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Genetic diversity and structure of Benin pineapple (*Ananas comosus* (L) Merr.) germplasm collection using Simple Sequence Repeat (SSR) markers

Original paper

Chalotte Abike Oladjo Adjé[1,2*], Antoine Abel Missihoun[2], Paulin Sedah[2], Hubert Adoukonou Sagbadja[2], Enoch Gbenato Achigan-Dako[1], and Clément Agbangla[2]

1. Genetics, Biotechnology and Seed Science Unit (GBioS), Faculty of Agronomic Sciences, University of Abomey-Calavi, Abomey-Calavi, Republic of Benin
2. Faculty of Sciences and Techniques (FAST), University of Abomey-Calavi, 01 BP 526 Tri postal, Cotonou, Republic of Benin.

Abstract: Pineapple stands as a cornerstone among Benin's vital fruit crops, playing a pivotal role in enhancing both household food security and income. This delectable fruit is prominently featured among the key crops advocated for cultivation within the country. Despite the critical role this crop plays, our understanding of the diversity within genetic resources is remains limited. This study aimed to assess genetic variation and infer the population structure of 57 pineapple accessions collected from Benin's national core collection, using 10 simple sequences repeat (SSR) markers. The result showed a total of 23 alleles, ranging from 2 to 4, with a mean of 2.3 alleles per locus. The polymorphic information content was 0.34 whereas the mean expected heterozygosity was 0.43. The UPGMA dendrogram revealed two main clusters. The collection was determined to exhibit a structured composition comprising two distinct groups based on genetic analysis. This grouping was further validated by AMOVA, affirming its existence. Our work offers valuable insights into the genetic diversity within Beninese pineapple germplasm, thereby guiding strategic conservation efforts. Moreover, these findings open avenues for leveraging the genetic variation present in Benin's pineapple germplasm for future pineapple breeding programs, thereby enhancing pineapple cultivation and resilience.

Keywords: *Ananas comosus*, SSR, structure, germplasm, Benin.

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Correspondence

Dr. Chalotte Abike Oladjo Adjé
abikecharlotte@gmail.com

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Introduction

Pineapple (*Ananas comosus* (L) Merr.) is one of the most economically important tropical fruits, widely cultivated with the production estimated at 27.82 in 2020 (Shahbandeh, 2022). Dubbed the "queen of fruits" for its excellent flavour and taste, pineapple ranks as the third most significant tropical fruit globally, following Banana and Citrus (Hossain et al., 2015). Recently, pineapple consumption has surged due to its edible value and high nutritional and medicinal properties (*i.e.*, sugar, protein, digestive enzyme, bromelin, vitamins and acids). The fruit is highly perishable but adds value from its processing capacity (juice, wines, cakes, syrup, vinegar). Major producer countries are the Philippines, Costa Rica, Brazil, Indonesia, and China supplying more than 50% of the total output followed by India, Nigeria, Thailand, Mexico, and Colombia which provide most of the remaining. Other countries have contributed to pineapple production such as Benin.

In Benin, pineapple production has grown steadily from 215,000 tons in 2015 to 440,178 tons in 2020 in the past few years (DDAEP et al., 2021). Pineapple production is based on two leading cultivars Smooth Cayenne and Sugarloaf, or Perola recently included for exportation. The Benin Central Government has put effort into increasing the national production and increasing the export destination since 2016, but the export rate to Europe, the main international fresh pineapple market available, is still limited (less than 2% of the national production) (Fassinou Hotegni et al., 2012). Many constraints including fruit heterogeneity reduce the export potential. It was reported that the heterogeneity observed in fruit production can be caused by several factors such as planting material heterogeneity, and poor agronomic practices (Fassinou Hotegni et al., 2015). However, the confusion noted in pineapple cultivars due to variations in naming customs among researchers or farmers can also lead to this problem. Pineapple cultivars are often grouped according to their leaves and fruit characteristics. Cultivar groups including Cayenne, Spanish, Queen, Abacaxi, Perola, and Maipure have been documented (Noyer and Lanaud, 1997). Morphological characterization of Benin pineapple germplasm revealed five cultivars including Smooth Cayenne, Cayenne de Rothschild, Perola, Singapore Spanish, and Green Spanish. Those cultivars were grouped into three clusters: Cayenne, Spanish and Perola with some variations based on morphological variation (Adje et al., 2019). Morphological characterization of germplasm collections around the world triggered the establishment of cultivar groups based on morphological descriptors which are often influenced by the environmental, epistatic and pleiotropic effects (Leal and Antoni, 1981; Coppens d'Eeckenbrugge et al., 1997; Duval et al., 1997). Consequently, it is important to explore molecular markers to decipher the cultivar identification. More accurate

