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RESEARCH ARTICLE

Exploring the genetic diversity of jackfruit (*Artocarpus heterophyllus* Lam.) grown in Uganda based on SSR markers

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Abstract *Artocarpus heterophyllus* Lam. is an economically important tree crop that is widely cultivated in Uganda for its fruit. Despite its economic importance, little is known about the genetic diversity of jackfruit in the country. This puts the crop's genetic resource at risk as farmers selectively grow varieties based on market demand. The study analyzed the genetic diversity of *A. heterophyllus* trees from 12 districts belonging to three agro-ecological zones and three political regions of Uganda. Ten SSR loci were used to assess the genetic relationship among 200 trees, 197 from Uganda and 3 out-group individuals. All SSR loci were polymorphic with an average of 10.9 alleles per locus. STRUCTURE analysis proposed two genetic clusters: Cluster 1 was composed of samples from Eastern and neighboring Central districts, and Cluster 2 which constituted out-groups and samples from Western and neighboring Central districts. Results of STRUCTURE analysis were confirmed by PCoA. Mbarara District exhibited the highest genetic diversity (He = 0.79, I = 1.71), while Kamuli (He = 0.61, I = 1.08 and Pallisa (He = 0.59, I = 1.12) displayed the lowest genetic diversity despite high abundances of jackfruit trees. Molecular variation was higher within populations than among populations. Moderate and significant genetic differentiation was registered among geographical zones, while varietal differences displayed little insignificant genetic differentiation. Soft and white pulped varieties, considered inferior on the market, harbored private alleles which may be genetically valuable resources. Therefore, sustainable utilization and conservation efforts of the jackfruit genetic resource should consider preserving inferior varieties for future crop improvement.

Keywords Crop improvement · Conservation · Genetic resource · Microsatellites

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Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.), is one of the economically important members of the genus

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Artocarpus of family Moraceae (Witherup et al. 2013; Zerega et al. 2015) which is increasingly becoming a source of livelihood in many countries in the Indian subcontinent, Australia, the Neotropics and East Africa (APAARI 2012; Witherup 2012). Owing to the big size of its fruits, in Uganda, jackfruit is called Ffenensi or Ffene. This name is derived from a Luganda Phrase "*Ffena ensi tulya kuno*", literally meaning that the fruit can feed the whole world. Borneo has been reported as the biodiversity and evolutionary hotspot of *Artocarpus* (Williams et al. 2017) however, the origin of jackfruit still remains unclear. It is thought to have originated from the Western Ghats of India (Samaddar 1985) and then spread to Malaysia and East Africa (Dutton 1976). Therefore, the presence of jackfruit in Uganda is probably attributed to the settlement of Asians in the country in the 1890s (Okoth 1971; Atieno Odhiambo et al. 1977).

Several studies have reported the importance of jackfruit ranging from food, medicine to excellent wood including the numerous value-added products such as wine, chips, ice cream, jellies etc. (Haq 2006; APAARI 2012; Rahman et al. 2016). Despite the numerous benefits the plant offers, it remains listed among the least known and underutilized crops (Haq 2006) in many countries, Uganda inclusive. However, the status of jackfruit has gradually changed in Uganda to the extent that it is now widely grown, marketed and consumed in different parts of the country (unpublished survey data).

The increased demand however has come with the desire for better quality hence compelling jackfruit farmers to cut down trees that are presumed to be of poor quality. Farmers are clearing the white pulped jackfruit as well as the soft variety in preference for the firm variety with red or yellow pulps (unpublished survey data). This is an indicator of the fact that the crop is under selection pressures by farmers especially towards market desired attributes. Unfortunately, in the long run, these selection pressures may lead to erosion of the crop's genetic resources before they are documented and used for crop improvement.

Despite being an underutilized crop, efforts have been made by researchers in other countries to improve the plight of jackfruit by establishing its diversity using both morphological characteristics and molecular markers. Studies that utilized morphological characteristics (e.g. Rai et al. 2003; Khan et al. 2010; Ali et al. 2015; Phaomei et al. 2017; Chandrashekar et al. 2018), reported high diversity within jackfruit. However, morphological diversity is highly influenced by environmental conditions and therefore need to be evaluated by molecular markers.

The molecular techniques that have been utilized in analysis of genetic diversity of jackfruit are RFLP (Kanzaki et al. 1997), AFLP (Shyamalamma et al. 2008; Schnell et al. 2001; Ying-Zhi et al. 2010), RAPD (Krishnan et al. 2015), ISSR (ChunHai et al. 2009), and SSR (Wang 2011; Witherup 2012; Witherup et al. 2013; De Bellis et al. 2016). Inspite of the fact that studies have been conducted to establish the diversity of jackfruit, no such study has been done in Uganda and with the increase in demand, the genetic resource of the plant remains vulnerable in the hands of farmers.

Among the molecular markers, SSR or microsatellites present several unique features that make them more advantageous and popular than others. The popularity of microsatellite markers is attributed to their codominant nature, abundance and even distribution in the eukaryotic genome, high polymorphism loci, being easily amplified by PCR, suitability for automated allele size and above all cross-species transferability which reduces costs of primer development. Due to their advantages, microsatellites have been widely utilized in population genetics, genetic finger printing as well as cultivar characterization (Arif et al. 2011; Flores-Renteria and Krohn 2013; Zong et al. 2015; Zerega et al. 2015). Therefore, this present study was based on cross species transferability of SSR molecular markers to utilize primers developed for breadfruit [*Artocarpus altilis,* (Parkinson) Fosberg] by Witherup et al. (2013) and De Bellis et al. (2016) to determine the genetic diversity of jackfruit grown in Uganda so as to guide its production, conservation and monitoring of the genetic resource.

