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Genetic diversity and relationships among populations of jackfruit, an underutilized nutrient-rich climate-smart fruit tree crop in Kenya and Uganda

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ABSTRACT

Jackfruit (*Artocarpus heterophyllus* Lam.) is an underutilized fruit tree crop in East Africa. Despite its importance for food and nutritional security, only limited information exists on the extent of genetic variability among Ugandan and Kenyan jackfruit populations. This study was aimed at analyzing the extent of genetic diversity in jackfruit germplasm from selected regions of Kenya and Uganda. A total of 30 mature fruit samples were collected randomly from three districts (Kampala, Wakiso and Mbale) in Uganda and five counties (Siaya, Kwale, Mombasa, Busia, and Murang'a) in Kenya. Genetic characterization was using six simple sequence repeat (SSR) and nine sequence-related amplified polymorphism (SRAP) markers. The results revealed that 62.50% of the loci for SSR and 82.14% for SRAP markers were polymorphic. The average polymorphism information contentwas 0.48 for SSR and 0.56 for SRAP markers. The Jaccard's similarity coefficient ranged from 0.55 to 1.0 for SSR and from 0.33 to 0.93 for SRAP markers, indicating considerable genetic diversity among jackfruit germplasm. Based on combined SSR and SRAP data, analysis of molecular variance revealed greater genetic diversity within the populations (76%) than among the eight populations (24%). Dendrogram and principal coordinate analysis separated the germplasm into two clusters, with several intermediates. The germplasm distribution among the clusters and sub-clusters was not associated with the geographical region. The significant genetic variability existing in Ugandan and Kenyan jackfruit germplasm can be used to improve jackfruit through breeding. The substantial genetic diversity should enable jackfruit breeders to develop high-yielding varieties with improved quality traits.

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Supplemental data for this article can be accessed here.

Introduction

Jackfruit (*Artocarpus heterophyllus* Lam.) is a highly nutritious tree crop that has the potential to alleviate food and nutritional insecurity, especially in developing countries (Williams and Haq 2002; Khan et al. 2010; Jagtap and Bapat 2010; Swami et al. 2012). It thrives well under marginal environmental conditions, such as poor soil fertility and erratic rainfall conditions. Thus, it is an essential crop during famine and seasonal food scarcity periods. However, the fruit tree is currently underutilized as a source of food and livelihoods to populations in eastern Africa (Balamaze, Muyonga, and Byaruhanga 2019).

The population of the continent of Africa is projected to be 2.49 billion in 2050, which will be about 25% of the world's population in 2050 (World Population Prospects 2019). The food demand, on the other hand, is expected to rise by 70% to meet the nutritional needs of this population. The current world's population is over-dependent on a few crops, namely rice (*Oryza sativa*), maize (*Zea mays*), wheat (*Triticum aestivum*) and potato (*Solanum tuberosum*), for daily calorie needs and these crops contain only a portion of the nutrients and lack other essential nutrients and micronutrients (FAO, 2017). Hence, there is a need to find alternative sources of nutrition, such as the underutilized crops like jackfruit. For genetic improvement of this fruit crop, the extent of its genetic diversity is an essential prerequisite. The knowledge of the genetic diversity can facilitate the preservation and propagation of superior germplasm that are at risk of extinction (Govindaraj et al. 2015).

There is a need to tap into the valuable jackfruit germplasm for breeding programs and to improve food and nutrition security in eastern Africa. Availability of well-characterized, genetically diverse jackfruit germplasm would facilitate targeted improvement of the crop. Jackfruit accessions have been characterized on the basis of morphological characteristics (Tulyathan et al. 2002; Odoemelam 2005). The diversity generated based on morphological characterization is not highly useful because it is based on very few distinctive features, which are affected by environmental conditions (Shyamalamma et al. 2008). Accurate evaluation and identification of jackfruit germplasm based on molecular markers to complement the use of morphological traits is of great importance. During the past two decades, there have been reports from different countries on the genetic variation in *A. heterophyllus* germplasm based on molecular markers. For example, investigators have used amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD) and simple sequence repeat (SSR) markers for characterizing jackfruit collections from the USA, India, Bangladesh, China and Uganda (Schnell et al. 2001; Shyamalamma et al. 2008; Wang et al. 2011; Gopalsamy et al. 2012; Nakintu et al., 2020). These

studies revealed two genetic clusters of jackfruit. Other studies observed four genetic clusters for jackfruit diversity in Bangladesh using isozymes and SSR (Azad, Jones, and Haq 2007; Witherup 2012) and in India using RAPD markers (Krishnan et al. 2015).

