System Based on a Simple PCR Reaction: Its Application to Mapping and Gene Tagging in Brassica. *Theoretical and Applied Genetics*, **103**, 455-461. <https://doi.org/10.1007/s001220100570>
- [16] Maia, V.M., Pegoraro, R.F., Aspiazú, I., Oliveira, F.S. and Nobre, D.A.C. (2020) Diagnosis and Management of Nutrient Constraints in Pineapple. In: Srivastava, A.K. and Hu, C., Eds., *Fruit Crops*, Elsevier, 739-760. <https://doi.org/10.1016/b978-0-12-818732-6.00050-2>
- [17] Mansora, A.M., Lima, J.S., Anib, F.N. and Hashima, H. (2018) Ultimate and Proximate Analysis of Malaysia Pineapple Biomass from MD2 Cultivar for Biofuel Application. *Chemical Engineering Transactions*, **63**, 127-132.
- [18] McDermott, J. (1993) Gene Flow in Plant Pathosystems. *Annual Review of Phytopathology*, **31**, 353-373. <https://doi.org/10.1146/annurev.phyto.31.1.353>
- [19] Murariu, D., Plăcintă, D.D. and Simioniu, V. (2019) Assessing Genetic Diversity in Romanian Maize Landraces, Using Molecular Markers. *Romanian Agricultural Research*, No. 36, 3-9. <https://doi.org/10.59665/rar3601>
- [20] Nam, V.T., Hang, P.L.B., Linh, N.N., Ly, L.H., Hue, H.T.T., Ha, N.H., et al. (2021) Molecular Markers for Analysis of Plant Genetic Diversity. *Vietnam Journal of Biotechnology*, **18**, 589-608. <https://doi.org/10.15625/1811-4989/18/4/15326>
- [21] Nei, M. (1972) Genetic Distance between Populations. *The American Naturalist*, **106**, 283-292. <https://doi.org/10.1086/282771>
- [22] Onyuma, S., Icart, E. and Owuor, G. (2006) Testing Market Integration for Fresh Pineapples in Kenya. Research in Agricultural and Applied Economics. International Association of Agricultural Economists (IAAE), No. 1004-2016-78885.
- [23] Palinkas, L.A., Horwitz, S.M., Green, C.A., Wisdom, J.P., Duan, N. and Hoagwood, K. (2013) Purposeful Sampling for Qualitative Data Collection and Analysis in Mixed Method Implementation Research. *Administration and Policy in Mental Health and Mental Health Services Research*, **42**, 533-544. <https://doi.org/10.1007/s10488-013-0528-y>
- [24] PEAKALL, R. and SMOUSE, P.E. (2005) GENALEX 6: Genetic Analysis in Excel. Population Genetic Software for Teaching and Research. *Molecular Ecology Notes*, **6**, 288-295. <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- [25] Perrier, X. and Jacquemoud-Collet, J. (2009) DARwin Software. <http://darwin.cirad.fr/>
- [26] Rakshit, S. and Swapna, M. (2015) DNA Markers in Diversity Analysis. In: Madhusudhana, R., Rajendrakumar, P. and Patil, J., Eds., *Sorghum Molecular Breeding*, Springer, 23-46. [https://doi.org/10.1007/978-81-322-2422-8\\_2](https://doi.org/10.1007/978-81-322-2422-8_2)

- [27] Rao, V.R. and Hodgkin, T. (2002) Genetic Diversity and Conservation and Utilization of Plant Genetic Resources. Kluwer Academic Publishers, 1-19.
- [28] Robarts, D.W.H. and Wolfe, A.D. (2014) Sequence-Related Amplified Polymorphism (SRAP) Markers: A Potential Resource for Studies in Plant Molecular Biology. *Applications in Plant Sciences*, **2**, Article 1400017. <https://doi.org/10.3732/apps.1400017>
- [29] Safavi, S.M., Safavi, A.S. and Safavi, S.A. (2015) Assessment of Genetic Diversity in Sunflower (*Helianthus Annus L.*) Genotypes Using Agro-Morphological Traits. *Journal of Biodiversity and Environmental Sciences*, **6**, 152-159.
- [30] Singh, B.D. and Singh, A.K. (2015) Polymerase Chain Reaction-Based Markers. In: *Marker-Assisted Plant Breeding: Principles and Practices*, Springer, 47-75. [https://doi.org/10.1007/978-81-322-2316-0\\_3](https://doi.org/10.1007/978-81-322-2316-0_3)
- [31] Valleser, V.C. (2019) Growth and Fruit Physicochemical Characteristics of 'MD-2' Pineapple (*Ananas comosus L.*) at Varying Seedbed Configurations. *Pertanika Journal of Tropical Agricultural Science*, **42**, 377-386.
- [32] Wardy, W., Saalia, F.K., Steiner-Asiedu, M., Budu, A.S. and Sefa-Dedeh, S. (2009) A Comparison of Some Physical, Chemical and Sensory Attributes of Three Pineapple (*Ananas comosus*) Varieties Grown in Ghana. *African Journal of Food Science*, **3**, 94-99.
- [33] Yeh, F., Yang, R., Boyle, T., Ye, Z. and Mao, J. (1997) PopGene, the User-Friendly Shareware for Population Genetic Analysis. Molecular Biology and Biotechnology Centre, University of Alberta.
- [34] Zagorcheva, T., Rusanov, K., Bosmali, E., Madesis, P., Tsvetkov, I. and Atanasov, I. (2020) Sequence-Related Amplified Polymorphism (SRAP) Markers, an Efficient and Affordable Tool for Evaluation Genetic Diversity in Forest Areas. *Silva Balcanica*, **21**, 41-46. <https://doi.org/10.3897/silvabalcanica.21.e54699>
- [35] Zhao, H. and Qin, Y. (2018) Genetic Diversity of Pineapple. In: Ming, R., Ed., *Plant Genetics and Genomics: Crops and Models*, Springer International Publishing, 51-59. [https://doi.org/10.1007/978-3-030-00614-3\\_4](https://doi.org/10.1007/978-3-030-00614-3_4)
- [36] Zommita, S., Yildiz, G.G., Zencirci, N., Özbek, Ö., Ünüvar, Ö.C. and Ünlü, E.S. (2023) Assessment of Genetic Diversity Based on Agro-Morphological Traits and ISSR Molecular Markers in Einkorn Wheat (*Triticum Monococcum ssp. Monococcum*) Landrace Populations from Turkey. *International Journal of Secondary Metabolite*, **10**, 86-105. <https://doi.org/10.21448/ijsm.1084853>
- [37] Wang, J.S., He, J.H., Chen, H.R. Chen, Y.Y. and Qiao, F. (2027) Genetic Diversity in Various Accessions of Pineapple [*Ananas comosus (L.) Merr.*] Using ISSR and SSR Markers. *Biochemical Genetics*, **55**, 347-366. <https://doi.org/10.1007/s10528-017-9803-z>
- [38] Silva, D.C., Krause, W., Arantes, D.S.O., Freitas, A.P., Santos, E.A., de Araújo, D.V., *et al.* (2024) Pineapple Breeding: Development of New Pineapple Cultivars without Leaf Spines and Resistant to Fusariosis. *Euphytica*, **220**, Article No. 135. <https://doi.org/10.1007/s10681-024-03363-w>