Genetic Diversity in Jojoba (*Simmondsia Chinensis* L.) using Simple Sequence Repeats in Semi-Arid Areas of Kenya

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Abstract
Jojoba is a dioecious desert shrub which produces oil of high quality and is used mainly in the cosmetic and lubrication industry. There is limited work on genetic characterization of Jojoba globally, and there is only one known Kenyan plantation which is not yet characterized. An experiment was setup with the aim of molecular characterization of Jojoba in semi-arid areas of Kenya using SSR markers. The experimental design was a Latin Square Design with 8 treatments replicated 8 times. The experiment consisted of 8 blocks where 8 Jojoba bushes per block were sampled. Stratified random sampling was used to select 8 bushes, which consisted of 4 males and 4 females based on height and crown diameter. Six leaves were harvested per bush. CTAB was used for DNA extraction whereas gel electrophoresis was performed together with agarose gel for visualization of DNA. Ten SSR primers were used to amplify sample target sequence using PCR. PCR products were determined by comparison with a DNA ladder ranging 100-1000 base pairs. Diversity data analysis was carried out using XLSTAT software while binary code was used to designate band presence or absence. PIC distinguished the different genotypes whereas DARwin5 software was used in construction of dendrogram. The results showed that the Jojoba bushes had low genetic diversity shown by PIC of 0.2583-0.3748. Due to the low genetic diversity, it is recommended that superior genotypes be imported to increase genetic diversity of Jojoba in semi-arid areas of Kenya.

Keywords: *Genetic diversity; Jojoba; SSR markers*

Introduction

Over 80% of Kenya is arid and semi-arid (ASAL) (KARI, 2009) with only a few crops being grown mainly for subsistence purposes. These areas lack proper cash crops which are drought tolerant while irrigation systems are poorly developed. They experience frequent drought leading to crop failure hence overdependence on food relief (Barrow, 1996).

In recent years, there has been considerable interest in using ASALs more productively by promoting crops which can tolerate these conditions such as *Jatropha curcas* (Ngethe, 2007) and Jojoba (*Simmondsia chinensis*) (Thagana *et al*., 2004). These are multipurpose crops, and have a potential use for rehabilitation as well as provision of income to the poor communities. However, the existing Jojoba plantations are less known hence neglected and poorly managed.

Jojoba is a high value shrub growing in ASALs (Hogan, 1979; Ahmad, 2001) and is a promising cash crop for the ASALs throughout the world. It is a native shrub of Sonoran desert of Arizona, Southern California and North Western Mexico. Jojoba is grown in many

other countries including Argentina, Brazil, India, Israel, Egypt, Saudi Arabia, Australia, South Africa, Peru, Chile and Iran (Muthana, 1981; Undersander *et al*., 1990).

It is dioecious with male and female plants in the ratio 1:1 in the field when raised from seed. A Jojoba stand can be in production for 100-200 years depending on management (Martin, 1983) and has a deep rooting habit (Forster and Wright, 2002). Jojoba produces nuts with 45-55% of its weight as oil. The oil from Jojoba is similar to that obtained from Sperm Whale which is threatened with extinction (Hogan and Bemis, 1983). It is used in cosmetics, lubricant industry, pharmaceuticals, electronics and computer industries ((Muthana, 1981; Undersander *et al*., 1990; Amarger and Mercier, 1996; Ward, 2003).

Dioecious plants have high genetic diversity within populations due to out-crossing (Hamrick and Godt, 1996). However, domestication and breeding has resulted to large scale cultivation of genetically uniform cultivars. This, in turn has led to an increasingly narrow genetic base for the crops, leading to genetic vulnerability (Rao and Hodgkin, 2002). Vegetative propagation of elite lines/clones is mainly used to establish large Jojoba plantations in order to overcome high male to female ratio and increase production.

There is limited work on genetic characterization of Jojoba using modern molecular marker techniques (Sharma *et al*., 2009a; Bhardwaj *et al*., 2010) and there is only one known Kenyan plantation which has not yet been reported to have been characterized and, therefore, forming the objective of the present study. DNA markers offer unlimited potential to uncover differences at the DNA level and hence an ideal tool to differentiate individuals and genotypes (Graner and Wenzel, 1992; Tonukari *et al*., 1997; Sivaprakash *et al*., 2004; Sharma *et al*., 2009a).

Genetic diversity determination is necessary in order to characterize Jojoba plantations using molecular markers for improved production. First generation of markers became available in the 1970s which included Random Filament Length Polymorphism (RFLP) which are relatively slow and cumbersome to use (Wambugu, 2001). Second generation came up in the 1990s where Polymerize Chain Reaction (PCR) technique gave rise to Random Amplified Polymorphic DNA (RAPDs), Amplified Filament Length Polymorphism (AFLP) and microsatellites (Simple Sequence Repeats - SSR). These are more powerful and accurate since they allow the precise identification of individual genes.