In Kenya and Uganda, numerous *A. heterophyllus* cultivars have been characterized based on nutritional content and phytochemical characteristics (Ojwang et al. 2017; OJwang et al., 2018). However, limited genetic information is available on *A. heterophyllus*, which could underpin future breeding programs and genetic studies. Simple sequence repeats (SSRs) and sequence-related amplified polymorphism (SRAP) markers have been used to characterize fruit trees in other regions (Chladova et al. 2019). There are several reasons for the preference of SSR markers in plant genotyping. They are generally multiallelic, codominant, transferable, highly reproducible, and have extensive genome coverage (Kumar et al. 2013). They have been extensively used for genetic characterization of mango (*Mangifera indica*) in India (Kumar et al. 2013), watermelon (*Citrullus lanatus*) in South Korea (Hwang et al. 2011), baobab (*Adansonia digitata*) in Sudan and Kenya (Wiehle et al. 2014; Chládová et al. 2019), mulberry (*Morus alba*) in India (Mathithumilan et al. 2013) and jackfruit (*A. heterophyllus*) in Uganda (Nakintu et al. 2019). The SRAP markers are important molecular markers for high-throughput diversity analysis, because they are codominant and reliable. They amplify the open reading frames (ORFs) in a genome (Li et al. 2001) and have been used successfully in the genetic characterization of several crop plants, including *Brassica oleracea* in the USA (Li and Quiros 2001), sugarcane (*Saccharum officinarum*) in India (Mirajkar et al. 2017), soybean (*Glycine max*) in southern China (Sun et al. 2013) and cucurbits (*Cucurbita pepo*) in Spain (Ferriol, Pico, and Nuez 2003).

In the study of most plant germplasm resources, SSR or SRAP markers are generally used separately. In the case of *A. heterophyllus*, no reports are available on the genetic variability of accessions from Kenya and only one study has been conducted to determine the genetic diversity of accessions from Uganda using SSR markers (Nakintu et al. 2019). The SRAP markers have not been applied in the analysis of genetic diversity of *A. heterophyllus* from Kenya and Uganda. There is no history of jackfruit breeding programs in Kenya and Uganda and the national crop improvement programs in both countries have depended on introductions of jackfruit as the crop is not indigenous (Dutton 1976). The knowledge of genetic diversity in jackfruit germplasm collections can significantly ease the reliable categorization of genotypes and identification of the ones with potential utility in different breeding programs. Therefore, the objective of this study was to determine the genetic diversity among *A. heterophyllus* germplasm from Kenya and Uganda using SSR and SRAP markers. The information obtained should be useful for efficient utilization of germplasm and conservation of

A. heterophyllus genetic resources for sustainable development of cultivars and initiation of breeding programs in Kenya and Uganda.

Materials and methods

Plant material and sampling sites

Fruit samples of jackfruit (Figure 1(a)) were obtained from selected counties and districts in Kenya and Uganda, respectively (Table 1). In Kenya, the fruits were sampled from Kwale (Lunga, Kivulini, Kikoneni, and Msambweni constituencies), Mombasa (Kisauni and Ukunda), Muranga, Busia, and Siaya (Ugenya constituency) counties. In Uganda, mature fruits (Figure 1(b)) were sampled from Kampala, Wakiso, and Mbale districts. The fruits were obtained from farms/homesteads that were 5 km apart. A total of 30 fruits were collected, of which 9 were from Uganda and 21 from Kenya. The Kenyan regions were selected on the basis of availability of the trees and

Figure 1. (a) Jackfruit tree from Kwale County, Kenya, in 2017 during the collection of fruit samples. (b) Fruits samples collected from Kwale County, Kenya. (c) Fleshy pulp region and the seeds obtained from the fruits. (Photos were taken by Redemtor Ojwang).

Table 1. The latitudes and longitudes of the different geographical locations and the number of fruits sampled.

Country	County/ District	Latitude	Longitude	Number of trees sampled per district/ Sub-counties	Annual Temperature	Annual Precipitation	Altitude
Kenya	Siaya	0° 03′ 40.18″ N	34° 17′ 17.63″ E	4 (Ugenya)	21.4°C	2155 mm	1,400 m
Kenya	Kwale	4° 10′ 25.50″ S	39° 27′ 7.42″ E	1 (Kikoneni) 2 (Lunga Lunga), 1 (Msambweni), 1 (Kivulini)	25.4°C	1060 mm	403 m
Kenya	Mombasa	4° 2′ 12.7608″ S	39° 40′ 10.4556″ E	2 (Kisauni), 3 (Ukunda)	26.1°C	997 mm	50 m
Kenya	Busia	0° 27′ 36.18″ N	34° 06′ 42.08″ E	4 (Samia)	21.8°C	2291 mm	1,227 m
Kenya	Murang'a	0° 43′ 0.01″ N	37° 06′ 60.00″ E	3 (Murang'a town)	19.7°C	996 mm	1255 m
Uganda	Kampala	0° 20′ 51.3456″ N	32° 34′ 57.0720″ E	3 (Kampala)	21.4°C	1747 mm	1,200 m
Uganda	Wakiso	0° 24′ 15.98″ N	32° 27′ 33.98″ E	3 (Wakiso)	20.8°C	1834 mm	1,200 m
Uganda	Mbale	1° 04′ 55.52″ N	34° 10′ 30.11″ E	3 (Mbale)	20.3°C	7139 mm	1,156 m

mature fruit, since they are sparsely populated. Seeds (Figure 1(c)) were extracted from the fruits, soaked overnight in distilled water, and germinated in a glasshouse in plastic pots containing sterile soil. Young leaves from four-week-old seedlings were used for genomic DNA extraction.

