AFRICA ENVIRONMENTAL REVIEW Journal
ISSN: 1727-8341

AER Journal Volume 5, Issue 1, pp. 224-232, June, 2022

RESEARCH ARTICLE
Available Online at *http://ojs.uoeld.ac.ke/*

Genetic Diversity of African Nightshade (*Solanum nigrum L.*) Complex Accessions from Western Kenya

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Abstract

African nightshade is one of the most important leafy vegetable crops grown in Western Kenya. Despite its importance as a nutritious vegetable and having inherent medicinal value, African nightshade has been neglected, few farmers are growing it, there is potential reduction of food supply and these may be as a result of few studies carried out to improve the crop. Recently, growing demand is necessary for the development of improved, high yielding varieties, intensify diversity studies of interest, conservation and utilization of genetic resources especially in Western Kenya. The simple sequence repeats (SSR) technique was used to assess the genetic relationships among 30 african nightshade accessions collected in Bungoma, Kakamega and Trans nzoia counties of Western Kenya. 6 SSR primers were used and each primer generated 1polymorphic band. Polymorphic Information Content ranged from 0.4215 to 0.8212 with a mean of 0.5881. The average heterozygosis' He=0.9111 for SSR markers used. The dendrogram showed that the accessions grouped into three main clusters showing richness in diversity, it also revealed that the coefficient distance that separated most of the accessions was less than 79.56. These findings show that there were possibilities of cross ability among the accessions, Variation among regions was not genetically evident.

Keywords: Simple Sequence Repeat, *Solanum nigum* Complex, African Nightshade, Genetic Variation, Taxonomy, Western Kenya

INTRODUCTION

The development of new African nightshade cultivars (*Solanum nigrum L*) is an important strategy to increase crop productivity of this important vegetable crop. African nightshade is an important indigenous leafy vegetable in Kenya which originated in Eurasia (Jagatheeswari et al., 2013). The vegetable occupies an important place in the Kenyan economy as it contributes significantly to nutritional security and medicinal value (Nandhini et al., 2014) as well as generate income for small holder farmers.

The rise in consumer awareness on the nutritional, economical and medicinal value of African nightshade has simultaneously led to increased utilization which has in turn increased its market demand and value (Osei et al., 2013). Despite its importance as a nutritious vegetable and having inherent medicinal value, african nightshade has been neglected, few farmers growing it, there is potential reduction of food supply and these may be as a result of few studies carried out to improve the crop, their correct taxonomic identification is yet to be established. This is because continued inter and intraspecific hybridization do occur naturally among african nightshade species as well as due to inconsistent genetic variation.

The susceptibility of morphological traits to phenotypic plasticity and the existence of many ploidy series have also caused problems to their taxonomic identification. Different communities use different local names to identify African nightshade species creating further confusion in the differentiation of one species from the other (Poczai & Hyvonen, 2011; Ojiewo et al., 2013a).

There is need that efforts be made to improve on African nightshade production through increased cultivation for commercial purposes so as to try and meet the demand in the market. Rise in demand and low supply of african nightshade has resulted into an increase in their market prices.

The production and use of molecular markers in genetic diversity studies was a key development in molecular genetics. The markers used for diversity studies include Restriction Fragment Length Polymorphism (RFLP), Single Nucleotide Polymorphism (SNPs), Random Amplified Polymorphic DNA (RAPD), Amplified Fragment Length Polymorphism (AFLP), Sequence characterized amplified regions (SCARs), Arbitrarily primed polymerase chain reaction (APPCR), DNA amplification fingerprinting (DAF), Simple sequence repeats (SSRs), Expressed sequence tags (ESTs), Cleaved amplified polymorphic sequence (CAPS), and sequence tagged sites (STSs) (Idrees & Irshad, 2014) . Molecular markers are suggested for diversity studies since they have many advantages over morphological characterization because they are stable, found in all tissues and are not affected by the environment. An ideal molecular marker should be polymorphic, abundant, reliable, simple, quick, provide enough resolution of genetic variation and should use only amount of DNA for amplification (Mondini , 2009). The aim of this study was to evaluate molecular characteristics of 30 African nightshade accessions using Simple sequence repeat from Western Kenya.