In the late 1990s, third generation of even more accurate markers called DNA expression arrays became available. These reveal whether or not a gene is expressed or switched on at a given moment in the development of an organism. More recent studies by Heikrujam *et al*. (2015) using CAAT box-derived polymorphism (CBDP) markers have been able to classify male and female Jojoba separately by use of dendrograms.

A number of molecular markers have been used on Jojoba mainly for sex determination in mature stands such as RAPDs (Agrawal *et al*., 2008; Mohasseb *et al*., 2009; Hosseini *et al*., 2011; Yadav *et al*., 2012), AFLP (Agarwal *et al*., 2011) and Inter-Simple Sequence Repeat (ISSR) (Sharma *et al*., 2008; Heikrujam *et al*., 2014). Genetic diversity and association mapping relies on the availability of high numbers of markers distributed throughout the entire genome.

Diversity studies have commonly been done with SSR markers which have a high Polymorphic Information Content (PIC) and several authors have used SSR markers to characterize various crops including cassava (Dominick, 2008) and sorghum (Sallu, 2008). Similarly, Zhou *et al*. (2012) and Katoch *et al*. (2013) used SSR for genetic diversity in *Saruma henryi* Oliv and *Picrorhiza kurrooa* respectively. Gene diversity was termed as the probability of two random chosen alleles being different from a population (Weir, 1996) while PIC was defined as the measure to calculate the discrimination power of markers (Botstein *et al*., 1980). SSR have a high mutational rate and new alleles can easily be created

during DNA replication process.

Microsatellites or SSR regions of DNA are composed of short (<6bp) sequences repeated in tandem (Gardner *et al*., 2011). They are usually co-dominant and a very high number of alleles can be detected at same loci (Echt *et al*., 1996). Since the assay is easy to perform and produces very polymorphic markers, microsatellites, are a highly attractive tool for genetic studies at population level (Morgante and Olieveri, 1993; Plaschke *et al*., 1995). The SSR markers are able to distinguish among closely related plant cultivars (Davila *et al*., 1998). More recent studies by Inoti *et al*. (2015) have reported that Jojoba seedlings can be characterized into males and females using morphological traits. Similarly, Heikrujam *et al*. (2014) validated the use of male sex specific ISSR marker, UBC-807on a Jojoba population comprising of 330 females and 255 males.

The study hypothesized that SSR markers can determine the amount of genetic variation in Jojoba bushes growing in semi-arid areas of Kenya. The objective of the study was to determine genetic diversity of Jojoba bushes using SSR molecular markers in semi-arid areas of Kenya. This will provide a data base for the amount of variation among the existing Jojoba bushes.

Materials and methods

Location and Site description

The research was conducted at Rukinga Wildlife Works Ltd, Maungu, Voi, where Jojoba bushes have been established. It is located 20 km east of Voi urban centre, Taita Taveta County, Voi District, and Coast Province of Kenya. It is located 140 km (near Buchuma) North West of Mombasa port along the Mombasa-Nairobi road highway, at an altitude of 892 m above the sea level. It lies between latitudes 3^0 23'60'' to 3^0 24' 26'' S and Longitudes 37^0 40' 60'' to 38^0 35' 25'' E.

The study site lies in the semi-arid savannah which receives an average annual rainfall of 458 mm with a bimodal pattern of distribution. Temperatures range from 16-37^0C with an average of 25^0C with moderate relative humidity of 59% and annual number of rainy days being 42.7 (TTDP 2008). Soils are moderately fertile with sandy loam and gravel texture and pH of 5-7 (Thagana *et al*., 2003).

Experimental design and sampling procedures

The experimental design was a Latin Square Design (LSD) with 8 treatments replicated 8 times (Gomez and Gomez, 1984). The experiment consisted of 8 blocks where the first 4 blocks were established earlier in 1981 using materials from Israel and USA. Later after 5 years since the establishment of the earlier materials, blocks 5, 6, 7 and 8 were planted using both seed and cuttings selected from the already established blocks.

Eight Jojoba bushes per block were sampled from 8 blocks consisting of different genotypes each. Stratified random sampling procedure was used to select 8 bushes, which consisted of 4 males and 4 females. Among the 4 bushes of each sex, the first was large, second medium, third small (based on height and crown diameter) while the fourth had horizontal (plagiotropic) growth habit.

Five to six fresh succulent leaves equivalent to 2 g in weight were harvested per bush and placed in polythene bags where 10 g of silica gel crystals were added to absorb the moisture before transporting to the laboratory. The trial was carried out between June 2012 and June 2014.