Materials and methods

DNA extraction and molecular analysis

Leaf samples were collected from Central, Eastern and Western regions of Uganda in three agro-ecological zones that is, Lake Victoria Crescent and Mbale Farmlands (LVCMF), Southern and Eastern Lake

Kyoga Basin (SELKB), and Southwestern Grass-Farmlands (SWGF, Wortmann and Eledu 1999). Each agro-ecological zone was represented by four districts, LVCMF (Iganga, Jinja, Masaka and Mityana), SELKB (Pallisa, Kamuli, Kayunga and Luweero), and SWGF (Ibanda, Mbarara, Mubende, and Sembabule, Fig. 1). Samples were selected based on different fruit textures (firm or soft) and pulp colors (red, yellow and white). Fruit texture and pulp colour were used by farmers to categorize jackfruit and were confirmed through a morphological study survey using a fruit hardness tester (FHT 1122, Guangzhou Landtek Instruments Co., Ltd) and Munsell plant tissue colour book (Munsell colour company 2012), respectively. The leaf samples were dried using silica gel in which they were preserved and transported to the laboratory until further processing. Three additional samples were collected from botanical gardens in Germany, BG 300 (Accession Number = 39132-7-206, IPEN Nr. = PH-0-BONN-39132, originating from Philippines) BG 301 (Accession Number = 38775-5-2016, IPEN Nr. = VN-0-BONN-38775, originating from Vietnam) and VW 302 (Accession Number = 1987/295, IPEN Nr. = XX-0-MB-1987/295, origin not known) which served as out-groups (Supplements Table 1). Geographical coordinates which were used to obtain geographical distances (Tables 1 and 2) and elevations of all populations were recorded in the field. Climatic data (annual average precipitation and temperature) for the sampled districts was obtained from dataafrica.io/profile/Uganda accessed on 29th July, 2018.

Dried leaf material was ground into fine powder (Tissue lyser II Retsch, Germany). Genomic DNA was isolated using the DNeasy Plant Mini Kit (QIAGEN®) following the manufacturer's instructions. DNA quantity and quality were checked using a Nanophotometer (IMPLEN) and 2% agarose gel electrophoresis. In a pre-test, 16 SSR marker loci developed by Witherup et al. (2013) and De Bellis et al. (2016), were screened for amplification, polymorphism and their annealing temperatures stringently optimized until a single band was obtained on the agarose gel (Table 3). The selected oligonucleotides, were fluorescently labeled by incorporating a fluorescent dye on the 5' end of the forward primers. This was achieved by using modified phosphoramidite on the oligosynthesizer (metabion international AG, Semmelweisstr. 3, 82152 Planegg, Germany). The reverse primers remained unaltered. Various fluorescent dyes such as 6-FAM = blue,

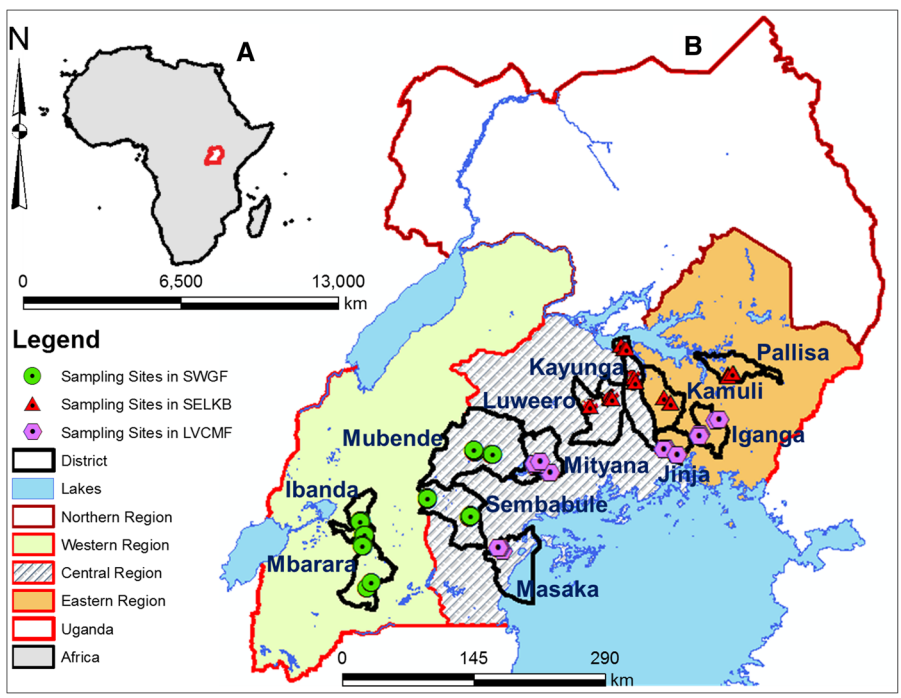


Fig. 1 A Map of Africa showing the location of Uganda. B Map of Uganda showing jackfruit sampling sites according to districts, agro-ecological zones and political regions

Table 1 Pairwise geographical distances (km) between the sampled districts

	Pallisa	Kamuli	Kayunga	Jinja	Iganga	Luwero	Mubende	Mityana	Sembabule	Masaka	Ibanda	Mbarara
Pallisa	0											
Kamuli	64.44	0										
Kayunga	103.74	36.77	0									
Jinja	98.15	55.81	43.56	0								
Iganga	66.87	53.69	64.18	35.81	0							
Luwero	139.64	70.96	47.67	89.66	108.28	0						
Mubende	266.97	198.11	169.19	202.02	232.29	127.33	0					
Mityana	203.95	134.7	101.16	128.91	160.71	70.14	74.83	0				
Sembabule	287.28	218.03	184.19	207.98	246.23	151.55	53.35	83.33	0			
Masaka	273.88	208.07	171.44	182.6	218.77	153.84	106.02	87.18	61.78	0		
Ibanda	385.76	314.79	288.41	306.71	341.62	246.04	123.63	180.84	98.73	141.03	0	
Mbarara	392.97	324.54	301.67	306.61	341.47	260.5	152.73	190.88	109.82	126.11	56.94	0

Table 2 Pairwise geographical distances (km) between the sampled political regions and agro-ecological zones

Political regions	Central	Eastern	Western
Central	0		
Eastern	43.56	0	
Western	109.82	306.61	0
Agro-ecological zones	LVCMF	SELKB	SWGF
LVCMF	0		
SELKB	55.81	0	
SWGF	74.83	169.1	0

HEX = green, NED = yellow, TET = yellow, TAMRA = red or yellow, ROX = red (mainly for size standard), VIC = Green, PET = red and LIZ = orange (mainly for size standard) can be used to fluorescently label primers. For this study, 6-FAM and HEX were used because their spectra were not overlapping when their PCR products were mixed. To ensure that desired alleles were amplified, five test samples were sequenced at the Seqlab GmbH a subsidiary of Microsynth AG|Hannah-Vogt-Str. 1|37085 Göttingen|Germany. For final analysis, ten SSR markers which were highly polymorphic with reproducible amplicons were selected for genotyping all the jackfruit samples (Table 3). Primers mAaCIR0019, mAaCIR0154, MAA219 consistently produced non-specific bands, mAaCIR003 failed to amplify while mAaCIR0204 and MAA 201 selectively amplified in a few samples and for those reasons, they were not included in the final analysis (Table 3).

