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Genetic diversity of *Solanum nigrum* cultivated in Kenya

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Abstract. *Wagio RS, Runo S, Muchugi A. 2019. Genetic diversity of* Solanum nigrum *cultivated in Kenya. Asian J Agric 2: 63-71. Solanum nigrum* L., sometimes known as black nightshade, is a worldwide weed found on arable land, in gardens, and garbage dumps. It has been utilized in the pharmaceutical industry to produce analgesics, ointments, and vasodilators. Using morphological and Simple Sequence Repeats (SSR) markers, this study aimed to assess the genetic diversity of *S. nigrum* populations growing in specific regions of Kenya. One hundres-twenty (120) samples from four populations were evaluated in total. Morphological characterization utilized four aerial characters. leaf surface (smooth and hairy), leaf margin (whole and sinuous), leaf form (ovate, lanceolate, and diamond), fruit color (red, purple small, and purple large). Tukey's HSD test demonstrated substantial variation among the evaluated attributes ($P \leq 0.05$). Four SSR primers produced 63 polymorphic bands, ranging from 14 to 17 per primer set. The range of Polymorphic Information Content was 0.1429 to 0.1855, with a mean value of 0.1704. Heterozygosity (using SSR markers) averaged out to be He=0.1370. Axis 1 and 2 of the PCoA explained 44.65% of the population distribution variance. The analysis of multi-level variance (AMOVA) showed that there was only 14% variation between populations and 86% variation inside the populations. Nei's genetic distance varied between 0.010 (Kipkaren and Matanya) and 0.020 (Makuyu and Mauche). Using DARwin 6.0.5 and Unweighted Neighbor Joining with 1000 bootstraps, a dissimilarity study was conducted. The absence of morphological and genetic distinctions in the dendrogram is indicative of the absence of genetic divergence between groups. Variance in the Agro-Ecological Zone is not indicative of variation in the *S. nigrum* cultivars. Comparing morphological and molecular data revealed leaf surface and fruit color grouping with genetic data. Using the same data, however, there was no grouping between leaf shape and leaf margin. The observed physical distinctions are primarily the effect of human selection. This study demonstrates that the *S. nigrum* complex displays limited genetic diversity but considerable morphological heterogeneity. Low genetic distance and the absence of clustering patterns indicate that *S. nigrum* is a closely related group of plants. The results of this study will have far-reaching implications for future breeding efforts of the *Solanum* complex, as they will be utilized to produce markers connected to crucial agronomic features. The findings will contribute further to resolving the taxonomic ambiguity surrounding *S. nigrum*.

Keywords: Genetic diversity, Kenya, *Solanum nigrum*

INTRODUCTION

Africa's indigenous leafy vegetables are a diverse set of unrelated species whose leaves are consumed by numerous local cultures. These veggies are essential to food security (Nandhini et al. 2014). Their leaves and fruit are widely consumed as food, mainly in Africa and Southeast Asia (Jagatheeswari et al. 2013). For example, *Solanum nigrum* L. is a common vegetable in Kenya. The majority of these veggies are produced by smallholders who cultivate them in kitchen gardens for subsistence. Due to decreased soil fertility and poor agricultural practices, yields have been falling (Juma 1989).

In developing countries, agricultural development and production are mostly dependent on subsistence and edible wild species. However, little attention has been paid to the dietary usage of non-domesticated plants, and a drastic contraction of the food base in many traditional societies has occurred (Tindall 1977). Wild edible vegetables are essential in Africa's agricultural and dietary systems. Chweya (1997) enumerated a variety of green vegetables consumed in Kenya. Some vegetables are considered weeds in some parts of the country, while others are considered native veggies. It is the case with *Solanum* species, which are linked to black nightshades and are the focus of this study.

Black nightshades are extensively dispersed in many global environments, from tropical to temperate regions and from sea level to altitudes greater than 3,500 meters. Their wide habitat tolerance, capacity to flower while young, and prolific seed production contributes to their success as invasive species (Jennifer and James 1977). *S. nigrum* is a vital source of germplasm for crop development, as it supplies resistant genes against several diseases, including Early blight (Olet et al. 2011).

Unfortunately, there is a great deal of misunderstanding over the precise identification of the taxa involved, particularly in regions where the species are most frequently utilized as food resources (Grieve 2012). In Africa, this misunderstanding is likely exacerbated by the usage of vernacular names, in which one name may apply to multiple species, or multiple names may apply to the same species. Biosystematic research on *S. nigrum* is required to comprehend the relationship between wild and domesticated plants (Chweya 1997). About 200 native plant species are utilized as leafy vegetables in Kenya, of

which just four are fully domesticated, more than fifteen are semi-domesticated, and the balance is wild. The *S. nigrum* is one of the most commonly consumed green vegetables in East Africa, consumed by both Nilotes and Bantus (Edward and Charles 1990).