Genomic DNA

Genomic DNA was extracted from young leaves of four-week-old jackfruit seedlings using the cetyltrimethylammonium bromide (CTAB) protocol described by Dellaporta, Wood, and Ticks (1983) and Doyle and Doyle (1990) with some modifications. The modifications included the exclusion of liquid nitrogen and β-mercaptoethanol in the extraction procedure. The quality and integrity of the extracted DNA was determined by electrophoresis on 0.8% agarose gel stained with ethidium bromide and viewed under a UV-transilluminator. The concentration of genomic DNA was determined by the use of a spectrophotometer (UV-visible, Elico Spectrophotometer, India).

SSR and SRAP markers analysis

Polymerase chain reaction (PCR) amplification was carried out using SSR and SRAP markers. The sequences of the SSR and SRAP primers used in this study were obtained from Liu et al. (2016) and Li and Quiros (2001),

respectively. There was an initial screening of 20 SSR primer pairs and 16 SRAP primer combinations. Six SSR primer pairs and nine SRAP primer combinations (Table 1), which showed clear and distinct bands, were used for genetic analysis of DNA extracted from jackfruit accessions. The PCRs were performed using a thermocycler (Veriti Applied Biosystems, Waltham, MA, USA). Each reaction consisted of 5× PCR buffer (4 µl), $MgCl_2$ (1 µl), 10 mM dNTPs (0.5 µl), forward and reverse primer (1 µl of each), Taq DNA polymerase (0.2 µl) (Biolabs, Hitchin, England), DNA (2 µl of 10 ng) and sterile water added to a final reaction volume of 20 µl. The SSR PCR profile was an initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for 1 min, primer annealing at 46–50°C depending on the primer pair used for 45 sec (Table 2), extension at 72°C for 1 min and a final extension at 72°C for 7 min. The PCR profile for SRAP markers, on the other hand, was as follows: An initial denaturation at 94°C for 5 min, followed by five cycles of denaturation at 94°C for 1 min, primer annealing at 35°C for 45 sec and an extension at 72°C for 1 min. The other 30 cycles were run at an annealing temperature of 50 °C using the same conditions and a final extension at 72°C for 5 min. The PCR products were separated

Table 2. List of simple sequence repeat (SSR) primer pairs and sequence-related amplified polymorphism (SRAP) primer combinations used and their optimized annealing temperatures.

Marker	Primer code	Forward sequence (5′ – 3′)	Reverse sequence (5′ – 3′)	Reference	Ta (°C)
SSR	AH14	GCTTGTGGGTTCTGGGATCTAT	CAGACACTAGTTTGGATGTACT	Liu et al. (2016)	50
	AH46	GGAGAGGGCGGTGCAGTAGAA	GCAGAGCAGACACTACAGTAGC	Liu et al. (2016)	50
	AH59	TCTCCTCCACCTCCTCCATTGT	GACCTTGGGACCCGCACTTCTT	Liu et al. (2016)	48
	AH76	GAACGGCAGATTTCACCATTTT	AGGATCAACTTAGCCCACTATA	Liu et al. (2016)	48
	†AH31	TCCTCTAACGTGCGCCCCTAAG	AAACCCAGCGTGCCACCATTG	Liu et al. (2016)	46
	†AH77	CGAGAAGGTTCCGAGCCAGATT	CCCGACCAAGACCCGGAGTATA	Liu et al. (2016)	46
SRAP	ME5-EM7	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCAA	Li and Quiros (2001)	35, 50
	ME5-EM9	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCGA	Li and Quiros (2001)	35, 50
	ME5–EM10	TGAGTCCAAACCGGAAG	GACTGCGTACGAATTCAG	Li and Quiros (2001)	35, 50
	†ME8-EM 7	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTCAA	Li and Quiros (2001)	35, 50
	ME8-EM9	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTCGA	Li and Quiros (2001)	35, 50
	ME8-EM10	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTCAG	Li and Quiros (2001)	35, 50
	†ME11-EM7	TGAGTCCAAACCGGAAC	GACTGCGTACGAATTCAA	Li and Quiros (2001)	35, 50
	ME11-EM9	TGAGTCCAAACCGGAAC	GACTGCGTACGAATTCGA	Li and Quiros (2001)	35, 50
	ME11-EM10	TGAGTCCAAACCGGAAC	GACTGCGTACGAATTCAG	Li and Quiros (2001)	35, 50

†indicates SSR and SRAP primers that were not used in the final analysis because they amplified only a single monomorphic band in all the samples

through electrophoresis in ethidium bromide-stained agarose gel (1.5%) and viewed in a gel doc XR (Bio-Rad, Hercules, CA, USA).