Each PCR reaction mixture (total volume = 12.5 µl), contained 1.25 µl of 2 mM dNTP, 1.25 µl of 25 mM $MgCl_2$, 0.25 µl of 10 pmol/µl forward and reverse primers respectively (Table 1), 0.25 µl of 10 ng/µl BSA, 1 µl of 99% DMSO, 1.25 µl of 10× buffer, 0.2 µl of 5 U/µl Taq DNA polymerase (Thermo Fisher Scientific), 5 ng DNA and ddH_2O.

The PCR amplification conditions were as follows: initial denaturation at 94 °C for 5 min; 35 cycles at 94 °C for 45 s, optimized primer annealing temperature (Ta, see Table 1) for 45 s, primer extension at 72 °C for 1 min and final extension at 72 °C for 10 min. Fragments were analyzed on an ABI 3130 XL Genetic Analyzer (Applied Biosystems) at LGC Cologne/Germany. The alleles were scored using

Table 3 Applied primers and their optimized annealing temperatures (Ta)

No.	Primer name	Primer sequence (F/R)	Source	Ta (°C)
Primers used for final analysis				
1.	mAaCIR0048	F: CGAAATCGGAACAGAAAAC	De Bellis et al. (2016)	53
		R: GTCCTTGGCTACTATAATCCCT		
2	mAaCIR0049	F: TACATACAAGCCAACTTCCA	De Bellis et al. (2016)	55
		R: CCTTTGTGAGGAAGACCA		
3	mAaCIR0141	F: TCAAGCCCCTCACTCAA	De Bellis et al. (2016)	59
		R: ATGGCATAGCACAACACAA		
4	MAA105	F: GTTGGGACACTGTGAACTATTC	Witherup et al. (2013)	54
		R: AAAAGCTAGTGGATTAGATGCA		
5	MAA122	F: CTGGCCTTCAGTTTTGTCAAC	Witherup et al. (2013)	54
		R: CACCAGGCTTCAAGATGAAA		
6	MAA135	F: TGCATCATAAGGTTGCTCTG	Witherup et al. (2013)	59
		R: TGGGCTTTTTCTGGAAAC		
7	MAA140	F: CCATCCCCCATCTTTCCT	Witherup et al. (2013)	53
		R: TCCTCGTTTGCCACAGTG		
8	MAA145	F: CCAACGCATAGCCAAATC	Witherup et al. (2013)	49
		R: AAATCCCAAACCCAACGT		
9	MAA156	F: CTGGTGCTTCAGCCTAATG	Witherup et al. (2013)	53
		R: TCAGCGTCAAAGATAACTCG		
10	MAA182	F: TACTGGGTCTGAAAAGATGTCT	Witherup et al. (2013)	50
		R: CGTTTGCGTTTGGATAAAT		
Primers not used for final analysis				
11	mAaCIR0019	F: TGACATTCCCGCAAAA	De Bellis et al. (2016)	NA
		R: AAGTCTTCTGTTCCTACTGACAA		
12	mAaCIR0033	F: CGGGTACAGGGTATTGGT	De Bellis et al. (2016)	NA
		R: AGGAGAGCGTTTGAGGAA		
13	mAaCIR0154	F: TCGAGGCCCTTGTTG	De Bellis et al. (2016)	NA
		R: GGAAATTCACCTTTCCTTG		
14	mAaCIR0204	F: TTTAGGGTCCGTTGAAGA	De Bellis et al. (2016)	NA
		R: GAAGTCTTGTTATTTGTGGAAG		
15	MAA201	F: GGTTCAATTCACACATACAGG	Witherup et al. (2013)	NA
		R: TTGAGGCTAAAAGAATATGAGG		
16	MAA219	F: ATTTGCATCATGTAGGACA	Witherup et al. (2013)	NA
		R: GGACACAACGACATTGAC		

NA = Ta not obtained since, primers mAaCIR0019, mAaCIR0154, MAA219 had un-specific amplification, mAaCIR003 failed to amplify while mAaCIR0204 and MAA 201 selectively amplified in a few samples

GeneMarker version 2.6.3 (SoftGenetics LLC, State College, PA, USA).

Data analysis

Genetic diversity indices such as number of alleles (Na), number of effective alleles (Ne), observed heterozygosity (Ho), expected heterozygosity/gene diversity (He), Shannon's information index (I), number of private alleles (Pa), percentage of polymorphic loci (PPL), inbreeding coefficient (F) and pairwise F_{ST} values were computed using GenAlEx version 6.503 (Peakall and Smouse 2012). To test for the adaptability of the crop for the prevailing conditions in the country, correlations between environmental variables (geographical distance, precipitation,

temperature, elevation) and genetic distance, were computed using mantle tests in GenAlEx version 6.503. The polymorphic information content (PIC) of the primers was calculated using the formula, $PIC = 1 - \sum (P_i)^2$ where P_i is the proportion of samples carrying the ith allele.