DNA extraction procedure

Cetyl trimethylammonium bromide (CTAB) protocol was used in the extraction of DNA

from the leaf samples according to modified method by Charles (2001). A weight of 0.1 g of each sample was transferred to a flame sterilized mortar and 1400ul of pre-heated CTAB extraction buffer added and crushed using a pestle and mortar. The substance was transferred to fresh labelled micro centrifuge tubes of 1.5mls and incubated in water bath for 10 minutes at 65^0C. A volume of 700ul chloroform: isoamylalcohol (24:1) was added to each sample. The tubes were centrifuged at 13000 revolutions per minute (rpm) for 10 minutes. A volume of 500ul of aqueous layer was transferred to micro centrifuge tube and 0.7 ml of isopropanol was added (samples stored in -20^0C overnight). The tubes were centrifuged at 13000 rpm for 15 minutes. The supernatant was discarded DNA and pellets were washed with 200ul of alcohol (70%). The DNA pellet was air dried for 30 minutes and then re-suspended in 70mls mineral water.

DNA Quality Assessment

Gel electrophoresis was performed. A volume of 100 ml of 1X TBE was added to 0.1 g of agarose to prepare 1% agarose. This was heated in a microwave for 2 minutes to dissolve the agarose. The gel was allowed to set after which 5µl of ethidium bromide was added into it. Agarose gel visualization under ultra-violet (UV) light was used to estimate the quantity of the DNA.

Polymerase Chain Reaction (PCR) Amplification and Gel Analysis

Polymerase chain reaction (PCR) was carried out in a reaction volume of 10 µl in small reaction tubes of 0.5mls in a thermal cycler make Gene Amp PCR System 9700, version 3.08. Ten SSR primers, sourced from Iqba East Africa, were used according to Ince *et al*. (2010) (Table 1) to amplify a target sequence from the samples. These 10 SSR primers were chosen because they are useful for diversity studies and are specific for Jojoba.

Table 1: Characteristics of Ten Markers Used in Jojoba Diversity Studies

Markers	Primer Forward	Primer Reverse	Repeat mortif	bps	Annealing temp.
JMA01	ACACCAGATTCCAGAGGCATA	ATTCGTCAAAGGGGATGATG	[CT]8	198	52° C
JMA02	AGAGTACGCGGGAAGCAGT	TGCTGGCAAGGGAGGTAATA	[AG]8	600	55° C
JMA03	AGTCGTTTCCCCTGCTTTTC	CTTCTGCTTATCCCCCTCATC	[CT]7	320	52° C
JMA04	GGACCTCTGCCCTTCTTCTT	TGGCGTCTTCACTGCTACTC	[GT]11	500	57° C
JMA05	CGGGGATTTATAGTCTTCACTCTC	GTCCAGGCTTCAGACCAGAG	[TC]13	214	56° C
JMA06	GCATCTGCCATTTTATGTTCAG	AACCCAGTTCCAGCTTCATC	[AAT]5	180	52° C
JMA07	GCCAAGTGGGGATGTAGAGA	GGGGACTGAACTCCACCAA	[GA]8	165	52° C
JMA08	GGAACCACAATGGCAACG	CGCAGGAAGGTCGTAAACTG	[TCT]9	185	52° C
JMA09	GCGGGGAAAGTGTTACGC	GATTAGCAGAGAAACCAAGGGACT	[AG]15	190	52° C

JMA10	AGTCAGAGTCACAGA GCAATGAA	AAGAGATTAGCAGAGA AACCAAGG	[TCT] 5	70 0	55° C

Source: Ince *et al*. (2010)

The set gel was placed in the buffer tank of 1X TBE and 5 µl of each sample re-suspended with 2 µl of bromophenol blue in each well. The samples were left to run for 30 minutes at 100 voltages at 400 mA. Agarose gel electrophoresis was employed for size separation whereas the size of the PCR products was determined by comparison with a DNA ladder which ranged between 100-1000 base pairs. The DNA fragments of known sizes run on the gel alongside the PCR products and the allele scores of each fragment was scored on a data sheet.