Data analysis

Only the bands that were clear, distinct, and reproducible from SSR and SRAP-PCR amplifications were scored. The presence of a band was assigned 1 and absence 0. Nei's gene diversity (H), polymorphic information content (PIC), Simpson's diversity index, and heterozygosity for each SSR and SRAP marker were determined using the PowerMarker V3.25 software. The DendroUPGMA server (Garcia-Vallvé et al. 1999) was used to calculate matrix distances (Jaccard's similarity and dissimilarity coefficients). The dendrograms were constructed using the FigTree software (Version 1.4.2). The correlation of genetic diversity parameters between SSR and SRAP markers was determined via SPSS version 19. Principal coordinate analysis (PCoA) was performed using the GenAlex 6.5 software (Peakall and Smouse 2006). This software was also used to calculate the among population genetic differentiation coefficient (F_{ST}). Mantel's test with 1000 permutations in GenAlex 6.5 software was used to determine the correlation between geographic and genetic distance as well as the correlation between genetic distance and climatic factors (Mantel 1967; Peakall and Smouse 2006). The corresponding data (altitude, precipitation and temperature) were acquired from Climate-Data.org website and UTM Geo Map Application (http://www.yogantara.com/).

Results and discussion

SSR-marker polymorphism and genetic diversity

The SSR primers used amplified a total of 234 bands, 62.50% of which were polymorphic across the evaluated jackfruit accessions. The Nei's gene diversity values ranged from 0.48 to 0.63, and marker AH76 had the highest Nei's gene diversity of 0.63, followed by marker AH 46 (0.59). The PIC values were relatively high for the SSR markers used in the study, ranging from 0.37 to 0.56, with a mean of 0.48, which indicated their informativeness and reliable discriminatory power based on the description by Botstein et al. (1980). The PIC values indicated that there was moderate genetic diversity as well as variation in the allelic frequencies of the jackfruit accessions at the SSR loci (Pervaiz et al. 2009; Vu et al. 2016). The high observed heterozygosity (Ho) (0.42) and expected heterozygosity (He) (0.48) (Table 3) were indicative of the moderate level of genetic diversity in jackfruit from Uganda and Kenya. This showed that the jackfruit accessions had distinct heterotic groups desirable for initiation of breeding.

Table 3. Diversity characteristics of simple sequence repeat (SSR) loci used in the characterization of jackfruit samples.

SRAP loci	Total number of bands	Number of polymorphic bands	Polymorphism (%)	Genetic parameter†				
				Ho	He	H	I	PIC
AH14	2	1	50	0.43	0.49	0.49	0.43	0.37
AH 76	3	2	66.67	0.50	0.50	0.63	0.52	0.57
AH 59	3	2	66.67	0.30	0.42	0.48	0.46	0.45
AH 46	3	2	66.67	0.43	0.49	0.59	0.49	0.53
Mean			62.5	0.42	0.48	0.55	0.48	0.48

†Ho = Observed heterozygosity, He = expected heterozygosity estimated with computer program GenAlex 6.503 (Peakall and Smouse, 2012), H = Nei's gene diversity, I = Simpson's diversity index, and PIC = polymorphic information content.

Grouping of genotypes on the basis of their genetic similarity aids in the identification and selection of diversified parents for specific breeding programs. The evaluated samples had high genetic similarity coefficients that ranged from 0.55 to 1.0, suggesting that high genetic similarity was present in the repeat sequence regions. Jackfruit samples from a collection site showed higher genetic similarity values compared with those from different sites. This indicated that jackfruit accessions from different collection sites (regions) were genetically diverse for hybridization to exploit heterosis to develop adaptable and productive hybrid varieties for each region. The samples from Busia County in Kenya showed the highest genetic similarity (coefficient of 1.0) with those from Mbale and Wakiso districts in Uganda. The high genetic similarity among the jackfruit accessions in Busia (in Kenya) and Mbale (in Uganda) is attributed to the sharing of seeds between farmers from the two regions and selling of jackfruit produce from one region to another, where the seeds could then be planted. This emphasizes the need to explore and exploit additional sources to study genetic variation for jackfruit genetic improvement.

The dendrogram constructed among the 30 jackfruit samples based on binary data revealed three major clusters (Clusters I, II, and III) (Figure 2), indicative of genetic variation among the assessed jackfruit accessions. Majority of jackfruit samples from the same geographical region grouped into different clusters (Figure 2). The clusters II and III were complex, containing 50% and 43% of the samples, respectively, and were further subdivided into sub-clusters. The ability to delineate the accessions into clusters and further sub-clusters is a significant step toward jackfruit improvement, as these accessions can form part of germplasm collection for use in initiating breeding programs. The jackfruit accessions in different clusters and/or sub-clusters can be utilized as suitable material to initiate breeding for favorable agronomic characters, such as abiotic stress-tolerance traits and high fruit yields. The clustering provides required guidance for selection of parents to initiate breeding in jackfruit. None of the clusters or sub-clusters contained *A. heterophyllus* accessions from the same geographical origins, i.e., Uganda