Population structure was studied using the model based program, STRUCTURE 2.3.4 (Pritchard et al. 2000) with 10 iterations for each genetic cluster, K = 1 − 12. Individual membership to genetic clusters was assessed by employing the admixture model. Each iteration was executed with a burn-in period of 50,000 and 100,000 Monte Carlo Markov Chain replicates. To determine the most suitable K, model distribution, delta K (Evanno et al. 2005) was determined using Structure harvester (Earl and von Holdt 2012). Resultant graphs were created with Distruct (Rosenberg 2004). To confirm the results of STRUCTURE analysis, principal coordinate analysis (PCoA) was done in GenAlEx version 6.503 via a distance matrix with data standardization of genetic distance.

Analysis of Molecular variance (AMOVA) among populations and within populations for the sampled districts, agro-ecological zones, political regions, pulp colors as well as fruit textures and the two clusters obtained from STRUCTURE analysis with the outgroups was executed in ARLEQUIN 3.5.1.2 (Excoffier and Lischer 2010) with 10,000 permutations.

Results

SSR analyses with ten primer pairs and 197 analyzed Ugandan jackfruit samples resulted into 109 alleles with a size range of 90–340 base pairs. All the ten loci were polymorphic and significantly deviated from Hardy–Weinberg Equilibrium ($P < 0.01$). The total number of alleles per locus ranged from 6 (mAaCIR0048 and mAaCIR0141) to 18 (MAA145), with a mean of 10.9 (Table 4).

Observed heterozygosity (Ho) ranged from 0.21 (MAA135) to 0.92 (mAaCIR0048) with a mean of 0.52. The expected heterozygosity/genetic diversity (He) ranged from 0.60 (MAA135) to 0.77 (MAA 122) with a mean of 0.71. The polymorphic information (PIC) of the SSR primers used ranged from 0.65 (mAaCIR0141) to 0.81 (MAA122). Primers mAaCIR0048, mAaCIR0049, and MAA156 revealed excess of heterozygotes among Ugandan jackfruit samples while primers MAA135 and MAA182 manifested relatively high inbreeding among jackfruits in Uganda. Null alleles were detected for primers mAaCIR0048, mAaCIR0049 and MAA122. Overall, the ten SSR primers revealed high genetic diversity among Ugandan jackfruit samples as measured by Shannon's Information index (mean = 1.72, Table 4).

Among the districts sampled, Mityana registered the highest number of alleles (73) followed by Mbarara (68) while Kamuli and Jinja the lowest (40, Table 5). The highest number of private alleles (6) was observed among samples from Pallisa followed by

Table 4 Number of samples (N) and allelic diversity parameters for 10 SSR loci overall Ugandan *Artocarpus heterophyllus* samples

Locus	N	Na	Ne	I	Ho	He	F	PIC
mAaCIR0048	196	6	4.21	1.51	0.92	0.72	− 0.29	0.72
mAaCIR0049	195	9	4.54	1.69	0.89	0.68	− 0.36	0.74
mAaCIR0141	197	6	3.41	1.39	0.34	0.63	0.47	0.65
MAA105	197	11	5.08	1.61	0.55	0.75	0.29	0.77
MAA122	194	11	5.91	1.89	0.43	0.77	0.44	0.81
MAA135	197	14	2.78	1.39	0.21	0.60	0.68	0.59
MAA140	197	11	5.22	1.89	0.39	0.73	0.46	0.78
MAA145	197	18	5.2	2.10	0.50	0.73	0.31	0.77
MAA156	197	11	4.98	1.79	0.64	0.67	− 0.04	0.77
MAA182	197	12	4.26	1.72	0.33	0.64	0.50	0.73
Mean	196.4	10.9	4.56	1.72	0.52	0.71	0.25	0.73

Na, number of observed alleles; Ne, number of effective alleles; I, Shannon's Information Index; Ho, observed heterozygosity; He, expected heterozygosity estimated with computer program GenAlex 6.503 (Peakall and Smouse 2012); PIC, polymorphic information content

Table 5 Number of samples (N) and genetic diversity indices for *Artocarpus heterophyllus* from different geographical zones (districts, political regions and agro-ecological zones) and varietal differences (fruit texture and pulp colour)

	N	Na	Ne	Pa	I	Ho	He	F
Districts								
Pallisa	16.00	47.00	26.89	6.00	1.12	0.50	0.59	0.19
Kamuli	16.00	40.00	27.11	0.00	1.08	0.54	0.61	0.10
Kayunga	16.00	58.00	35.00	3.00	1.39	0.61	0.69	0.11
Jinja	18.00	40.00	28.00	0.00	1.12	0.60	0.63	0.19
Iganga	16.00	43.00	29.29	0.00	1.14	0.47	0.61	0.26
Luwero	19.00	50.00	32.73	2.00	1.13	0.64	0.68	0.03
Mubende	16.00	63.00	38.88	4.00	1.49	0.70	0.72	0.41
Mityana	16.00	73.00	47.36	3.00	1.69	0.56	0.77	0.26
Sembabule	16.00	63.00	43.80	1.00	1.56	0.43	0.74	0.42
Masaka	16.00	65.00	42.92	0.00	1.57	0.47	0.74	0.36
Ibanda	16.00	59.00	42.53	2.00	1.53	0.50	0.74	0.33
Mbarara	16.00	68.00	51.21	1.00	1.71	0.58	0.79	0.27
Mean					1.39	0.52	0.69	0.25
Political regions								
Central	99.00	100.00	50.11	18.00	1.79	0.53	0.79	0.33
Eastern	66.00	61.00	30.69	6.00	1.26	0.50	0.65	0.22
Western	32.00	75.00	51.59	3.00	1.73	0.54	0.79	0.29
Mean					1.59	0.52	0.74	0.06
Agro-ecological zones								
LVCMF	67.00	72.00	34.06	16.00	1.36	0.57	0.68	0.14
SELKB	66.00	78.00	41.14	4.00	1.60	0.49	0.74	0.33
SWGF	64.00	87.00	53.33	12.00	1.77	0.48	0.79	0.39
Mean					1.58	0.52	0.74	0.29
Fruit texture								
Firm	181	105	45.48	43.00	1.72	0.51	0.77	0.34
Soft	16	66	41.96	4.00	1.57	0.59	0.74	0.21
Mean					1.64	0.55	0.76	0.28
Pulp colour								
Red	69.00	85.00	45.02	3.00	1.67	0.52	0.76	0.32
White	49.00	93.00	46.45	12.00	1.74	0.53	0.77	0.32
Yellow	79.00	92.00	43.69	7.00	1.66	0.51	0.76	0.32
Mean					1.69	0.52	0.76	0.32