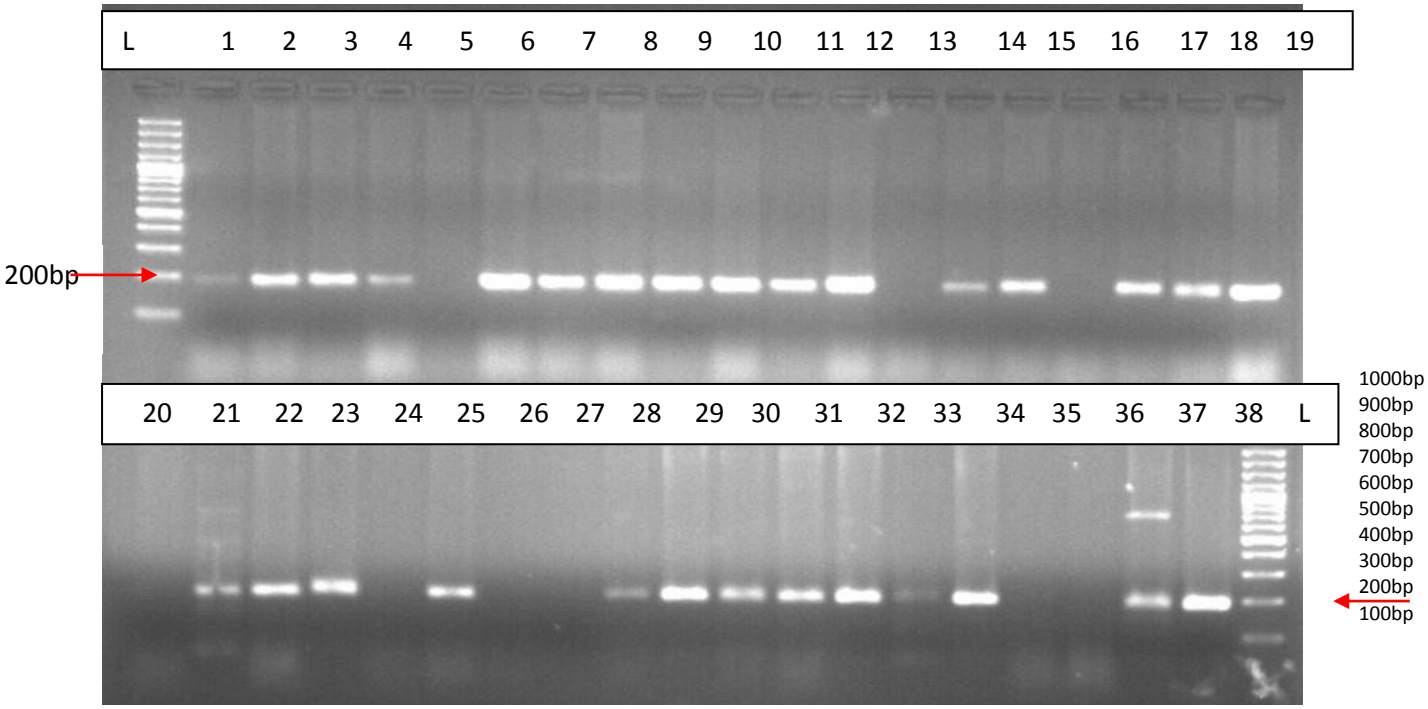
Data analysis

Genetic diversity data was analysed using XLSTAT software (2009) whereas diversity data was obtained by scoring shared bands between pairs of different Jojoba genotypes and only the strongest bands were considered as recommended by Wu and Tanksley (1993). The binary code (1) was used to designate the presence and (0) for absence of a band at a specific location for each genotype. Coded data of genetic diversity was computed and arranged in similarity matrix as described by Tonukari *et al.* (1997).

The Polymorphic Information Content (PIC) values were used to reveal the ability of each primer to distinguish the different Jojoba genotypes and their sex which was calculated for each primer used. Data was further analyzed using DARwin5 software (2011) programme and this enabled the construction of a dendrogram through neighbour-joining method which revealed the relationship among the Jojoba genotypes.

Results

Ten SSR markers were used and eight were able to amplify the DNA with clear bands that could be clearly scored at 200 base pairs using a ladder of 100-1000 base pairs (Fig. 1).