Several DNA markers have been applied to assess genetic diversity and relationship among pineapple accessions among them, the SSR markers. The SSR markers are most commonly used because they are ubiquitous, hypervariable, co-dominant, robust, specific chromosome and multi-allelic in nature (Rakshit et al., 2012). Microsatellite markers are widely used for the assessment of genetic diversity in several cultivated crop species such as sweet sorghum (Ali et al., 2008; Missihoun et al., 2015), onion (Mallor et al., 2014), rice (Ravi et al., 2003), and wheat (Salehi et al., 2018). Previous studies have reported the development of SSR markers capable of amplifying the entire genome of *A. comosus* and their use to look into a genetically diverse pineapple (Feng et al., 2013; Rodrígueza et al., 2013; Zhang et al., 2014). So far, few studies have explored the genetic diversity of pineapple germplasm in West Africa, despite the proliferation of cultivars produced over time. In this study, we employed SSR molecular markers for the first time to investigate the genetic diversity among fifty-seven pineapple accessions collected from various regions of Benin. We hypothesize that these accessions exhibit considerable variation and anticipate a robust correlation between pineapple morphological groups and the genetic groups.

Materials and Methods

Plant Materials

Fifty-seven (57) accessions were collected from the Benin pineapple core collection. The Benin pineapple core collection results in prospecting and

collection across different regions of the country. The samples used included 20 accessions of Perola group, 21 accessions of Cayenne group, 15 accessions of the Spanish group, and one of var. *bracteatus* (Table 1).

Genomic DNA extraction and SSR genotyping

Total genomic DNA was extracted from 200 mg of fresh leaves from each accession using the CTAB (Cetyl Trimethyl Ammonium Bromide) extraction protocol as previously described (Adjé et al., 2016). The quality of the extracted genomic DNA was checked by electrophoresis 1% agarose gel. All the accessions underwent genotyping using ten (10) SSR primers (listed in Table 2) distributed throughout the pineapple genome. These primers were developed by Kinsuat and Kumar (2007) and selected from the sequence information previously screened by Rodrígueza et al. (2013) for molecular diversity analysis in pineapple. The PCR reactions were performed in a total volume of 25 µl reaction solution containing 3 µl of 50 ng/µl the genomic DNA, 1.5 µl of 10 pmol of each primer, 1 µl of 1 mM of dNTPs mix, 0.2 µl 5U of Taq DNA polymerase, 5 µl of 5X PCR buffer, and 1.25 µl of 25 mM Magnesium Chloride (MgCl2). DNA amplification was performed in a Mastercycler nexus gradient (Eppendorf AG 22331 Hamburg), using the following program: 30s initial denaturation at 94 °C, followed by 35 cycles, each consisting of 94°C for 30s, primer annealing at 55 °C for 30 seconds; an extension at 72 °C for 1 min. The amplification is terminated by a final extension at 72 °C for 5 min. Amplification products were electrophoresed on a 3% agarose gel in 1x TBE solution at a constant power of 100V for 1 hour and viewed under UV light in E-Box gel documentation.

Data analysis

The markers' resolution and the discriminatory power were determined by calculating the Polymorphic Information Content (PIC), which offers an estimate of the discriminating power of each locus. The PIC value of a locus, which ranges from 0 (monomorphic) to 1 (highly informative), was calculated using PowerMarker software (Liu, 2005) according to the following formula:

$$PIC = 1 - \sum_{i=1}^{n} F_i^2$$

where: F_i is the frequency of the i^{th} allele in a locus. The dataset was analysed with MicroChecker v2.2.3 software (Van Oosterhout et al., 2004) to identify genotyping errors due to null alleles (nonamplified alleles), short allele dominance (large allele dropout), and the scoring of stutter peaks at each locus. The genetic diversity measure included percentage of polymorphic loci (P), number of alleles per locus (A), observed heterozygosity (Ho), expected heterozygosity (He), Shannon's diversity index (I), allele frequencies, were performed using GenAlEx software package vs. 6.5 (Peakall and Smouse, 2006). Nei genetic distances between pairs of accessions were calculated based on each dataset to investigate the genetic relationship between accessions. The clustering of accessions was performed based on the Nei genetic distance matrix using the unweighted pair group method with arithmetic averaging (UPGMA) with Power Maker software. For population genetic structure analysis, the Bayesian model-based clustering method of Structure V.2.3.3 software. Correlated allele frequencies were applied for the estimation of ancestry fractions of each cluster. The value of k (range 1–10) was performed. The Structure Harvester software version 0.6.92 was used to determine the optimum k value using the log probability of data, LnP(D) based on the rate of change in LnP(D) between successive K with burn of 100000 until 100000 iterations. Analysis of Molecular Variance (AMOVA) was performed to partition genetic variation among and within cultivars groups. Wright's F_{st} was used to estimate the cultivar's group differentiation and was calculated using the GenAlEx software package vs. 6.5 (Peakall and Smouse, 2006).