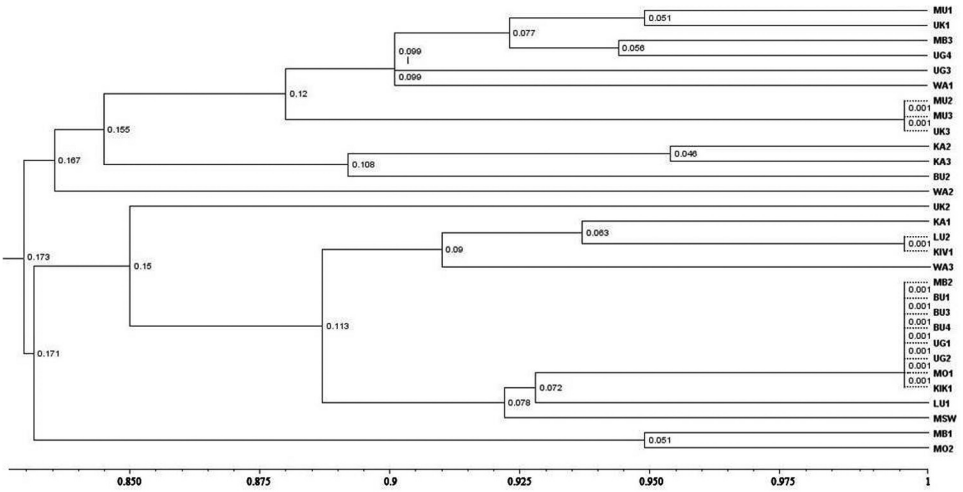

Figure 2. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram indicating the genetic relatedness of the samples using SSR markers. Abbreviations indicate the geographical origin of each sample; Mu – Murang'a, Ug – Ugenya, Mb – Mbale, Wa – Wakiso, Lu – Lunga Lunga, Bu – Busia, Mo – Mombasa, Kik – Kikoneni, Msw – Msambweni, Kiv – Kivulini and Uk – Ukunda.

and Kenya. These observations could be attributed to the presence of high genetic diversity within varieties of the same geographic origins. This could facilitate easy identification of divergent parents for breeding to develop hybrid varieties for each region.

SRAP-marker polymorphism and genetic diversity

The SRAP markers generated 82.14% polymorphic bands, with Nei's gene diversity values ranging from 0.39 to 0.81, with a mean of 0.61, indicating a moderate level of genetic diversity, similar to what Govindaraj, Vetriventhan, and Srinivasan (2015) reported. The findings were also consistent with reports by Shyamalamma et al. (2008) using AFLP markers and Gopalsamy et al. (2012) with RAPD markers, which found that jackfruit from selected regions in India, had moderate genetic diversity based on Nei's gene diversity. The results showed that the germplasm investigated in this study exhibited adequate genetic variation that can be exploited for jackfruit improvement. The variation is important for breeding as it avails accessions with diverse response to abiotic and biotic stresses and some of the accessions could harbor resistance genes. The high gene diversity also indicates that the SRAP markers used were highly polymorphic. The PIC values for the SRAP markers ranged from 0.36 to 0.78, with a mean of 0.55 (Table 4), which indicated that the *A. heterophyllus* accessions used in the study were diverse. It also showed that the markers were efficient in discriminating the

Table 4. Genetic diversity parameters of sequence-related amplified polymorphism (SRAP) markers.

SRAP loci	Total number of bands	Number of polymorphic bands	Polymorphism (%)	Genetic parameter†				
				Ho	He	H	I	PIC
ME5-EM10	4	4	100.00	0.77	0.36	0.81	0.87	0.78
ME5-EM9	3	2	66.67	0.37	0.46	0.54	0.48	0.50
ME5-EM7	3	2	66.67	0.40	0.48	0.48	0.65	0.37
ME8-EM9	3	2	66.67	0.23	0.36	0.39	0.42	0.38
ME8-EM 10	3	3	100.00	0.43	0.49	0.62	0.58	0.59
ME 11-EM9	4	3	75.00	0.57	0.49	0.71	0.66	0.68
ME11-EM 10	3	3	100.00	0.53	0.50	0.68	0.63	0.63
Mean			82.14	0.47	0.49	0.61	0.61	0.56

†Ho = Observed heterozygosity, He = expected heterozygosity estimated with computer program GenAlex 6.503 (Peakall and Smouse, 2012), H = Nei's gene diversity, I = Simpson's diversity index, and PIC = polymorphic information content

accessions. The results indicated that SRAP could be efficiently applied in the analysis of genetic diversity and could identify the *A. heterophyllus* accessions for breeding.