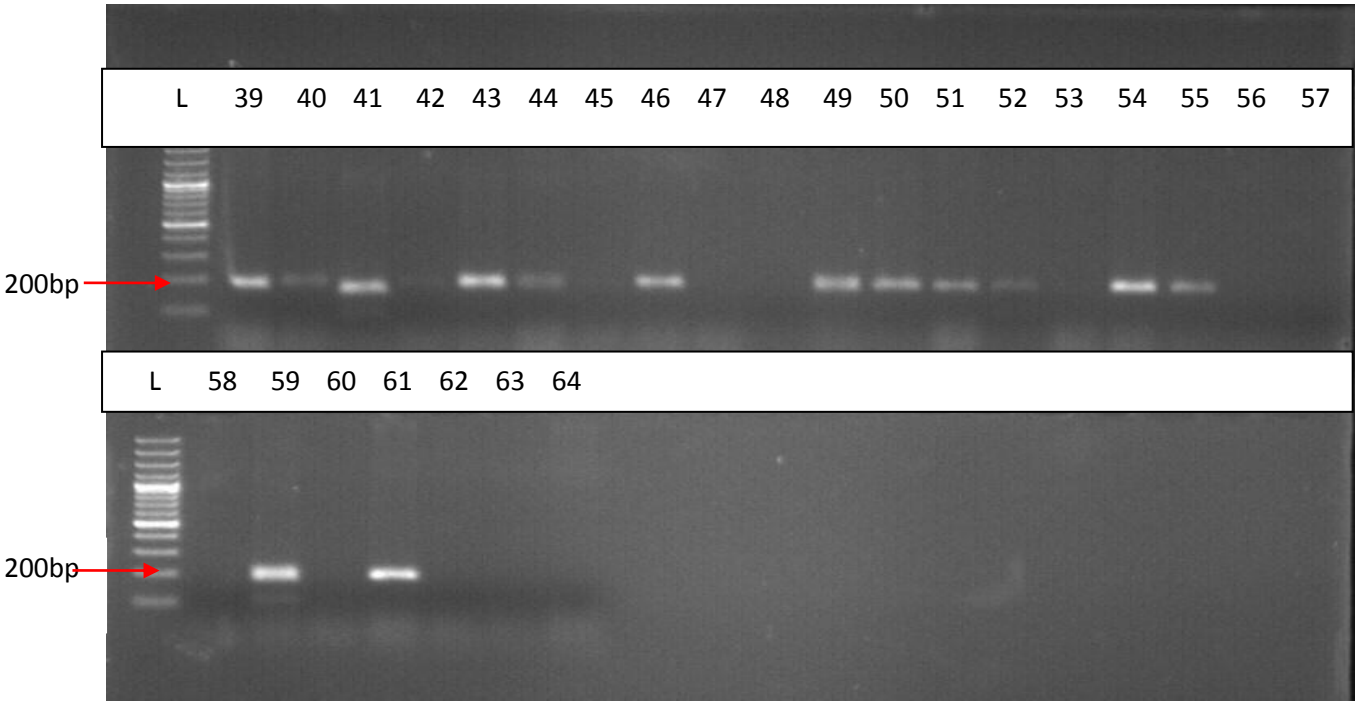


Figure 1: Polymorphic band pattern generated by JMAO8 primer on Jojoba genotypes. The standard ladder is 100-1000 base pairs (bp) and arrow points a polymorphic band at 200 bp.

Among the 8 blocks, block 8 had the highest number of genotypes that were polymorphic (100%) while block 6 had the least (50%) (Table 2).

Table 2: Morphological Characteristics and Genetic Diversity of 64 Jojoba Genotypes Used in the Study

Sample No	Block No	Bush No	Bush sex	Height (cm)	Crown diameter (cm)	Size characteristics	No. of loci	Genotypes showing polymorphism
7	1	1488	Male	222	310	Horizontal	4	
12	1	1501	Male	112	170	Small	7	
13	1	1495	Male	137	267	Medium	1	
15	1	1431	Female	42	68	Small	6	
45	1	1438	Female	142	222	Medium	0	
48	1	1430	Female	200	350	Large	0	
52	1	1443	Female	149	267	Horizontal	4	
60	1	1493	Male	250	315	Large	4	
Total				**1254**	**1969**		**26**	**75%**
Mean				**156.75**	**246.125**		**3.25**	
4	2	1511	Male	145	185	Medium	3	

6	2	1451	Female	196	300	Horizontal	6	
14	2	1458	Female	52	95	Small	2	
27	2	1465	Female	228	325	Large	0	
43	2	455	Male	102	122	Small	6	
46	2	1506	Male	288	335	Large	4	
59	2	1507	Male	178	340	Horizontal	6	
61	2	1459	Female	130	225	Medium	7	
Total				**1319**	**1927**		**34**	**87.5%**
Mean				**164.875**	**240.875**		**4.25**	
3	3	1517	Male	205	240	Medium	7	
17	3	1472	Female	240	243	Large	7	
30	3	1518	Male	232	282	Large	6	
33	3	1516	Male	120	150	Small	5	
38	3	1475	Female	142	137	Small	7	
47	3	1474	Female	146	230	Medium	0	
51	3	1471	Female	237	465	Horizontal	5	
56	3	1513	Male	215	322	Horizontal	1	
Total				**1537**	**2069**		**38**	**87.5%**
Mean				**192.125**	**258.625**		**4.75**	
11	4	1525	Male	335	335	Large	5	
18	4	1485	Female	194	220	Horizontal	8	
28	4	1481	Female	95	168	Small	5	
31	4	1480	Female	135	210	Medium	6	
54	4	1524	Male	165	165	Medium	6	
62	4	1515	Male	56	80	Small	0	
63	4	1484	Female	235	395	Large	0	
64	4	1520	Male	212	405	Horizontal	0	
Total				**1427**	**1978**		**30**	**62.5%**
Mean				**178.375**	**247.25**		**3.75**	
22	5	1406	Male	150	145	Medium	6	
29	5	1420	Male	150	242	Horizontal	6	
36	5	645	Female	204	280	Large	0	