Results

SSR markers polymorphism and genetic diversity

All the ten markers used in this study generated a total of 23 alleles across the 57 accessions of pineapple with an average of 2.3 alleles per locus. Nine of the primers showed polymorphisms for all the accessions. The locus ACPCT124BM was monomorphic. The fragment sizes ranged from 105 pb (ANBR75) to 290 pb (ANBR73).

Table 1. Pineapple accessions used in this study. Cultivar names and groups identified by Adje, et al. (2019).

Accession code	Cultivar	Group	Accession code	Cultivar	Group
EAD1644	Smooth Cayenne	Cayenne	EAD1821	Perola	Perola
EAD1751	Baronne de Rothschild	Cayenne	EAD1678	Perola	Perola
EAD1784	Baronne de Rothschild	Cayenne	EAD1757	Perola	Perola
EAD1708	Smooth Cayenne	Cayenne	EAD1845	Perola	Perola
EAD1724	Smooth Cayenne	Cayenne	EAD1474	Perola	Perola
EAD1730	Smooth Cayenne	Cayenne	EAD1862	Perola	Perola
EAD1580	Smooth Cayenne	Cayenne	EAD1562	Perola	Perola
EAD1606	Smooth Cayenne	Cayenne	EAD1330	Perola	Perola
EAD1774	Smooth Cayenne	Cayenne	EAD1340	Perola	Perola
EAD1840	Smooth Cayenne	Cayenne	EAD1351	Perola	Perola
EAD1859	Smooth Cayenne	Cayenne	EAD1411	Perola	Perola
EAD1445	Smooth Cayenne	Cayenne	EAD1430	Perola	Perola
EAD1494	Smooth Cayenne	Cayenne	EAD1850	Singapore Spanish	Spanish
EAD1648	Smooth Cayenne	Cayenne	EAD1855	Green Spanish	Spanish
EAD1834	Smooth Cayenne	Cayenne	EAD1456	Green Spanish	Spanish
EAD1673	Smooth Cayenne	Cayenne	EAD1463	Singapore Spanish	Spanish
EAD1502	Smooth Cayenne	Cayenne	EAD1481	Green Spanish	Spanish
EAD1550	Smooth Cayenne	Cayenne	EAD1871	Green Spanish	Spanish
EAD1358	Smooth Cayenne	Cayenne	EAD1831	Green Spanish	Spanish
EAD1441	Smooth Cayenne	Cayenne	EAD1837	Singapore Spanish	Spanish
EAD2020	MD2	Cayenne	EAD1667	Green Spanish	Spanish
EAD1687	Perola	Perola	EAD1525	Green Spanish	Spanish
EAD1734	Perola	Perola	EAD1571	Singapore Spanish	Spanish
EAD1698	Perola	Perola	EAD1369	Singapore Spanish	Spanish
EAD1719	Perola	Perola	EAD1383	Green Spanish	Spanish
EAD1593	Perola	Perola	EAD1402	Singapore Spanish	Spanish
EAD1623	Perola	Perola	EAD1436	Green Spanish	Spanish
EAD176	Perola	Perola	EAD2222*	*bracteatus*	
EAD1679	Perola	Perola			

* The local name for this species code is unknown

The number of alleles detected per locus varied from 2 to 5. The marker ACLR179BMb amplified the high number of alleles whereas the minimum number was observed at ANBR81 (Supplementary Figure 1). The discriminative power of the markers was assessed by calculating the Polymorphism Information Content (PIC) provided the value of the nine polymorphic primers ranging from 0.033 to 0.658 with an average of 0.340. The lowest PIC value was observed for the locus ANBR81, in contrast the maximum was observed for the locus ACLR179BMb. According to the allelic frequencies, we considered 1 allele (4%) as rare (P<0.5), 4 (18%) as common alleles (0.05<P <0.2) and 18 (78%) as

most frequent alleles (P>0.2). The expected heterozygosity (*He*) values ranged from 0.095 (ANBR81) to 0.744 (ACLR179BMb) with a mean value of 0.413. The observed heterozygosity (H_0) varied from 0.1 (ANBR81) to 0.950 (ACPCT651BM) with the mean of 0.425 showing an excess of heterozygosity (Table 3). Analysis with MicroChecker software (at 95% of confidence level) highlighted the existence of null alleles at microsatellite marker ANBR81 (Table 2).