MATERIALS AND METHODS

Study Sites

The study was conducted out in Bungoma, Kakamega and Trans nzoia counties of Western Kenya.

Sampling Criterion

Stratified random sampling was done based on prior information on species grown and sampling partnership with farmers. Consultations were done with area Agricultural officers before collection of materials. Sampling was done in a 0.5km radius in each location. Five farms where African nightshades were growing were selected at random. The selected farms ranged in sizes between 0.3ha and 2ha and production of African nightshade done at a small scale. Five mature plants having mature fruits were randomly selected in each farm and also seeds of the same accession in the farm were collected from the farmers. A total of 30 seed samples of the accessions were collected from the three selected counties constituting of 10 samples each. These materials were labelled based on the area and farm (farmer) number of collection as Bungoma (B), Kakamega (K) and Trans nzoia (T), the envelopes were placed in glass Jars with tight lids and the Seeds were kept dry using a Silica Gel Packet.

Table 1: African nightshade accession used in the study

(Accessions) Name	Local Name	Source	GPS Coordinates	
			Longitude	Latitude
Bungoma 1	Namasaka	Lugulu	34.751° E	0.665
Kakamega1	Esucha	Ingotse	34.696 ° E	0.356
Trans Nzoia 1	Kisoyet	Kwanza	35.003 ° E	1.163
Bungoma 2	Namasaka	Maeni	34.750 ° E	0.792
Kakamega 2	Irisuza	Lukuyani	35.103 ° E	0.711

Bungoma 3	Namasaka	Mabanga	34.619 º E	0.601 º N
Kakamega 3	Liisucha	Chimoi	34.826 º E	0.580 º N
Kakamega 4	Esucha	Navakholo	34.681 º E	0.407 º N
Kakamega 5	Lisutsa	Shinyalu	34.766 º E	0.274 º N
Bungoma 4	Namasaka	Mayanja Vitunguu	34.544 º E	0.528 º N
Bungoma 5	Namasaka	Chwele	34.581 º E	0.737 º N
Kakamega 6	Liisucha	Lubao	34.807 º E	0.332 º N
Bungoma 6	Namasaka	Bokoli	34.660 º E	0.712 º N
Trans Nzoia 2	Managu	Kiminini	34.927 º E	0.884 º N
Trans Nzoia 3	Namasaka	Sikhendu	34.830 º E	0.884 º N
Bungoma 7	Namasaka	Sang'alo	34.593 º E	0.528 º N
Bungoma 8	Namasaka	Kimilili	34.727 º E	0.792 º N
Trans Nzoia 4	Managu	Kiungani	34.951 º E	0.95 º N
Bungoma 9	Namasaka	Ndalu	34.987 º E	0.818 º N
Kakamega 7	Liisucha	Malava	34.855 º E	0.454 º N
Kakamega 8	Liisucha	Kaburengo	34.801 º E	0.578 º N
Trans Nzoia 5	Kisocheet	Saboti	34.838 º E	0.931 º N
Trans Nzoia 6	Osoig	Endebesi	34.852 º E	1.086 º N
Kakamega 9	Liisucha	Lwandeti	34.849 º E	0.607 º N
Bungoma 10	Namasaka	Kamukuywa	34.784 º E	0.799 º N
Trans Nzoia 7	Managu	Mucharage	34.856 º E	0.818 º N
Trans Nzoia 8	Namasaka	Bidii	35.035 º E	1.033 º N
Kakamega 10	Liisucha	Matete	34.805 º E	0.555 º N
Trans Nzoia 9	Managu	Bondeni	34.902 º E	0.991 º N
Trans Nzoia 10	Ksoyo	Cherang'ani	35.234 º E	0.988 º N