39	5	710	Female	165	243	Medium	7	
42	5	1395	Male	145	157	Small	2	
44	5	1377	Male	254	317	Large	4	
50	5	709	Female	101	80	Small	3	
53	5	758	Female	160	137	Horizontal	4	
Total				**1329**	**1601**		**32**	**87.5%**
Mean				**166.125**	**200.125**		**4**	
5	6	644	Female	235	197	Large	0	
8	6	539	Female	107	102	Small	5	
20	6	1285	Male	180	213	Horizontal	1	
21	6	1199	Male	183	213	Medium	2	
26	6	504	Female	162	232	Horizontal	0	
49	6	1367	Male	253	215	Large	5	
57	6	1289	Male	93	145	Small	0	
58	6	577	Female	117	155	Medium	0	
Total				**1330**	**1472**		**13**	**50%**
Mean				**166.25**	**184**		**1.625**	
2	7	1566	Male	204	240	Large	6	
16	7	1154	Male	118	175	Small	0	
19	7	366	Female	102	173	Small	7	
32	7	466	Female	125	153	Horizontal	7	
35	7	1161	Male	188	240	Horizontal	0	
37	7	1150	Male	135	162	Medium	7	
40	7	371	Female	165	185	Medium	3	
41	7	365	Female	283	224	Large	6	
Total				**1320**	**1552**		**36**	**75%**
Mean				**165**	**194**		**4.5**	
1	8	41	Female	185	200	Horizontal	2	
9	8	811	Male	157	244	Horizontal	8	
10	8	883	Male	174	200	Medium	8	
23	8	56	Female	230	256	Large	4	
24	8	882	Male	120	140	Small	4	

25	8	141	Female	58	120	Small	4	
34	8	816	Male	223	224	Large	7	
55	8	119	Female	203	305	Medium	3	
Total				**1350**	**1689**		**40**	**100%**
Mean				**168.75**	**211.125**		**5**	

Ten SSR markers used in this study yielded 20 polymorphic bands. Eight of the SSR markers showed polymorphism while the remaining two; JMA02 and JMA05 did not show polymorphism while JMA04 showed the highest percent polymorphism (13.32) with JMA10 being the least (9.18) (Table 3). PIC and gene diversity showed a similar trend with the highest 0.3748 and 0.4995 respectively while the least was 0.2583 and 0.3047 respectively. However, the reverse trend was showed by major allele frequency where JMA10 was the highest with 0.8125 while JMA04 was the least with 0.5156.

Table 3: Summary of gene statistics showing PIC and percent polymorphism for ten SSR markers

Marker	Sample Size	Major Allele Frequency	Allele No	Gene Diversity	PIC	% Polymorphism
JMA01	64.0000	0.5313	2.0000	0.4980	0.3740	13.29
JMA02	64.0000	-	-	-	-	-
JMA03	64.0000	0.6094	2.0000	0.4761	0.3628	12.89
JMA04	64.0000	0.5156	2.0000	0.4995	0.3748	13.32
JMA05	64.0000	-	-	-	-	-
JMA06	64.0000	0.5781	2.0000	0.4878	0.3688	13.11
JMA07	64.0000	0.6250	2.0000	0.4688	0.3589	12.75
JMA08	64.0000	0.6719	2.0000	0.4409	0.3437	12.21
JMA09	64.0000	0.5469	2.0000	0.4956	0.3728	13.25
JMA10	64.0000	0.8125	2.0000	0.3047	0.2583	9.18
Total	**640.0000**	**4.8906**	**16.0000**	**3.6714**	**2.8140**	**100.0000**
Mean	**64.0000**	**0.6113**	**2.0000**	**0.4589**	**0.3518**	**12.5000**

The mean allele number was 1.6 while the total was 16. The most informative primers were JMA01, JMA04 and JMA09 with PIC of 0.3740, 0.3748 and 0.3728 respectively. The least informative among the polymorphic primers was JMA10, with a PIC of 0.2583.

The cluster dendrogram phylogenic tree (Fig. 2) clustered genotypes into 3 groups. A few genotypes did not cluster with the main groups including 20, 53 and 60 which remained distinct from the other genotypes but clustered together. The clusters were not formed by male or female, neither by specific blocks hence not very definite. These genotypes which did not group together with others, hence can be considered distinct and most diverse, however, they constituted only 4.7% of the sampled genotypes which is negligible.