Phylogenetic analysis

The genetic relationship between pineapple accessions was assessed by computing the genetic distance within pairs of accessions. Nei's genetic distances between pineapple genotypes varied from 0 to 0.3, with a mean value of 0.15. The genetic distances within the pineapple cultivar group varied from 0 to 0.09, 0 to 0.12, and 0 to 0.16 respectively within the Spanish, Perola, and Cayenne groups. The UPGMA clustering analysis based on Nei's coefficient showed two main groups (Figure 1). There was no group made up exclusively of accessions from the same region. The first group (I) contained 31.57% of the accessions (14 accessions of Perola, 2 accessions of Cayenne, and 2 accessions of Spanish).

Table 2. Single Sequence Repeat (SSR) primers used for genetic diversity assessment of 57 pineapple accessions of Benin.

N°	Locus	Repeat Motif	Sequences	Fragments size	Na	Ne	I	H0	He	PIC
1	ACLR179BMa**	$(GTA)_4$	CCTTTGTTTTGTTACTTTTTAT CCAGTTATTTTTAGTAAAGTCC	227-243	2.000	1.471	0.56	0.400	0.320	0.269
2	ACLR179BMb**	$(TAA)_4$	GGACTTTACTAAAAATAACTGG ATACTAACAACACCTCTTTCAC	239-241	5.000	3.902	0.62	0.750	0.744	0.658
3	ACPCT124BM**	$(CCT)_8$	GTAGCAACAGCTATGAAAAC GATACAACGACAAGTACTACG	211-227	1.000	0.68	0.000	0.000	0	
4	ACPCT651BM**	$(GAA)_{13}$	GATACATAACAGTGTATTGGAG TAACTACTCTATGTTGTGACCA	210-220	3.000	2.266	0.69	0.950	0.559	0.518
5	ANBR58***	$(CT)_{21}$ $(CA)_{21}$	ATATGATAGGACTTACTTTTGG AAGGCTACAGATAGTTAAAGAG	147-268	2.000	1.835	0.65	0.300	0.455	0.388
6	ANBR72***	$(GA)_{27}$	TGCACCTTCTTACTTCTATAAT ACAACTAGCAAAACTTTGTATC	240-268	2.000	1.923	0.58	0.300	0.480	0.398
7	ANBR73***	$(CT)_{17}$	CATTAGATTAGTTCACAAACAA AGAATATTATGGAAAAATTGAG	280-288	2.000	2.000	1.52	0.700	0.500	0.379
8	ANBR75***	$(GA)_{30}$	ATGATCTCCTAAAAATCATAAG CTTAATTAGGGTTTTATTTGTC	109-110	2.000	1.995	1.10	0.750	0.499	0.397
9	ANBR80***	$(GA)_8$	GTTTAAGCAATAATTCCTAGAG TATAATCATGATGGAACATCTA	273-287	2.000	1.923	0.95	0.000	0.480	0.358
10	ANBR81***	(CT)21	TTAATCAAGTTCTTTAAAGGTT CTAGTAAAGTCTCTTTCCATTG	219-245	2.000	1.105	0.59	0.100	0.095	0.033
	Mean				2.3	1.94	0.806	0.425	0.413	0.340
	Standard Error				0.00	0.76	0.096	0.320	0.207	0.016

The following abbreviations represent Na: Mean value of alleles number; Ne: Number of effective alleles; I: Shannon information index; He: expected heterozygosity; Ho: observed heterozygosity; F: Fixation index and PIC: Polymorphic information content. The PIC values in bold represent the minimum, the maximum, and the mean values. **: Loci derived from *A. comosus* [modified from Kinsuat and Kumar (2007)]. ***: Loci derived from *Ananas bracteatus*. † Significant possibility of the presence of null alleles (95% confidence level) detected by Micro Checker (Van Oosterhout et al., 2004).

The second group (68.43%) was subdivided into 2 subgroups. The first subgroup IIa contained 14.28% of the accessions (8 accessions of Spanish) and the second subgroup contained 54.17% of the accessions (19 accessions of Cayenne, 6 accessions of Perola, and 5 accessions of Spanish). The accession Bracteatus EAD2222 diverged from the other accessions and constituted a separate branch on its own.