Na, number of alleles from the ten loci; Ne, number of effective alleles from ten loci; Pa, number of private alleles; I, Shannon Information Index; Ho, observed heterozygosity, He, expected heterozygosity and F, fixation index (inbreeding coefficient) computed from GenAlEx (Peakall and Smouse 2012)

those from Mubende (4) while four districts (Kamuli, Jinja, Iganga and Masaka) had no private alleles. The Shannon information index (I = 1.71) and the gene diversity (He = 0.79), portrayed samples from Mbarara to be the most genetically diverse while Kamuli (He = 0.61, I = 1.08 and Pallisa (He = 0.59, I = 1.12) registered the lowest jackfruit genetic diversity. High levels of inbreeding were observed among samples from Sembabule, (F = 0.42) while samples from Luweero revealed random mating (F = 0.03, Table 5). Genetic differentiation based on pairwise F_{ST} values showed that samples from Jinja and Iganga were genetically the closest (F_{ST} = 0.014) while samples from Kamuli and Mubende were the most distant

Table 6 Pairwise F_{ST} values of the sampled districts

	Pallisa	Kamuli	Kayunga	Jinja	Iganga	Luwero	Mubende	Mityana	Sembabule	Masaka	Ibanda	Mbarara
Pallisa	0.000											
Kamuli	0.050	0.000										
Kayunga	0.041	0.041	0.000									
Jinja	0.052	0.019	0.022	0.000								
Iganga	0.046	0.029	0.032	0.025	0.000							
Luwero	0.066	0.029	0.015	0.014	0.042	0.000						
Mubende	0.126	0.135	0.088	0.114	0.117	0.103	0.000					
Mityana	0.096	0.060	0.051	0.054	0.068	0.048	0.058	0.000				
Sembabule	0.100	0.079	0.067	0.073	0.088	0.067	0.065	0.024	0.000			
Masaka	0.098	0.074	0.071	0.075	0.086	0.069	0.061	0.024	0.016	0.000		
Ibanda	0.105	0.068	0.067	0.062	0.071	0.062	0.078	0.028	0.033	0.035	0.000	
Mbarara	0.087	0.064	0.051	0.062	0.075	0.052	0.046	0.023	0.024	0.025	0.029	0.000

F_{ST} is the measure of genetic differentiation among population, 0.0 means no differentiation while 1 refers to complete differentiation

(F_{ST} = 0.135). Mubende recorded the highest pairwise F_{ST} values with other districts ranging from 0.046 to 0.135 while Mbarara and Kayunga showed low genetic differentiation from other districts with all their pairwise F_{ST} values <0.1 (Table 6).

The Central region harbored the highest genetic diversity of jackfruit (Na = 100, I = 1.79, He = 0.79), followed by the Western region (Na = 75, I = 1.73, He = 0.79), while the Eastern region had the lowest (Na = 61, I = 1.26, He = 0.65, Table 5). Genetic diversity analysis for agro-ecological zones revealed that the SWGF harbored the highest genetic diversity (Na = 87, I = 1.77, He = 0.79), followed by LVCMF (Na = 78, I = 1.6, He = 0.74), while SELKB registered the lowest genetic diversity (Na = 72, I = 1.36, He = 0.79). The fixation index varied from 0.14 in SELKB to 0.39 in the SWGF.

Since farmers and traders in Uganda categorized jackfruit based on fruit texture and pulp color, the study also investigated the genetic diversity indices of these categories. According to fruit texture, the firm variety registered more alleles (105) with 43 being private than the soft variety (66) with only 4 being private (Table 5). The soft variety despite having a lower number of alleles, recorded a higher observed heterozygosity value (Ho = 0.59) than the firm variety (Ho = 0.51). However, the firm variety was more genetically diverse (I = 1.72, He = 0.77) than the soft one (I = 1.57, He = 0.74). Both soft and firm varieties had low inbreeding coefficients (F = 0.21 and 0.38) respectively. Of the pulp colors, white had the highest number of alleles (93) followed by yellow (92) and red had the lowest (85, Table 5). Similarly, the white pulped variety had the highest number of private alleles (12) while red had the lowest (3). The white variety had the highest Shannon information Index, observed heterozygosity and gene diversity (I = 0.74, Ho = 0.53, and He = 0.77, Table 5). Therefore the white variety was the most genetically diverse among pulp colored varieties. All the three pulp colors had the same inbreeding coefficient (0.32).

Bayesian analyses of population structure of *A. heterophyllus* revealed ΔK = 2 to best represent the data. Using 98% as the inclusion factor to a cluster, of the 197 jackfruit samples collected from Uganda, 89 individuals (45.2%) constituted only one cluster, and the other cluster accommodated 60 individuals (30.5%) while 48 individuals (24.3%) showed intermediate genetic pattern between the two clusters