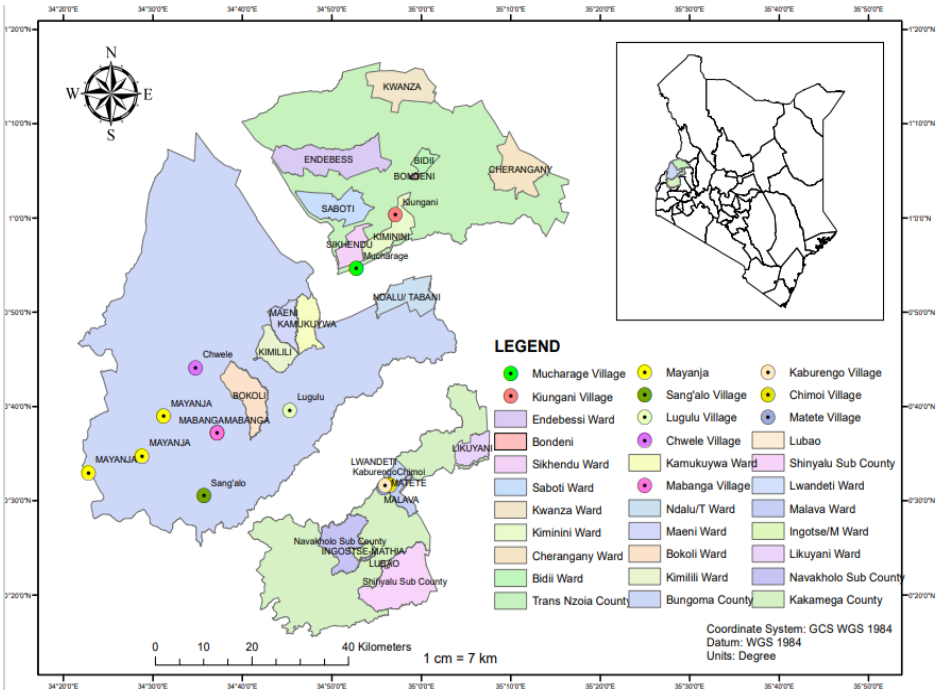


Figure 1: Map showing three regions in western Kenya where the African nightshade samples were collected.

Leaf Harvesting and DNA Extraction

Leaves of four week old young, tender and healthy African nightshade accessions were harvested and wrapped in foil paper and then immediately taken to the laboratory put in refrigerator at -20°C so as to retain its quality. The leaves were then rinsed in distilled water to remove soil particles on their surfaces, as done by Agbagwa et al., (2012). African nightshade accessions leaves were weighed and 200 mg of each accession leaves were gently ground into a fine paste in 500 µl of CTAB buffer using a motor and pestle. The paste was then transferred into a microfuge tube and incubated for 15 minutes at 55°C in a recirculating water bath. The CTAB/ plant extract paste was then centrifuged at 12000 rpm for five minutes so as to spin down the cell debris. The supernatant was then transferred into clean microfuge tubes, 250 µl of chloroform: Iso-Amyl Alcohol (24:1) was added into each tube and the solution mixed by slow and repeated inversion. The mixture was then centrifuged at 13000 rpm for one minute and the upper aqueous phase which contains the DNA was carefully transferred into a clean microfuge tube. 50 µl of 7.5 M ammonium acetate was added into each tube followed by addition of 500 µl of ice cold absolute ethanol. The tubes were then slowly and carefully inverted several times so as to precipitate the DNA. The precipitated DNA accumulated at the bottom of the tubes and the supernatant was carefully removed by slowly pouring it out of the tube while at the same time taking care not to dislodge the DNA pellets. The DNA pellet was then washed twice using ice cold 70% ethanol. The DNA was centrifuged at 13000 rpm for 1 minute after washing and the supernatant removed. The DNA was then dried by inverting the tube containing the DNA on a clean paper towel for 14 minutes and care was taken to make sure the DNA pellet does not fall out of the tube. The tubes with the DNA pellets were then turned upright and while still covered with paper towel left for 30 minute to ensure that the pellets were completely dry. The extracted DNA was then suspended in 400 µl of sterile DNase free water. 10 µl/ml (10 µl RNase in 10 ml H_2O) RNase was then added to remove any RNA that might have been present in the preparation. After re suspension, the DNA was incubated at 65°C for 20 minutes to destroy any DNases that might have been present. The DNA was then stored at 4°C for further use in Polymerase chain reaction.