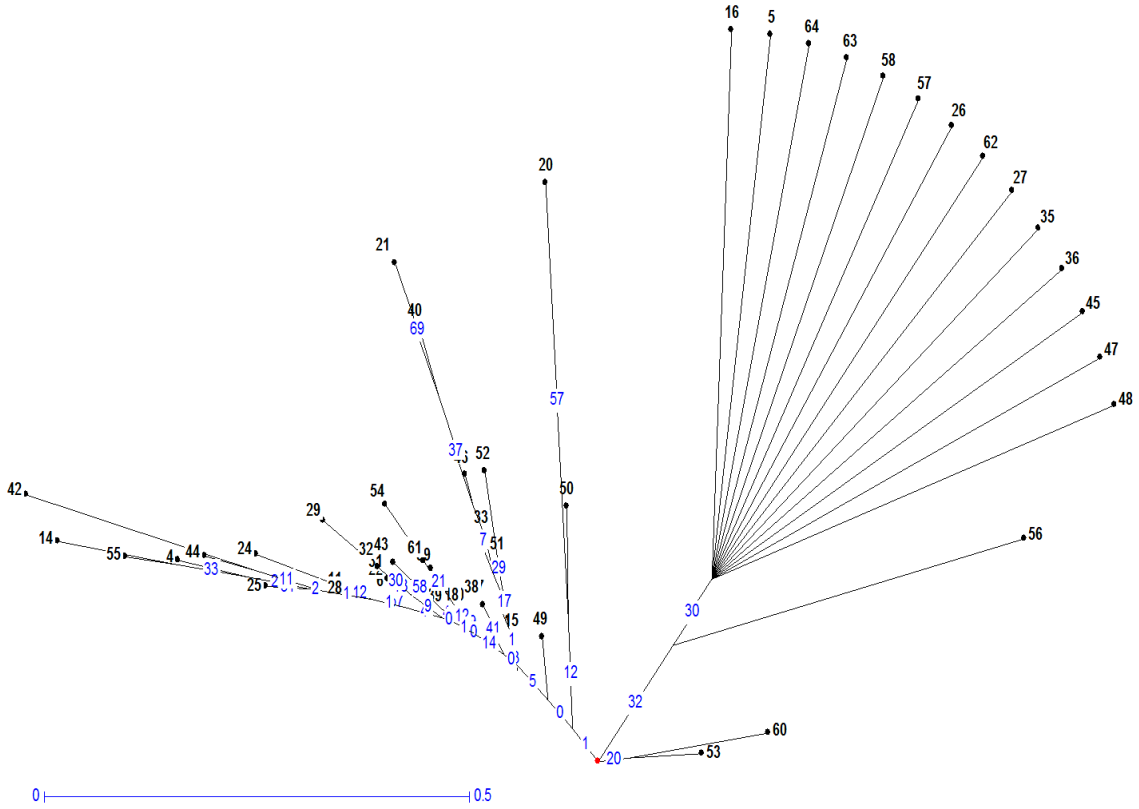


Figure 2: Cluster dendrogram showing the grouping of 64 Jojoba genotypes.

The results showed that the mature Jojoba bushes had a low genetic diversity which was shown by PIC range of 0.2583-0.3748.

Discussion

Genetic diversity that was mapped by the SSR markers in this study was narrow. SSR markers which were polymorphic produced a PIC range of 0.2583 and 0.3748 with an average of 0.3518 demonstrating low genetic diversity of the studied genotypes. Similar studies on papaya cultivars in Thailand by Ratchadaporn *et al*. (2007) reported a PIC range of 0.35 to 0.40 and concluded that all the cultivars were genetically closely related to each other. This is indicative of the posibility of similar pedigree of the genotypes in the studied plantation.

The cluster dendrogram also demonstrated closeness of the genotypes to each other and to the parentage. The observations in this study are in line with the findings by Nei (1987), who stated that to determine the nucleotide sequence of a DNA fragment is a reliable application at population and taxonomic levels and provides the ultimate solution for detecting genetic variation. Recent studies by Heikrujam *et al*. (2014; 2015) have classified Jojoba genotypes into males and females using dendrograms, which is divergent from the present study.

Dodds (1991) reported that transition from primitive to advanced cultivars has had the effect of narrowing the genetic base as observed in this study. Through selection over time, the average genetic constitution of the population changes (Wright, 1976). Broadening the genetic base is possible through increasing the number of superior shrubs for selection leading to an increase in the selection intensity (Balocchi, 1990). Introduction of new genotypes should be tested over a wide range of environments since there is a high genotype

by environment interaction. Jojoba in Voi was established through seedlings and cuttings from the primary block with a few introductions, all from a similar source (Thagana *et al*., 2003).