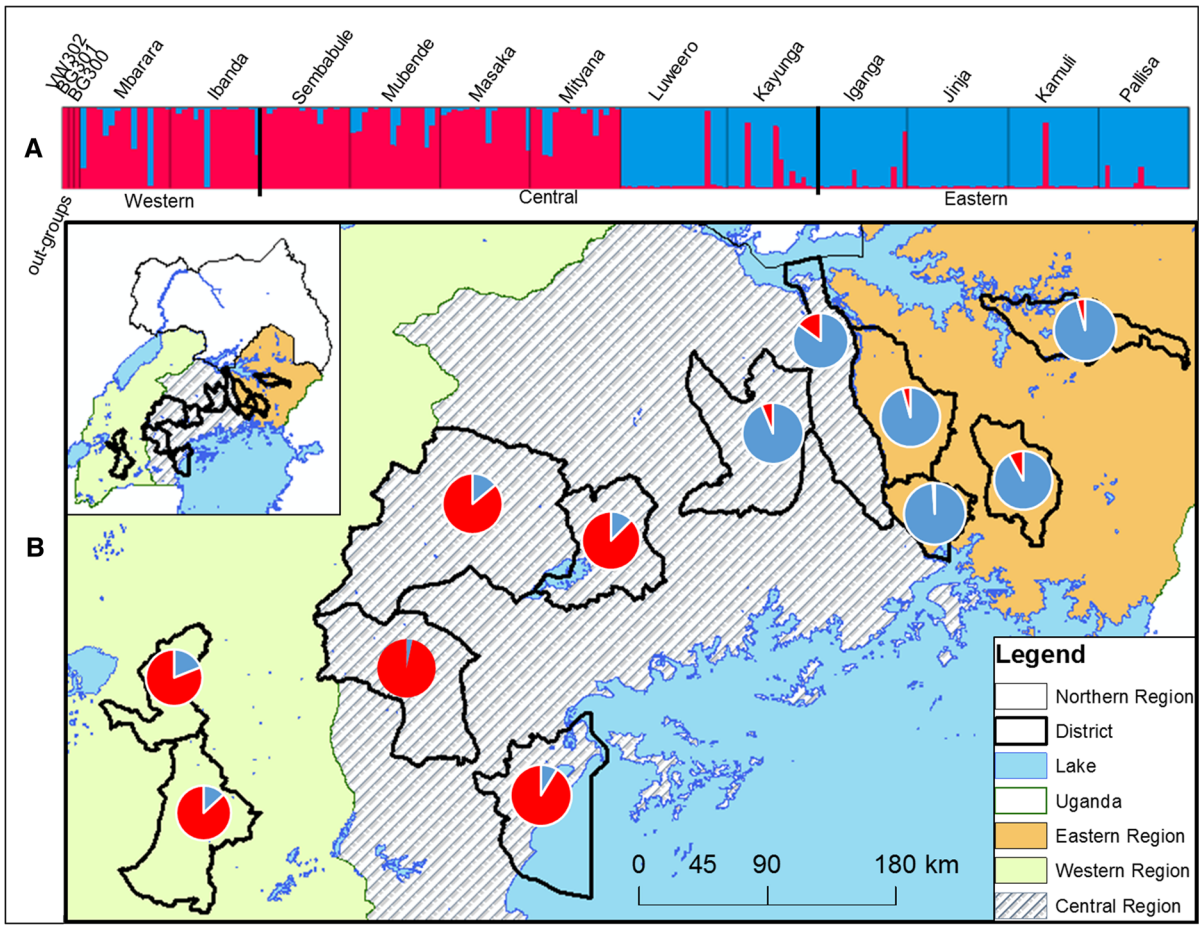


Fig. 2 A Population genetic structure of 197 jackfruit samples from 12 districts and three out-groups as revealed by STRUCTURE analysis using the admixture model for ΔK = 2. B Map of the Ugandan districts with pie charts indicating combined genetic cluster affiliations of *A. heterophyllus*

(Fig. 2). While a high proportion of samples from Pallisa, Kamuli, Kayunga, Jinja, Iganga, and Luweero are represented by one cluster, the other cluster is dominant in Mubende, Mityana, Masaka, Ibanda and Mbarara. Of the samples obtained from the Central region, 27.3% belonged to Cluster 1, 41.4% to Cluster 2 while 31.3% showed admixture between the two clusters. Samples from the Eastern region mainly belonged to Cluster 1 (89.4%) and only 10.6% overlapped between the two clusters. For samples from the Western region, 53% belonged to Cluster 2, 37.5% showed admixture between the two clusters and only 6.25% belonged to Cluster 1. The out-groups obtained from botanical gardens in Germany showed a high proportion of membership to Cluster 2 (Fig. 2). In addition, genetic data showed weak positive correlations with geographical distance (r = 0.25, $P = 0.01$), precipitation (r = 0.19, $P = 0.01$), temperature (r = 0.16, $P = 0.01$), and elevation (r = 0.23, $P = 0.01$) though the correlations were all significant ($P < 0.05$).

The PCoA (Fig. 3) coupled with STRUCTURE analysis, revealed the Central region as the hub of genetic diversity of jackfruit with its genotypes being in close association with those from the Eastern and Western samples. However, the Western and Eastern regions showed little association among genotypes. These results were further supported by pairwise F_{ST} values (Central and Western, $F_{ST} = 0.013$, Central and Eastern, $F_{ST} = 0.035$, Eastern and Western, $F_{ST} = 0.053$, Supplements Table 2).

Analysis of Molecular Variance (AMOVA) revealed that most of the genetic variation resided within populations (80.95–95.14%) and this was

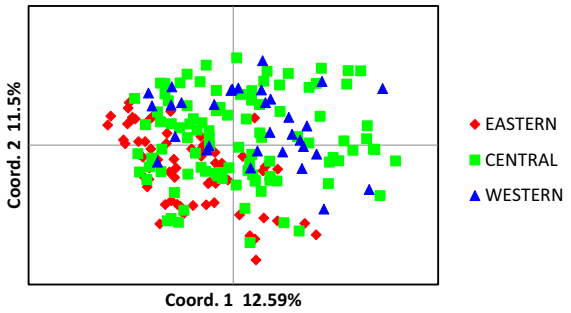


Fig. 3 Principal coordinates' analysis (PCoA) with individuals marked according to their origin (Central, Eastern and Western region). The first three components explained 34.39% of the cumulative variation

consistent with all the different groupings (districts, agro-ecological zones, political regions, pulp color, and fruit texture, Table 7). Districts and political regions registered moderate genetic differentiation with F_{ST} values 0.0857 ($P < 0.001$) and 0.0548 ($P < 0.001$) respectively. Little though significant genetic differentiation was recorded among agro-ecological zones ($F_{ST} = 0.0486$, $P < 0.001$). Varietal categorization based on pulp color $F_{ST} = 0.001$ ($P = 0.722$) and fruit texture, $F_{ST} = 0.003$ ($P = 0.552$) showed little genetic differentiation. AMOVA depicted great genetic differentiation between samples in Cluster 1 and out groups ($F_{ST} = 0.191$, $P < 0.001$) while Cluster 2 and the out groups showed moderate genetic differentiation ($F_{ST} = 0.064$, $P = 0.134$) which supports the results of STRUCTURE analysis (Fig. 2).