DNA Quantification

DNA quantity and quality of each accession was determined by running samples on 1% (w/v) agarose gels for 1 hour at 80 volts diluted in 100 ml 1 x TAE buffer (0.89 M Tris base, 0.89 M boric acid, 20 Mm edta pHm8.0) and 900 mL of distilled water. A standard undigested lambda DNA with a range variation of 10, 20, 50, 80 and 100 ng was used as a comparison to determine the DNA concentration of the African nightshade accessions by comparing band sizes and intensities. The gel was stained in ethidium bromide (10 mg/ml) for 30 minutes and later destained in distilled water for 20 minutes before viewing under ultraviolet trans illuminator. Between 0.5 µg and 1 µg of high quality DNA was obtained and was diluted to 0.01 µg/µl with deionized distilled water for PCR amplification.

The quality of DNA extracted was confirmed through agarose gel electrophoresis where 3% of agarose gel was prepared by weighing 3 g of agarose powder and pouring it into a conical flask containing 100 ml of 1x TBE buffer and then the mixture was placed into a microwave and heated for 3 minutes for it to melt (until the agarose is completely dissolved and there is a nice rolling boil). The conical flask containing the mixture was then removed and allowed to cool for five minutes. 0.5 µg/ml Ethidium bromide was then carefully added into the gel for visualization and stirred to mix evenly. Gel combs were then arranged into a gel tray for creation of wells where the DNA samples were to be loaded. The gel was then carefully cast into the tray and allowed to set for 20 minutes at room temperature on a flat surface until it completely solidified. During gel

casting care was taken to ensure no bubbles were formed in the gel since the bubbles could interfere with DNA movement during electrophoresis. The type of combs used were to create a minimum of 31 wells, one well for loading the ladder and the remaining wells for loading the 30 samples. The combs were then carefully removed after the gel had hardened and the gel was transferred into a gel electrophoretic tank filled with 0.5x TBE buffer for loading of the DNA samples.

10 µl of 1 kb ladder was loaded into the first well followed by a mixture of 5 µl sample (the DNA extracted for each ANS genotype), 5 µl water and 2µl 6x loading buffer which were loaded in the remaining wells. The loading enabled the DNA samples to settle at the bottom of the gel wells and not to diffuse into the buffer. Gel electrophoresis was then conducted for 30 minutes at 100 voltages and thereafter carefully removed from the gel tanks and exposed to ultra violet light after which it was photographed. The presence of high resolution molecular weight bands confirmed that the quality of DNA extracted was good.

PCR Amplification

PCR amplification was carried out in 25 µl volume of reaction mixture consisting of 2µl DNA sample template, 5 µl of 5x PCR buffer, 0.1 µl Taq polymerase, 0.5 µl reverse primer, 0.5 µl forward primer and 17 µl double distilled water. A total of six different primers were used for polymerase chain reaction with each primer pair (reverse and forward) being used per reaction. After an initial denaturation of 4 min at 94°C, 30 cycles of 1 min denaturation at 94°C, 1 min annealing at 49–55°C and 1 min of extension at 72°C were performed, followed by a final extension of 10 min at 72°C. The amplifications were carried out using Applied Bio systems 2720 Thermo cycler.