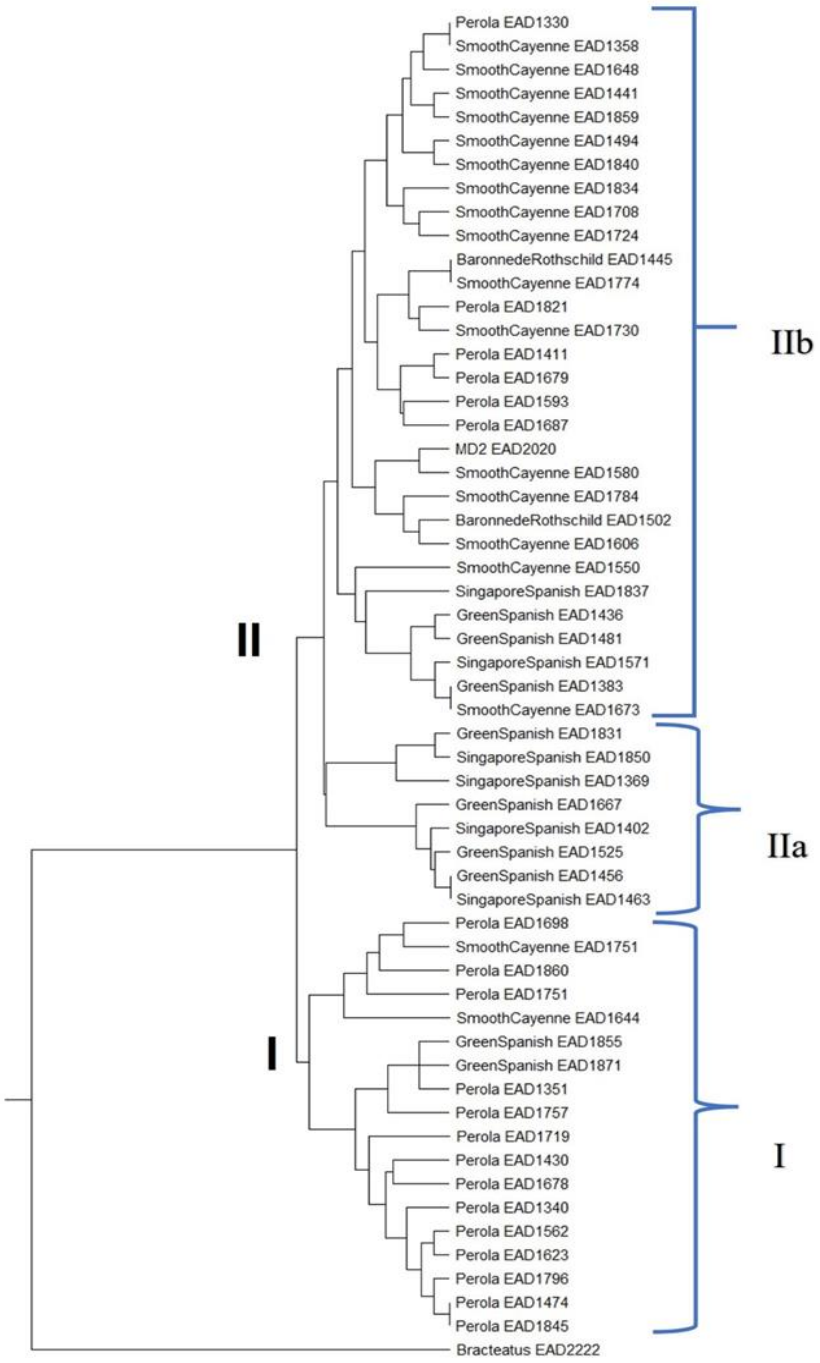

Figure 1. UPGMA cluster dendrogram (based on Nei genetic distance matrix) showing the relationships of 57 pineapple accessions based on 10 SSR markers.

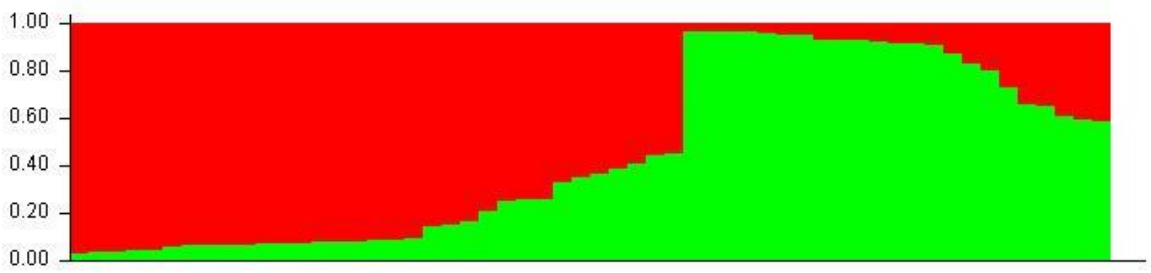

Figure 2. Bar plot structure of K = 2 obtained by population STRUCTURE analysis software version 2.3.4 depicting the genetic relationships. The plot shows two major clusters separated by a straight line.