Discussion

This study presents the first population genetic investigation of jackfruits grown in Uganda. Other crops that have been investigated earlier with population genetic tools in this country are e.g. coffee (Musoli et al. 2009; Aluka 2013); cassava (Turyagyenda et al. 2012), and sweet potatoes (Zawedde et al. 2014) using SSR markers and sesame using ISSR (Nyongesa et al. 2012) and revealed human mediated transfer of

Table 7 The genetic diversity among and within populations for geographical zones (districts, agro-ecological zones and political regions) as well as varietal categorizations (pulp color and fruit texture) as revealed by an Analysis of Molecular Variance (AMOVA)

Source of variation	df	Sum of squares	Variance components	% Variation	F_{ST}	*P* value
1. Districts						
Among populations	11	158.433	0.331	8.57	0.086	< 0.001
Within populations	382	1349.503	3.532	91.43		
2. Agro-ecological zones						
Among populations	2	57.156	0.189	4.86	0.049	< 0.001
Within populations	391	1450.781	3.710	95.14		
3. Political regions						
Among populations	2	58.954	0.215	5.48	0.055	< 0.001
Within populations	391	1448.982	3.706	94.52		
4. Pulp colour						
Among populations	2	8.760	0.004	0.11	0.001	0.722
Within populations	391	1499.176	3.834	99.89		
5. Fruit texture						
Among populations	1	4.578	0.012	0.33	0.003	0.552
Within populations	392	1503.358	3.835	99.67		
6. Cluster 1 and outgroups						
Among populations	1	12.566	0.790	19.05	0.191	< 0.001
Within populations	206	691.645	3.357	80.95		
7. Cluster 2 and outgroups						
Among populations	1	7.126	0.271	6.40	0.064	0.134
Within populations	196	778.005	3.969	93.60		

planting materials leading to weak correlations between genetic and geographical distances. This should be typical for jackfruit since it is a cultivated plant.

SSR markers were adopted in this study due to the high reproducibility of the data (Zerega et al., 2015) and polymorphism rendering them apposite for analyzing a large number of samples (200). According to Botstein et al. (1980), Venkatachalam et al. (2010) and Zawedde et al. (2014), PIC values > 0.5 are indicative of highly polymorphic loci with strong discriminatory power, which accounts for the here used SSR primers (PIC = 0.73, Table 4). Due to the strong discriminatory power of the SSR primers used and high genetic diversity within *A. heterophyllus,* the samples registered no duplicates.

The mean number of alleles per locus (Table 4, Na = 10.90), mean Ho (0.52) and He (0.71) obtained in this study were higher than the ones reported by Witherup (2012) from Bangladesh jackfruit (Na = 8.31, Ho = 0.29, He = 0.44) revealing the existence of relatively high genetic diversity and variation in Uganda in this food crop. The differences may be attributed to different genotypes in the two countries and multiple introductions to Uganda since the crop is not indigenous (Dutton 1976). In addition, slightly different molecular tools used in the study on genetic diversity of jackfruit in Bangladesh, may also account for some of the observed differences between the two studies. Nevertheless, the mean genetic diversity (He = 0.71) reported in this study is in agreement with the range of He values (0.640-0.814) reported among domesticated perennial fruits using SSR markers (Miller and Gross, 2011; Zerega et al. 2015; Ebrahimi et al. 2017; Pereira-Lorenzo et al. 2017).

The high genetic diversity of jackfruit in Mbarara is likely due to the fact that this district, and the Western region at large, especially the cattle keeping communities, had for long neglected the growing of jackfruit, due to mythical issues relating to jackfruit killing their livestock (unpublished survey data). However, with the realization of the commercial benefits the crop can offer, Mbarara picked up the growing of jackfruit and the genotypes in Mbarara are widely collected from different parts of the country. Hence, Mbarara covers most of the alleles and genotypic structures as revealed by the structure analysis (Fig. 2) and depicted by the PCoA (Fig. 3). This is further supported by the low pairwise F_{ST} values that Mbarara shared with both near and distant districts (Table 6). Pallisa and Kamuli as well as the Eastern region at large, registered low jackfruit genetic diversity despite harboring high abundances of jackfruit trees. This may be due to human selection of traits desired on the market to the extent that the soft pulp variety does not exist in these district (personal observation). Jackfruit trees in these districts with inferior traits were cut and used for timber and fuel. This form of reduction in genetic diversity among crop germplasms due to human selection has also been reported by Fregene et al. (2003) and Miller and Gross (2011). Human selection may also account for the genetic diversity differences observed among agro-ecological zones. SWGF harbors districts that have recently adopted the growing of jackfruit e.g. Mbarara and registered the highest genetic diversity among agro-ecological zones. The high jackfruit genetic diversity in this agro-ecological zone (SWGF) may be attributed to farmers not being acquainted with the qualities of different jackfruits and therefore may be planting any germplasm without selection while LVCMF and SELKB are dominated by districts where farmers have had more interaction with jackfruit. Therefore, farmers in these agro-ecological zones (LVCMF and SELKB) might be selecting jackfruits with superior qualities hence lowering the crop's genetic diversity in these agro-ecological zones.

The genetic diversity of the firm variety (He = 0.77, I = 1.75, Table 5) was higher than that of the soft variety (He = 0.74, I = 1.57, Table 5). The low genetic diversity of the soft variety can potentially be explained by genetic bottlenecks (i.e. small population sizes). These may occur due to strong human selection, since the farmers have severely substituted the soft variety by more consumer friendly jackfruits and its presence in the wild is uncertain. In addition, the soft variety is highly perishable and difficult to transport over long distances, hence limiting its cultivation, market value and geographic distribution. On the market, the white fleshed variety is less attractive and consumers easily get the impression of unripe fruits. Therefore, the white varieties are less consumed than the red and yellow fleshed ones. Consequently, farmers are opting to have it cut down in case there is need for timber or fuel, and might be replaced by red and yellow fleshed varieties. However, the whited fleshed jackfruits registered higher genetic diversity (He = 0.77, I = 1.74) than other pulp colors

(Yellow, He = 0.76, I = 1.66, Red, He = 0.76, I = 1.67) thus, depicting their contribution to the genetic diversity of the crop in the country. The high genetic diversity observed in the white fleshed variety may be attributed to a high number of private alleles observed (Pa = 12) which should be of special conservation concern to the National Agricultural Research Organization and National gene bank. These genotypic patterns might be indicative of additional unique alleles that may be necessary for optimizing food crop characters of the jackfruit populations in future.