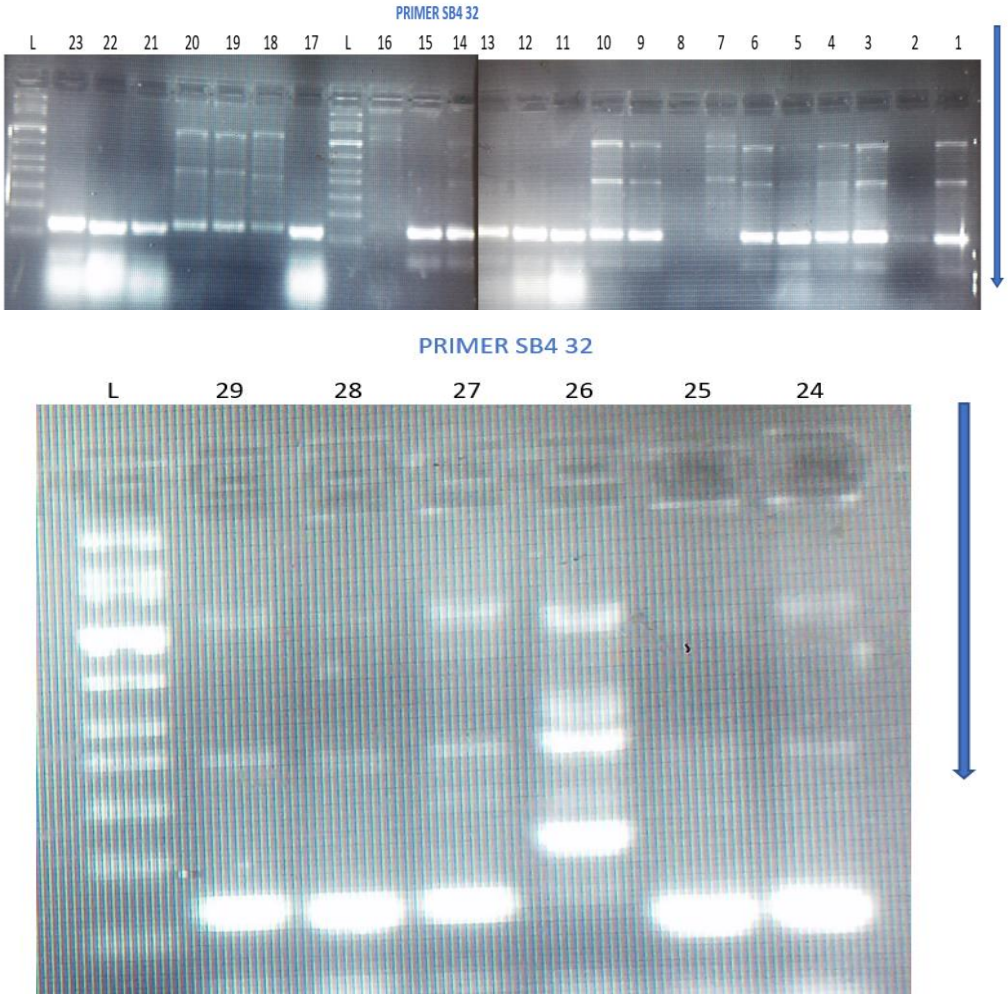
DNA Data Analysis

For each reproducible band visualized, the Allele sizes were scored manually using molecular size ladder and scored as 1 for each present band and 0 for absence of the band for each primer. The size of the band was determined by comparing the band with 100 base pair molecular size ladder. The matrix data generated was used for statistical analysis. Cluster analysis was performed to establish the genetic associations among accessions and Genetic distance dendrogram drawn using Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, version 2.1. The genetic associations were evaluated by calculating the genetic similarity matrix using Euclidean and subjected to Un weighted Pair-Group method (UPGMA) clustering using the sequential agglomerative hierarchical nested (SAHN) programme and tree plot analysis generated. Matrix data was also subjected to analysis using Power Marker (ver 3.0) to determine Major allele frequency, Genetic diversity and Polymorphic information content (PIC) indices