Black nightshades are worldwide weeds affecting 37 crop species in 61 countries (Falck 2010). Several studies have utilized DNA markers to evaluate the genetic diversity of numerous crops, including black nightshades (Anas and Yoshinda 2004; Wang et al. 2009; Mutegi et al. 2011; Sayed et al. 2012). Since SSR markers are highly polymorphic and plentiful in the genome, they are a potent tool for uncovering interspecific and intraspecific evolutionary relationships (Barkley et al. 2005). In Kenya, investigations on the diversity of *S. nigrum* using SSR markers have not been complete; therefore, this study provides a chance to evaluate the efficacy of these markers in diversity research. According to the study's findings, the *S. nigrum* species represents a collection of closely related plants, as earlier research conducted outside Kenya hypothesized.

The aims of this research were to assess the genetic diversity of black nightshades (*S. nigrum*) using SSR markers, to determine the morphological variations within black nightshades, and to infer the association between morphological and genetic changes in black nightshades.

MATERIALS AND METHODS

Collection sites

To conduct this research, researchers chose four locations in Kenya: Matanya (Nanyuki sub-county), Mauche (Njoro sub-county), Kipkaren (Kapsabet sub-county), and Makuyu (Muranga South sub-county) (Figure 1).

Site selection

The selection of venues was impacted by the following factors: (i) Agroecology; these regions belong to distinct agroecological zones. For example, Mauche and Kipkaren are found in Agroecological zone 3, which is restricted to heights between 900 and 1800 meters above sea level with annual precipitation between 950 and 1500 mm. On the other hand, Makuyu and Matanya are found in Agro-ecological zone 4, which has the same height as zone 3, but receives between 500 and 1000 mm less precipitation annually (www.infonet-biovision.org-AEZs- the Kenya system). Environmental well-being). (ii) Sociocultural; there are societies with distinctive cultures that utilize black nightshade as traditional veggies (Onyango 1993).

The Ndorobo population of Mauche consists primarily of hunters and gatherers who subsist on wild fruits and meat. However, after a huge portion of the Mau forest was cleared, this group transitioned from hunting and gathering to subsistence farming. Kipkaren is home to the Nandi, who are skilled animal caretakers. As a complement to Ugali and Mursik (fermented milk), their major food source, traditional vegetables like black nightshade, date back to the sixteenth century. Matanya is predominantly inhabited by pastoralists (Samburu) and farmers (Kikuyu). While pastoralists rely on livestock for subsistence, farmers cultivate food crops, including black nightshades, for sale. Makuyu is dominated by Kikuyu farmers who engage in commercial and subsistence agriculture (www.kenya information guide 2015). (iii) Over time, black nightshade consumption has expanded dramatically, and the crop is now cultivated commercially (Maundu et al. 2004).

Sampling criteria

A stratified random sample was conducted based on prior knowledge of the cultivated species and sampling cooperation with farmers. Before collecting materials, Agricultural officers in the region were consulted thoroughly. Each place was sampled within a 1-kilometer radius. Five farms where black nightshades were growing were chosen randomly to avoid duplication. The selected farms ranged in size from 0.5 ha to 2 ha, and their black nightshade production was on a small scale. Each farm selected six plants randomly and collected one mature stem for a total of six stems per farm. Each collected stem possessed ripe leaves and fruits. From the four designated sites, 120 mature plant samples were collected (every 30 plants). These samples were labeled according to the collection area: Makuyu (M), Matanya (N), Mauche (R), and Kipkaren (K).