Genetic similarity coefficients among the samples ranged from 0.33 to 0.93. The basis of the highest genetic similarity coefficients between the samples was not related to the geographical region of the fruit samples. The high genetic similarity coefficient (0.93) of jackfruit accessions collected from Busia in Western Kenya and Lunga Lunga in Coastal Kenya tended to be closely related, indicating a common genetic basis. Therefore, a wider range of jackfruit accessions should be introduced in these regions to diversify the current jackfruit germplasm for future breeding programs. The jackfruit accessions from Wakiso in central Uganda and Ukunda (from Kwale County Kenya) in Coastal Kenya had the lowest genetic similarity coefficient (0.33), suggesting the accessions were genetically distinct from one another, which could be used as parental lines to initiate breeding programs. Therefore, a wider range of accessions should be introduced to broaden the genetic base of the germplasm from Wakiso (in central Uganda) and Ukunda (from Kwale County Kenya) to strengthen breeding programs.

Cluster analysis based on the SRAP binary data separated the 30 jackfruit samples into two major clusters (Cluster I and II) at a similarity level of 77% (Figure 3), with the potential to contribute new beneficial alleles to jackfruit breeding programs. One sample (Ukunda2) collected from the Mombasa County in Kenya was an outgroup. Cluster I was more complex than cluster II since it consisted of 21 samples, which was further separated into two sub-clusters. Each cluster and sub-clusters contained jackfruit samples from different geographical regions, showing no location specificity among the jackfruit accessions. This indicated that the accessions were genetically

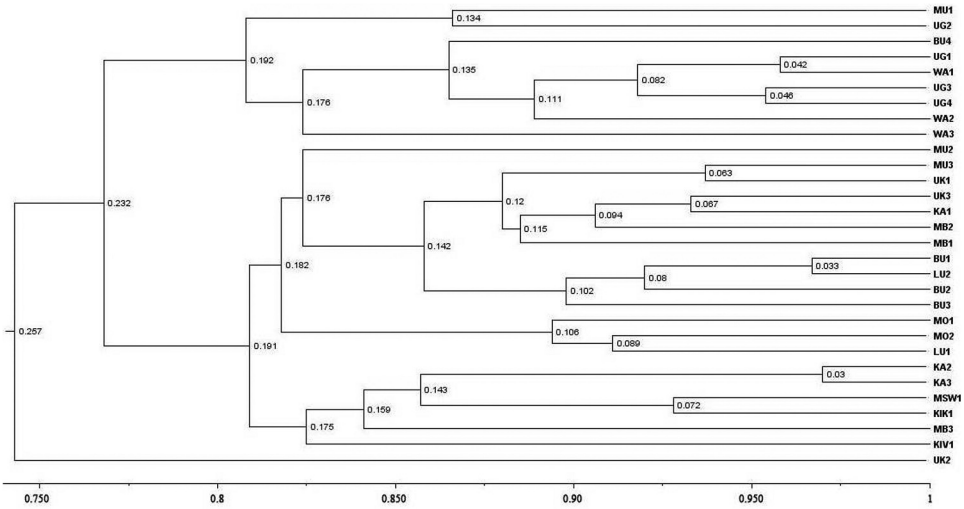

Figure 3. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of genetic relationships of jackfruits from different regions using SRAP markers. Abbreviations indicate the geographical origin of each sample; Mu – Murang'a, Ug – Ugenya, Mb – Mbale, Wa – Wakiso, Lu – Lunga Lunga, Bu – Busia, Mo – Mombasa, Kik – Kikoneni, Msw – Msambweni, Kiv – Kivulini and Uk – Ukunda.

distinct from one another, and could be used as complementary parents for crossing to obtain a heterotic response. Since the accessions in the same cluster had more genetic similarity than those from different clusters, the crossings should involve jackfruit accessions from different clusters.

Analysis of molecular variance

The analysis of molecular variance (AMOVA) utilized a combination of SSR and SRAP data (Table 5). The AMOVA revealed that 76% and 24% of the total SSR and SRAP marker variation was attributable to within and among populations, respectively. The presence of high genetic variability within the population signifies the divergence of jackfruit accessions within a single population, indicating that any region holds a particular important genetic diversity. Since genetic diversity within populations is higher than among populations, all populations can serve as a potential source of desirable characters for improvement programs. The pairwise genetic differentiation coefficient (F_{ST}) values indicated that variation existed among the populations, with the positive values ranging from 0.0212 to 0.2642 (Supplementary Table S1). The F_{ST} values showed that variation among populations from Wakiso and Mombasa, Kampala and Siaya, Mbale and Siaya, Busia and Siaya was significant at $p = 0.05$ (Supplementary Table S2). Pairwise F_{ST} is a good indicator of the degree of divergence among populations and is useful for parent selection; for example, parents could be chosen from divergent

Table 5. Analysis of molecular variance (AMOVA) for 30 jackfruit accessions in eight populations based on Simple sequence repeats (SSR) and sequence-related amplified polymorphism (SRAP) data.