Population structure within pineapple collection

After conducting a total of 20 runs for every k value ranging from 1 to 10, the analysis showed that the optimal value of k was determined to be 2 (k=2). This suggests that accessions from the germplasm can be segregated into two major genetic clusters. The Evanno table presents values of Δk for every k from 1 to 10, where the optimal number of k (k=2) is shown by the highest yield of Δk (10.64) as given in Table 3 and Supplementary Figure 2. A scatter plot was computed based on the value and confirmed the optimal number of k as 2. The optimum model of k=2 suggested the existence of two mean sup. Membership of all accessions to a particular group was based on a likelihood threshold of 0.6 (Figure 2). Based on the threshold > 0.6, the study did not reveal any admixtures among the evaluated accessions. Group k1 had the largest membership with 58.93% of the accessions while the smallest was k2 which gathered 41.07% of the accessions. The two groups identified K1 (red color) and K2 (green color) were respectively composed of 33 accessions (most of Cayenne and Spanish) and 23 accessions (most of Perola). This structure confirmed the result of the dendrogram.

Table 3. Evanno table output generated by Structure Harvester.

K	Reps	Mean LnP(K)	Stdev LnP(K)	Ln'(K)	|Ln''(K)|	Delta K
1	20	-696.340000	0.294511	—	—	—
2	**20**	**-676.055000**	**5.209452**	**20.285000**	**55.435000**	**10.641234**
3	20	-711.205000	29.748516	-35.150000	23.425000	0.787434
4	20	-722.930000	138.020594	-11.725000	40.760000	0.295318
5	20	-693.895000	51.479147	29.035000	61.200000	1.188831
6	20	-726.060000	72.533255	-32.165000	3.485000	0.048047
7	20	-761.710000	60.080831	-35.650000	18.510000	0.308085
8	20	-815.870000	130.744138	-54.160000	61.660000	0.471608
9	20	-931.690000	211.083426	-115.820000	236.880000	1.122210
10	20	-810.630000	128.057923	121.060000	—	—

Table 4. Analysis of molecular variance (AMOVA) among and within pineapple accession groups of Benin. Groups include Spanish, Cayenne, Perola.

Source	df	SS	MS	Est. Var.	%
Among groups	1	4.151	4.151	0.046	2%
Among individual within groups	45	91.115	2.025	0.050	2%
Within individual groups	47	90.500	1.926	1.926	95%
Total	93	185.766		2.021	100%

Genetic differentiation of the identified pineapple populations

The analysis of molecular variance (AMOVA) followed by a principal coordinate analysis (PCoA) was performed to appreciate the degree of differentiation among the groups identified by Structure package. From the AMOVA, we noted that only 2% of the total genotypic variation was explained by the difference among the groups, and 2% was caused by the difference among individuals within the groups (Table 4).

The maximum of the variation had been observed within individuals within groups (96%). To support the result of the AMOVA, a low F_{ST} value (0.027) was observed in the two identified groups, and the haploid was high (Nm=14.80) suggesting the high gene exchange among the groups. These results suggested a low genetic differentiation among groups and very high within groups.

Discussion

This work based on the screening of 57 pineapple accessions, is the first detailed overview of genetic diversity and population structure in a representative collection of pineapple accessions in Benin using SSR markers. This study provides results highly useful for pineapple genetic resources conservation and management and exploitation in the breeding program in Benin.

Microsatellites (SSRs) have been considered as markers of choice for genetic and breeding applications (Sharma et al., 2021). They have been previously used to assess genetic diversity in pineapple cultivars and relatives (Gioia et al., 2019). Here, the genotyping of the Benin pineapple germplasm collection confirmed that the primers used were informative for diversity analysis. The average value of 2.3 alleles per locus is similar to those reported by Rodrígueza et al. (2013) but lower than the 3.9 reported by Ismail et al. (2020). The lower number of alleles observed compared to the previous could be attributed to a narrow diversity of the accessions used in the current study since the other authors used accessions already existing in their research institution in Malaysia. The polymorphism rate was 90%, while 100% was reported by Rodrígueza et al. (2013). The polymorphism rate was higher than the value of 40% reported by Kinsuat and Kumar (2007) who used 50 SSR markers and 53% by Rodrígueza et al. (2013) who tested 66 SSR markers. The low polymorphism rate reported by Kinsuat and Kumar (2007) and Feng et al. (2013) can be explained by the high number of SSR markers newly developed and tested for the first time by those authors to assess genetic diversity in *Ananas comosus var. comosus*. The mean PIC value of the primers was 0.340, less than 0.5 suggesting the lower discriminating nature of those markers as reported by Rodrígueza et al. (2013). The observed heterozygosity was higher than the expected heterozygosity for all cultivars showing the excess of heterozygotes, in contrast the deficit of heterozygosity was reported by Rodrígueza et al. (2013) and by Feng et al. (2013). The high value of the observed heterozygosity could indicate a variability in the alleles. The variability within the alleles can explain the high variations observed within the genotypes and confirm the somaclonal variations observed in pineapple. This can also explain the failure of the morphological or mass selection which is not successful and still showed other forms in the offspring.