Studies on oil palm (Ting *et al*., (2010), sorghum (Smith *et al*., 2000; Ganapathy *et al*., 2012), millet and pigeonpeas (Singh *et al*., 2012) showed high PIC range. This is expected because these crops have not been exploited and thus have a broad gene pool. They are also established through seeds while Jojoba in this study was established through seeds and cuttings. Further studies using seed established stands may give divergent results. Matthew *et al*. (2011) working on common bean reported a PIC average of 0.31 for polymorphic markers and concluded that few differences were found among the bean cultivars. These findings corroborate with the results of this study. However, Bhardwaj *et al*. (2010) reported average PIC of 0.16 and 0.18 using RAPD and ISSR respectively in Jojoba germplasm which were lower than the findings of the present study indicating use of clonal cuttings in Jojoba propagation. Solliman *et al*. (2017) reported the use of tissue culture techniques in propagation of Jojoba.

A high degree of relatedness was demonstrated by the low PIC range found in Jojoba, which is the only species in Simmondsiaceae family. Other authors had reported similar observations on pulse crops (Kumar *et al*., 2004) and pigeonpeas (Panguluri *et al*., 2006; Yang *et al*., 2006; Odeny *et al*., 2009). However, PIC more than 0.5 are efficient in discriminating genotypes (Sharma *et al*., 2009b; Ganapathy *et al*., 2012; Sharifova *et al*., 2013), a factor that was missing in this study. This corresponds with the reports that the plantation may have been mainly made of clonal cuttings which were propagated from the earlier introductions (Thagana *et al*., 2003) and that the different blocks were made for management purposes and not to separate different clones. The present study is consistent with earlier studies by Stewart (2004) and Halkett *et al*. (2005) who reported that clonal plants generally have low genetic diversity similar to that found in self-pollinated plants such as soybeans.

More recent studies by Heikrujam *et al*. (2015) using CAAT box-derived polymorphism (CBDP) markers have classified male and female Jojoba separately by use of dendrograms and males showed higher PIC compared with the females. This was consistent with earlier studies by Sharma *et al*. (2009a) although contradictory to the present study, where dendrograms were not able to distinguish the sexes.

Gene diversity was termed as the probability of two randomly chosen alleles being different from a population (Weir, 1996) while PIC was defined as the measure to calculate the discrimination power of markers (Botstein *et al*., 1980). PIC in this study was low and so was gene diversity. This further confirms that the study population was relatively similar. The similalities did not classify the 64 genotypes into distinct groups, just like what was observed by Alves *et al*. (2013). The markers used may not have sampled the genomic control of observed phenotypes, as also reported by others (Sunil *et al*., 2011; Martins *et al*., 2011).

This study was divergent from other previous studies which showed high to moderate genetic diversity. A study on Jojoba involving five primers showed highly polymorphic nature of the studied plants in the natural habitat of Egypt, an indication that they belong to different genotypes (Gaber *et al*., 2007). Al-Soqeer (2010) investigated 8 Jojoba genotypes and clones in the central region of Saudi Arabia and reported high genetic variability among the Jojoba clones and this could permit improvement by selection and breeding for commercial plantation establishment. However, further investigations by Osman and AboHassan (2013) in western and northern Saudi Arabia showed moderate genetic variability among Jojoba clones.

The cluster analysis did not group the diffent genotypes by different blocks. This could be

due to the fact that they came from populations that may be related in other ways hence could not be picked by the variables studied. The character that led the populations to be classified into three groups using the pedigree tree need further investigation.

Conclusion

The Jojoba plantation in Voi, Kenya has a low genetic diversity shown by the PIC values which ranged from 0.2583 to 0.3748 and can be explained by the fact that the plantations were established mainly from cuttings after selection. This implies that the genotypes in the present plantations are closely related and cannot be used to interbreed for improvement.

Recommendations

Due to low genetic diversity in the Jojoba bushes, there is need to incorporate useful diversity in the breeding programmes through determination of the desirable traits that are missing, and to identify external sources of variation for improvement purposes.

However, since Jojoba is dioecious, plants derived from seeds are different from each other and therefore seedlings can be used to supply planting material in the short-run. Selected superior females should be pollinated using superior males in order to increase the level of genetic diversity. However, in the long term there is need to broaden the genetic base of the Jojoba plantation by importing diverse superior germplasm for future multiplication. This study will be useful to breeders, farmers and policy makers in scaling up Jojoba production in semi-arid areas.

Acknowledgement

The author wish to thank Wildlife Works Ltd, Maungu, for providing materials for data collection and also KALRO, Njoro for laboratory analyses. The author also wish to sincerely thank Prof. Miriam Kinyua, Department of plant breeding an biotechnology, University of Eldoret, Kenya and also Dr. Wilson Thagana, Department of agricultural sciences and biotechnology, Kenyatta University, Kenya for their positive comments and contribution towards improving this manuscript.

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