RESULTS

The clustering and sub clustering seen in the dendrogram (Fig 1) below indicated that there were possibilities of crossability among African nightshade accessions being studied. The dendrogram (Fig 1) revealed 3 main clusters joined at a Euclidean distance of 198.91. The first cluster comprises of Trans Nzoia 5 (*Solanum nigrum*). The second cluster comprised of Kakamega 10 (*Solanum nigrum*). The third cluster comprised of all the other 28 African nightshade accessions. The third cluster is further subdivided into two other clusters at a distance of 115.49. The third cluster comprised Trans Nzoia 7 (*Solanum villosum*), Trans Nzoia 6 (*Solanum nigrum*), Kakamega 7 (*Solanum nigrum*), Bungoma 9 (*Solanum villosum*), Trans Nzoia 4 (*Solanum scabrum* improved), Trans Nzoia 10 (*Solanum nigrum*), Trans Nzoia 9 (*Solanum nigrum*),Trans Nzoia 8 (*Solanum villosum*), Bungoma 10 (*Solanum villosum*), Kakamega 9 (*Solanum scabrum* improved), Bungoma 8 (*Solanum nigrum*), Bungoma 7 (*Solanum scabrum* improved), Bungoma 5 (*Solanum nigrum*), Kakamega 8 (*Solanum nigrum*) , Trans Nzoia 3 (*Solanum nigrum*),

Trans Nzoia 2 (*Solanum villosum*), Bungoma 6 (*Solanum nigrum*), Kakamega 6 (*Solanum nigrum*), Bungoma 4 (*Solanum nigrum*), Kakamega 4 (*Solanum villosum*), Kakamega 2 (*Solanum scabrum* improved), Kakamega 3 (*Solanum scabrum* improved), Trans Nzoia 1 (*Solanum nigrum*), Kakamega 5 (*Solanum villosum*), Bungoma (*Solanum nigrum*), Kakamega1 (*Solanum villosum*), Bungoma 1 (*Solanum villosum*). The third cluster comprises of two clusters linked at a Euclidean distance of 58.02 with eleven sub clusters linked at a Euclidean distance of 39.78.



1= Bungoma 1 (S.N), 2= Kakamega 1(S.V), 3= Trans nzoia 1 (SN), 4= Bungoma 2(SN), 5= Kakamega 2(SS), 6= Bungoma 3 (SS), 7=Kakamega 3 (SS),8= Kakamega 4(SV), 9= Kakamega 5(SV), 10= Bungoma 4 (SN), 11= Bungoma 5(SN), 12= Kakamega 6 (SN), 13= Bungoma 6(SN), 14= Transnzoia 2(SV), 15= Transnzoia 3(SN), 16= 2 Bungoma 7 (SS), 17= Bungoma 8 (SN),18= Transnzoia 4 (SS), 19=Bungoma 9(SV),20= Kakamega 7 (SN), 21=Kakamega 8 (SN)22= Transnzoia 5 (SN), 23= Transnzoia 6 (SN), 24= Kakamega 9(SS), 25= Bungoma 10 (SV), 26= Transnzoia 7(SV), 27= Transnzoia 8(SV), 28= Kakamega 10(SN0, 29= Transnzoia 9 (SN), 30=Transnzoia 10(SN).(SN-*Solanum nigrum*, SV-*Solanum villosum*, SS-*Solanum scabrum*)

Plate 1: PCR products amplified with Primer SB6 84 and visualized under UV light.