Based on our molecular data, Spanish accessions share more alleles with Cayenne accessions than Pérola accessions. Thus, the Spanish could be closer

to Cayenne. This may also indicate an exchange of genetic information between those two cultivars. The two clusters revealed by the UPGMA dendrogram closely follow the botanical classification of the pineapple cultivars in Benin (Adje et al., 2019). Feng et al. (2013) reported four clusters including Cayenne, Queen, and Spanish accessions. Population structure occurrence of Benin pineapple accessions assessed using Bayesian cluster analysis with the STRUCTURE software corroborated the previous result and revealed the existence of two subpopulations K1 (mostly Cayenne accessions) and K2 (Perola accessions) within the pineapple collection in Benin. Ismail et al. (2020) also reported two subgroups in Malaysia germplasm without any morphological characteristics associated.

The AMOVA performed on the subpopulations obtained revealed high differentiation within individuals within the two groups (96%). This high variation matched with the several morphological variations reported by Tossou et al. (2015) in Cayenne and Perola cultivars. This observed variation can be also attributed to the nonexistence of a standard classification in pineapple and the nomenclatural confusion in pineapple taxonomy. Previous morphological characterization of the same samples from Benin showed three groups (Adje et al., 2019). The results showed observed a high consistency between the morphological and molecular classification confirming cultivar classification (Noyer and Lanaud, 1997). Incongruence between morphological and molecular clusters was reported by Zhao and Qin (2018), Feng et al. (2013) and Shoda et al. (2012). Previous genetic diversity studies using AFLP also showed the relationship between morphological and molecular clustering (Kato et al., 2004). In pineapple, morphological characterization is usually based on traits such as "presence or absence of spine on the leaf, leaf, fruit and flesh colour, fruit shape, etc." It was reported that the presence or absence of leaf-spine is controlled by one single gene whereas the fruit skin colour is controlled by the accumulation of anthocyanin, carotenoids and chlorophyll degradation (Collins, 1960; Brat et al., 2004). The phenotypic differentiation observed in those organs was not only due to the variation in levels of accumulation of the different pigments (Samouelian et al., 2009), it could also be justified by the genetic background behind it. During plant pigment biosynthesis, the disturbance of one or a few genes can greatly affect the plant morphology (Samouelian et al., 2009).

The narrowest Nei's genetic distance was noted between Perola and Cayenne cultivars (0.04). The same trend was reported by Rodrígueza et al. (2013). The relationship within cultivar groups of *A. comosus* var. *comosus* was not well documented. Feng et al. (2013) reported four clusters instead of three expected from conventional classification. The close relationship between Cayenne and Perola can be because, Cayenne cultivars are known to be derived from the ancestral pineapple plants originated from French Guiana (Collins, 1960). Through vegetative reproduction, these plants generate many phenotypic variant forms because of the high somaclonal variation rate for some morphological traits (Collins, 1960). Mutations are the major source of variation used in the selection of new cultivars. The diversity analysis highlighted a relatively average level of observed heterozygosity (Ho) within the identified groups of pineapple. This observation is in line with a vegetative reproduction regime of the species and a low allelic diversity is also generally within cultivars.

This research is a strong argument to the ongoing debate on pineapple production in Benin and in West Africa about the morphotype observed within the established morphological groups (Baafi et al., 2015; Tossou et al., 2015). According to Tossou et al. (2015), there are ten morphological types in Perola and four morphological types in Smooth Cayenne. However, according to farmers, there are four morphological types in Perola (conical sessile form, conical non-sessile form supported by the extension of the heart, cylindrical non-sessile form supported by the extension of the heart, cylindrical sessile form) and two morphological types in Smooth Cayenne (conical and cylindrical shape). These variations are related to fruit shapes, length, weight, and colour. Variations in shape may reflect the effect of cultivation practices (planting density, fertilization, flower induction) and abiotic factors (night temperature, sunburn, drought). Some authors demonstrated the effect of the environment on the colour and shape of the fruits (Py et al., 1987; Bartholomew and Sinclair, 1993). However, the

effect of environmental factors on the appearance of different forms of fruit has been less documented. Malézieux et al. (2003) noticed that after a long dryness, the leaves take on a pale green colour which turns pale yellow and finally red. During dry periods, leaf growth is slowed down, and leaves resume growth when water becomes available. Sunburn during inflorescence development can produce fruits that are severely distorted; these fruits being more abundant in cold periods. Our results showed evidence of genetic variation among existing germplasm and confirmed the high variability of the pineapple genome. The number of variations found in the Perola and Cayenne groups should be considered a red flag for the need for selection within breeding programs. We recommend that further studies using increased numbers of SSR markers for large-scale sampling in Perola and Cayenne can provide robust data to definitively answer the origin of morphological variation within these cultivars.