Source of variance	Degrees of freedom	Sum of squared deviation	Mean squared deviation	Estimated variance	Percentage (%) of variation	P-value
Among Populations	7	58.300	8.329	1.225	24%	0.001*
Within Populations	22	85.167	3.871	3.871	76%	
Total	29	143.467		5.096	100%	

*Significant at $p \leq 0.001$

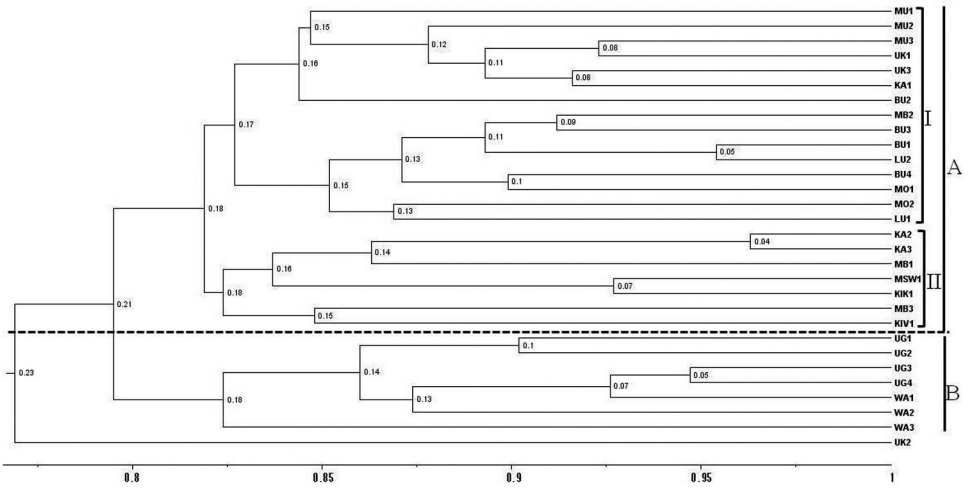

Figure 4. Unweighted pair group method with arithmetic mean (UPGMA) dendrogram of genetic relationships of jackfruits from different regions using combined SSR and SRAP genetic similarities. Abbreviations indicate the geographical origin of each sample; Mu – Murang'a, Ug – Ugenya, Mb – Mbale, Wa – Wakiso, Lu – Lunga Lunga, Bu – Busia, Mo – Mombasa, Kik – Kikoneni, Msw – Msambweni, Kiv – Kivulini and Uk – Ukunda.

population pairs, such as Kampala and Siaya, Kampala and Wakiso as pairwise F_{ST} between them was high ($F_{ST} > 0.20$). Moreover, the p values (at $p = 0.05$) of the Mantel test indicated that there was no significant correlation between genetic divergence and geographical distances among the different populations (Supplementary Table S3), suggesting that genetic differentiation in jackfruit does not follow a pattern of isolation by distance. The mean annual precipitation ($p = 0.007$) was correlated with genetic distance, whereas mean annual temperature was not ($p = 0.171$) (Supplementary Table S3). However, a partial Mantel test accounting for mean annual precipitation, used to estimate genetic distance, was not independently correlated with geographical distance ($p = 0.147$).

Cluster analysis of combined SSR and SRAP data

The cluster analysis of the 30-jackfruit germplasm based on combined SSR and SRAP data separated the germplasm into two clusters (A and B) (Figure 4). Cluster A was further subdivided into two sub-clusters, I and II (Figure 4). Sub-cluster I contained jackfruit samples from Murang'a, Kwale (Lunga Lunga), Ukunda, Mombasa, Busia, and Kampala, whereas sub-cluster II contained samples from Kwale (Kikokeni and Kivulini), Kampala and Mbale. Cluster B included germplasm from Siaya County (Ugunja, Kenya) and Wakiso district (Uganda). The presence of two clusters probably indicates that two distinct genotypic groups of jackfruit were introduced into