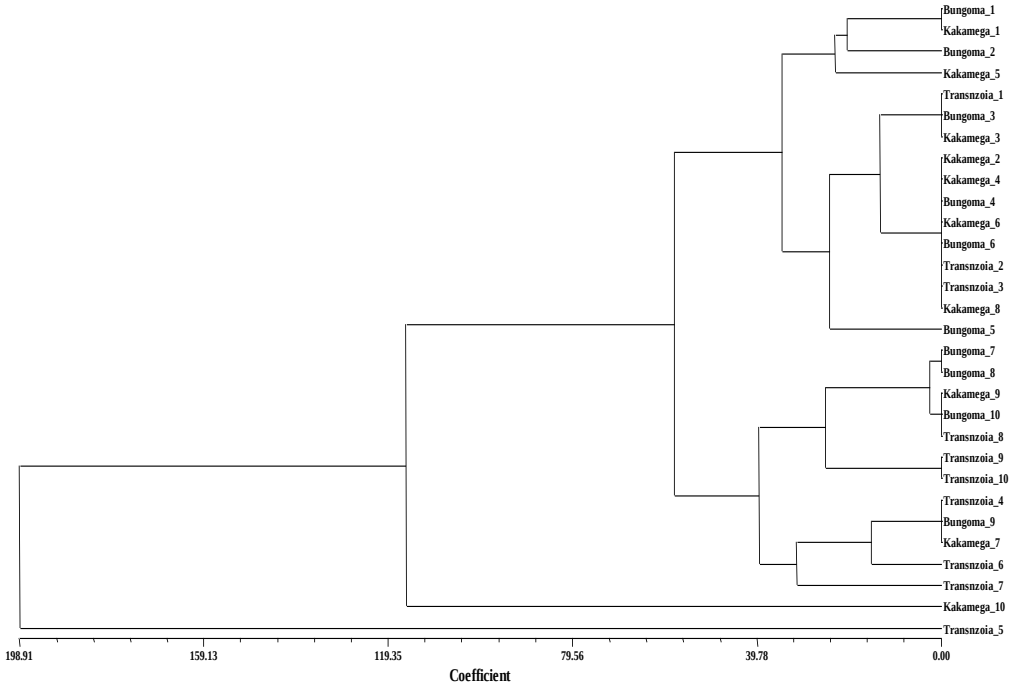


Figure 1: Genetic distance among accessions was estimated with using Numerical Taxonomy Multivariate Analysis System package (NTSYS-pc) software, version 2.1. The genetic associations were evaluated by calculating the genetic similarity matrix using Euclidean and subjected to Unweighted Pair-Group method (UPGMA) clustering using the sequential agglomerative hierarchical nested (SAHN) programme and tree plotanalysisgenerated.

Table 2: Allele Frequency, Allele Number, Genetic Diversity, Heterozygosity and PIC for SSR Primers used in DNA amplification

Marker	Major. Allele. Frquency	GenotypeNo	AlleleNo	GeneDiversity	Heterozygosity	PIC	f
CA158	0.4667	3.0000	4.0000	0.6239	0.9333	0.5515	-0.4831
SB432	0.2500	7.0000	9.0000	0.8406	0.8333	0.8212	0.0255
SB636	0.4667	4.0000	5.0000	0.5894	1.0000	0.5036	-0.6877
SB684	0.4833	2.0000	3.0000	0.5317	0.9667	0.4215	-0.8125
STWIN12	0.4833	2.0000	3.0000	0.5317	0.9667	0.4215	-0.8125
TMS29	0.2333	7.0000	9.0000	0.8311	0.7667	0.8092	0.0944
Mean	0.3972	4.1667	5.5000	0.6581	0.9111	0.5881	-0.3700

PIC-polymorphic information content

DISCUSSION

Molecular markers (SSR) have been used successfully to clarify genetic diversity within crops and their wild relatives, and between accessions of cultivated or semi-cultivated plants from different geographical or ecological areas, and as a source for selection and for conservation of genetic diversity (Dehmer 2001; Hammer et al., 2003; Lanteri and Barcaccia 2005; Mace et al., 1999; Muluvi et al., 1999; Perera et al., 1998; Potokina et al., 2002; Shan et al., 2005; Vergara and Bughrara 2003; de Vicente et al., 2005).

The present study applies SSR to study the genetic diversity in African nightshade accessions of Western Kenya. The clustering pattern exhibited by the African nightshade accessions in this study indicates that the genetic variation between accessions is high. The lack of clustering according to region provenance is an indication that accessions from different regions (Bungoma, Kakamega and Trans Nzoia counties) are not significantly different genetically either. A similar clustering pattern was reported between Ugandan, Indonesian and European material (Olet, 2004).

African nightshade accessions within a cluster consisted of more genetically similar accessions than those among different clusters similar results were obtained by (Osei et al., 2013).

The assessment of African nightshade accessions using SSR primers showed that there was genetic diversity recorded because accessions clustered into three different groups (I, II and III). The clusters and sub-clusters observed in the dendrogram indicated that there was rich genetic diversity within the African nightshade accessions collected from the three counties in Western Kenya.

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