Conclusion

Exploring the genetic diversity and structure in pineapple germplasm holds significance for both conservation and improvement efforts. This work demonstrates that SSR markers can effectively differentiate between various groups, offering utility in the conservation of pineapple genetic resources. The findings reveal that Benin pineapple exhibits substantial diversity within distinct two groups. The insights generated in this study provide a valuable resource for breeders, enabling them to identify promising genotypes for enhancing the Benin pineapple production and meeting demands in the international market.

Supplementary Materials

The supplementary material for this article can be found online at: https://www.jpmb-gabit.ir/article_712245.html.

Supplementary Figure 1. Pictures of the agarose gel showing PCR amplification of marker ANBR81 (a) and marker ACLR179BMb (b).

Supplementary Figure 2. Scatter plot to determine true K value using log probability (ΔK) method.

Author Contributions

Conceptualization: C.O.A.A., E.G.A.D.; project administration: E.G.A.D., Supervision: H.A.S.. C.A., E.G.A.D.; Methodology: C.O.A.A., A.A.M.; E.G.A.D; data curation: C.O.A.A, P.S.; formal analysis, funding acquisition: C.O.A.A., validation, visualization: C.O.A.A., E.G.A.D; writing—original draft: C.O.A.A., writing review and editing: C.O.A.A., A.A.M., P.S., H.A.S., E.G.A.D, C.A.

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Conflicts of Interest

The authors declare no conflict of interest.

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مقاله پژوهشی

بررسی ساختار و تنوع ژنتیکی ژرم‌پلاسم آناناس بنین (*Ananas comosus* (L.) Merr.) با استفاده از نشانگرهای SSR

شارلوت آبیک [۱،۲*]، آنتوان آبل میسیهون [۲]، پائولین سده [۲]، هوبرت آدوکونو ساگباجا [۲]، انوک گبناتو آچیگان-داکو [۱]، کلمان آگبانگلا [۲]

[۱] بخش ژنتیک، بیوتکنولوژی و علوم بذر (GBioS)،دانشکده علوم زراعی، دانشگاه آبومی کالاوی، آبومی کالاوی، جمهوری بنین
[۲] دانشکده علوم و فنون (FAST)، دانشگاه آبومی کالاوی،کوتونو، جمهوری بنین

چکیده: آناناس به عنوان محصولات مهم کشور بنین، نقشی اساسی در افزایش امنیت غذایی و درآمد خانوار برخوردار است. این محصول خوش طعم به دلیل مزه منحصر بفرد خود، در میان محصولات کلیدی مورد حمایت برای کشت در داخل کشور بنین قرار دارد. علیرغم اهمیت خاص این محصول، درک ما از میزان تنوع در منابع ژنتیکی آن محدود است. این مطالعه با هدف دسترسی به تنوع ژنتیکی و استنتاج ساختار جمعیت ۵۷ ژنوتیپ آناناس جمع‌آوری‌شده از کلکسیون ملی بنین، با استفاده از ۱۰ نشانگر (SSR) انجام گرفت. در مجموع ۲۳ آلل در این جمعیت شناسایی گردید. تعداد آلل‌ها در هر نشانگر بین ۲ الی ۴ متغیر بوده، که از میانگین ۲/۳ آلل در هر مکان نشانگری برخوردار بود. محتوای اطلاعات چندشکلی برابر با ۰/۳۴ بوده، در حالی که میانگین هتروزیگوسیتی مورد انتظار ۰/۴۳ بود. دندروگرام UPGMA دو خوشه اصلی را نشان داد. درکلکسیون آناناس مورد بررسی بر اساس تجزیه و تحلیل ژنتیکی، دو گروه مجزا با ترکیب ساختاری متمایز شناسایی شد. این گروه‌بندی توسط AMOVA نیز تأیید گردید. این مطالعه بینش ارزشمندی را در مورد میزان تنوع ژنتیکی در ژرم‌پلاسم آناناس بنین ارائه نموده که بواسطه آن فرایند حفاظت استراتژیک این گیاه را تسهیل می‌نماید. علاوه بر این، یافته‌های این تحقیق امکان استفاده از تنوع ژنتیکی موجود در ژرم پلاسم آناناس بنین را در برنامه‌های آتی به‌نژادی آناناس فراهم نموده که به نوبه خود موجبات افزایش کشت و سازگاری آناناس می‌شوند.

کلمات کلیدی: *Ananas comosus*، SSR، ساختار، ژرم پلاسم، بنین.

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نویسنده مسئول

Dr. Chalotte Abike Oladjo Adjé
abikecharlotte@gmail.com

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