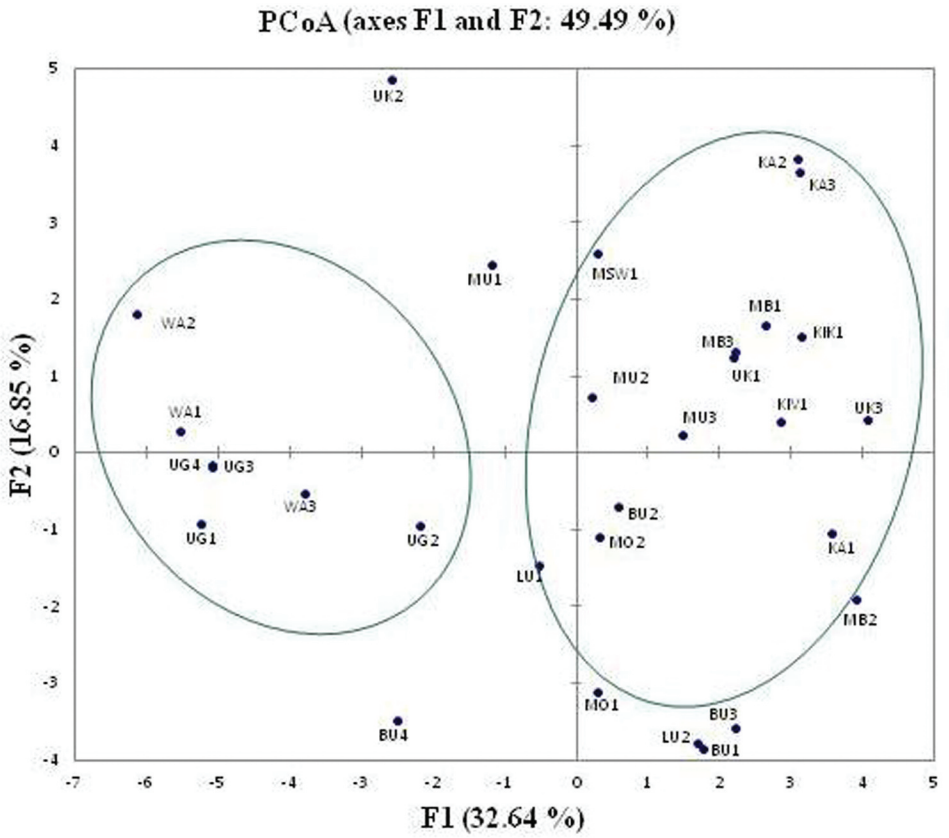

Figure 5. Principal co-ordinate analysis (PCoA) analysis based on combined SSR and SRAP markers. Abbreviations indicate the geographical origin of each sample; Mu – Murang'a, Ug – Ugenya, Mb – Mbale, Wa – Wakiso, Lu – Lunga Lunga, Bu – Busia, Mo – Mombasa, Kik – Kikoneni, Msw – Msambweni, Kiv – Kivulini and Uk – Ukunda.

Kenya and Uganda, since no jackfruit breeding has been carried out in the two countries. No clear association existed between the geographical origins of the accessions in the clustering pattern. This could be explained by the presence of extensive planting material (seeds) exchange, high level of gene flow and common ancestry of the populations. Plant material exchange from place-to-place leads to an increase in gene flow and distribution of alleles among different populations, regardless of their geographic distance. Although not all samples from the same region grouped in the same cluster or sub-cluster, the grouping of some samples was closely related to their region of origin. This suggests that genetic homogeneity and differentiation existed within and between the regions. Therefore, to develop breeding populations possessing desirable and superior agronomic traits, especially abiotic stress tolerance and high quality and quantity of fruits in jackfruit, crosses could be made between distantly related and complementary

accessions selected from different clusters and sub-clusters. The genetic homogeneity among the jackfruit accessions observed between regions concurs with earlier reports by ChunHai et al. (2009) on the genetic characterization of jackfruit from China using ISSR markers. However, our study contradicts the reports by Shyamalamma et al. (2008) in India, where jackfruit germplasm growing in regions with medium/heavy precipitation and in dry conditions grouped in different clusters and a good correlation between genetic and geographical regions was observed. Ours results indicate that the jackfruit accessions from Kenya and Uganda were adapted to a wide range of environmental conditions in the different districts and counties where samples came from. Therefore, a key indicator of genetic diversity is not necessarily the geographical origin of germplasm collections.

Principal coordinate analysis (PCoA) of combined SSR and SRAP markers

Principal coordinate analysis, based on combined SSR and SRAP data, grouped the thirty jackfruit accessions into two main clusters; the first cluster included accessions from all regions, except Ugenya and the second cluster included accessions only from Wakiso and Ugenya; and the remainder seven accessions were scattered across these clusters (Figure 5). These findings indicated that accessions of each cluster shared the same ancestor, whereas each of these intermediate accessions had a distinct origin. These results indicated that the geographical distance did not affect the genetic differentiation of the jackfruit samples. This indicated that the intermediate accessions were genetically distinct from one another, which could offer elite materials for breeding programs.

Conclusions

This study presents the first report on the genetic diversity of jackfruit populations collected from Kenya. The results of the genetic diversity parameters indicated that SSR and SRAP markers were efficient and useful in the genetic characterization of jackfruit samples. The study revealed a considerable genetic diversity among jackfruit germplasm in Uganda and Kenya. The results of genetic diversity and genetic distance are useful for parental selection to improve the breeding value of jackfruit accessions so that crosses between accessions that are farther apart can produce hybrids that are likely to have higher genetic potential than their parents. The present study grouped the accessions into two main genetic clusters with many intermediates and these two genetic clusters would be managed as two evolutionary units; hence, the selection of parents must be based on the wider intercluster distance. The UPGMA dendrogram and PCoA showed that there was no correlation between clusters and the geographical origin of the jackfruit samples. However, the majority of jackfruit samples

from the same geographic region were found to have a higher genetic similarity compared with samples from different regions. Therefore, crosses between the jackfruit accessions should be made from different geographical regions. These findings provide crucial information about jackfruit accessions in Kenya and Uganda, which is essential for the conservation of genetic resources and the initiation of germplasm improvement programs.

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Disclosure statement

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Authors' contributions

EKM, ENN, BNM, RAO and DOO conceived and designed the experiments. RAO performed all the experiments. EKM, ENN, BNM and DOO supervised the execution of the research. RAO analyzed the data with guidance from EKM and ENN. RAO, EKM and ENN processed the data and wrote the manuscript. All the authors revised and approved the manuscript.

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