The presence of two main genotypic clusters within the Ugandan jackfruits as revealed by structure analysis (Fig. 2a) reflects patterns already found earlier, e.g. by Wang (2011) among jackfruit germplasms in China using SSR and Schnell et al. (2001) among jackfruit germplasms in USA using AFLP. However, other studies found 4 genotypic clusters for jackfruit diversity e.g. Witherup (2012), Azad et al. 2007 in Bangladesh, and Krishnan et al. 2015 in India using SSR, Isozyme and RAPD technique respectively. The existence of two genetic clusters may point to different strains that were introduced to Uganda and which might have hybridized in recent times or even the hybrids themselves were introduced. Possibly two genotypes were introduced, one genotype dominated the Eastern region, the other genotype was common in the Western region while the Central region haboured both genotypes almost in equal proportions. According to Raji et al. (2009) and Turyagyenda et al. (2012), admixture of crop genetic resources may be explained by the social structure and interaction of communities. Therefore, with increased breakage of cultural barriers, increasing human interaction, travel and demand for jackfruit as fruit in Uganda, the admixture observed may be accounted for as the jackfruit genetic material crosses cultural and geographical barriers for commercial purposes. Since jackfruit is mainly dispersed by humans, genetic similarities among samples may be indicative of human interactions and can be used to trace the transition of jackfruit genotypes in the country. As revealed by PCoA, STRUCTURE analysis and pairwise F_{ST} values of political regions, the transitory route of jackfruit in Uganda has its center in the Central region of the country. The Central region harbors almost all if not all ethnic groups of Uganda and this increases inter-ethnic contacts in this region hence may be responsible for increased exchange of germplasm and the high genetic diversity observed. Genetic diversity differs between the Eastern and Western regions, with moderate genetic differentiation (F_{ST} = 0.053) between the two regions as revealed by cassava (Turyagyenda et al. 2012) and this may point to reduced inter-ethnic contacts and different cultural norms.

The out-groups from Vietnam and Philippines clustered with samples in Cluster 2 from Uganda suggesting Asia as a possible origin or close genetic relationship of one of the jackfruit genetic clusters.

The low genetic differentiation observed among varietal groups supports earlier findings by ChunHai et al. (2009), Ying-Zhi et al. (2010) and Wang (2011) where soft and firm varieties of jackfruit also only registered minor differences by using ISSR, AFLP and SSR markers respectively. Thus, changes in fruit structure may only be due to very few genetic characters, not detected by the here used molecular tools and these characters are not manifested in the total genome. Although, pollination in jackfruit is not clearly understood, other possible explanations for these findings are common hybridization events of jackfruit varieties through which the varieties might have lost their originality making it challenging to clearly discern them at molecular level.

Low genetic differentiation was observed among agro-ecological zones and this might be indicative of the broad ecological tolerance of the crop to a wide variety of conditions (temperature, precipitation, elevation) which may account for its wide cultivation and usage. This may also explain the weak correlation between genetic and geographic distance, which might reflect human mediated seed transport across the country rather than evolutionary forces that explain the genetic differentiation observed among agro-ecological zones. A similar trend was reported by Nyongesa et al. (2012), where human mediated seed transportation was responsible for weak correlations observed between sesame genetic and geographic distance. Therefore, conservation efforts of jackfruit germplasm should be based on human interactions with the crop.

In this study, a small number of molecular markers (10 SSR markers) was used, however, due to their multi-allelic nature, codominance, high polymorphism, high reproducibility and good coverage of the genome (Kawuki et al. 2009; Sree Lekha et al. 2010; Turyagyenda et al. 2012), 10 SSR pairs are sufficient

to trace movement of alleles between populations (Zawedde et al. 2014) and distinguish distinct individuals within a population (Yada et al. 2010; Zawedde et al. 2014). Moyib et al. (2007) reported comparable findings using 5 and 16 polymorphic SSR markers in cassava cultivars and landraces showing that utilization of a few polymorphic SSR markers is plausible for genetic variation studies. Therefore, since the SSR markers used in this study were highly polymorphic (PIC = 0.73, Table 4), the 10 SSR markers employed in this study were sufficient to reveal the genetic diversity of jackfruit in Uganda. This being the first study in the country to characterize the genetic diversity of jackfruit, more studies employing more molecular markers are recommended.

Conclusion

Overall, jackfruits grown in Uganda are genetically diverse and reflect two main genetic clusters with several intermediates. The study revealed high genetic diversity among districts with recent adoption of jackfruit cultivation e.g. Mbarara. From a conservation point of view the extremes of the genetic diversity related to fruit morphological features and genetic structures should be safeguarded for long term crop improvement. This is especially important for the soft and white varieties with their unique alleles which may not only be decisive for fruit flesh color and texture but might confer the crop some additional advantages which are not investigated yet. Weak correlations between environmental variables (temperature, precipitation, elevation) and genetic data indicate that jackfruit is widely adapted to grow in different conditions of Uganda and if given attention, *A. heterophyllus* can ensure food security. The study also revealed a genetic differentiation gradient between samples collected from the East and the West, pointing to reduced germplasm exchange between the two regions. Lastly, though the study cannot conclude that some jackfruits in Uganda are of Asian origin, the results provide evidence about evolutionary relationships of one cluster to the Asian individuals which served as out-groups.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interests.

Ethical approval The study went through ethical clearance procedures. It was reviewed by both the Institutional Review Board of Mbarara University of Science and Technology and Uganda National Council for Science and Technology. The jackfruit leaf samples were obtained after ascertaining farmers' consent.

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