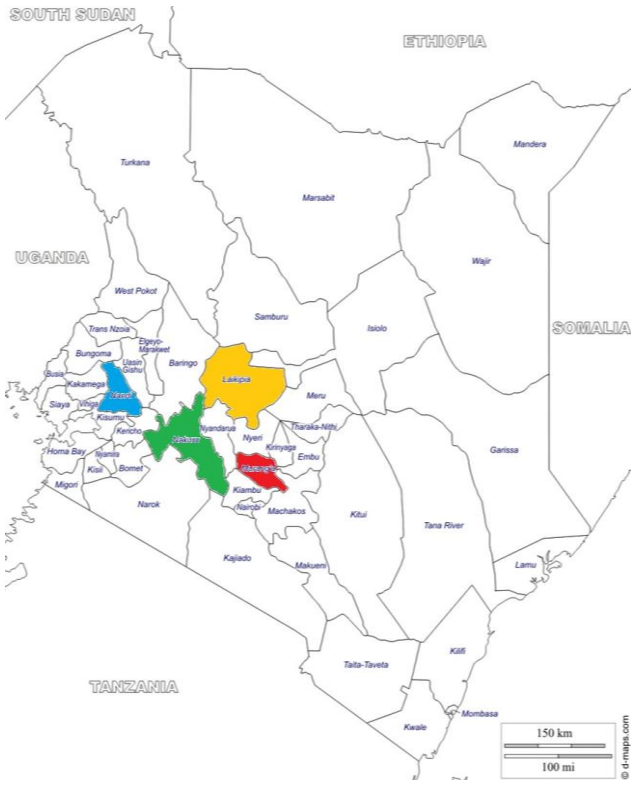

Figure 1. Map showing four counties in Kenya where the *S. nigrum* samples were collected. (Source www.d-map.org)

Morphological characterization

Samples were transported to the Plant Transformation Laboratory at Kenyatta University, where they were visually evaluated based on the criteria outlined in Table 1 by Jennifer and James (1977) and Nandhini et al. (2014). Each trait was evaluated, tabulated, and the means were determined.

DNA extraction

Prior to DNA extraction using the CTAB procedure, 50 mg of leaf material was taken from each participant and dried in a snap-lock bag containing silica gel (Sue et al. 1997). The leaves were crushed in liquid nitrogen using a pestle, 9.0 mL of warm CTAB buffer (65°C) was added and gently mixed by inversion, and the centrifuge tubes were incubated at 60°C for 60 minutes with continuous rocking in a water bath. After removing the tubes from the water bath and adding 4.5 mL of a chloroform/isoamyl mixture (24:1), they were centrifuged at 1500 revolutions per minute for 10 minutes at room temperature. Remove the supernatant and repeat the chloroform extraction. Next, the top aqueous layer was pipetted into fresh 15 ml tubes, 6.0 mL of isopropanol was added, and the mixture was inverted and gently stirred. The samples were cooled at 4°C overnight, then spun at 1500 revolutions per minute for 10 minutes, and the supernatant was discarded. Next, one milliliter of sterile distillate water and fifty microliters of sodium acetate were added. In succession, DNA was precipitated with 100% and 70% ethanol. Next, DNA was spooled out, air dried for 2 hours, and resuspended in 200 µL of sdH_2O before being stored at -20°C. The final DNA concentration was determined by electrophoresis in 1% agarose at 80V for 30 minutes in TAE relative to a known 1kb reference.

SSR marker analysis

DNA was utilized to identify highly polymorphic primers formerly employed in *Solanum* species. Angeline et al. (2010) employed seven SSR primers to perform PCR on Solanaceae and cross-species (Table 2). On a total reaction volume of 25 µL including 5 µL 10x PCR buffer [20mM Tris-HCL (pH 8), 50mM KCL], 17.875 l ddH2O, 1 µL (10pMole) of primer set, 0.125 µL *Taq* polymerase, and 50ng genomic DNA template, DNA amplification was done. Thermocycler amplifications were carried out under the following conditions: 5 minutes of denaturing steps at 94°C, followed by 35 cycles of 1 min at 95°C, 45°C (Annealing temperature) for 2 min, and 72°C (Elution temperature) for 2 min. The extension period at 72°C during the final PCR cycle was increased to 10 minutes. The optimal conditions varied for distinct primer pairs, with 45°C and 50°C annealing temperatures for the seven primer sets.

To separate the PCR product by electrophoresis, 2% agarose (Bioline) was loaded with 5 µL aliquots and gel loading dye and sybr green stain. In 0.5x TAE [0.438g/L Tris (pH 8), 0.11 mL/L acetic acid, and 0.029 g/L EDTA], electrophoresis was conducted for 1.5 hours at a constant voltage of 100 volts. As a molecular marker for determining fragment size, a 1-kb ladder (Bioline) was utilized. Finally, polymorphic bands were observed under a UV trans-illuminator and captured on a digital camera. Each band was rated based on its comparison to a 1-Kb size ladder. Heather and Matthew (2012) scored the profiles independently for each primer set so that each band at a given migration point in the gel was a character and was marked as present "1" or absence "0".

Data analysis

The analyzed *S. nigrum* data were log converted to comply with ANOVA's assumptions. The Tukey's Honestly Significant Difference (HSD) test was then used to calculate the HSD and determine whether there were significant differences between the groups. If the probability level was less than 0.05, it was judged to be significant.

The genetic closeness between samples and between populations was determined by using the band scores from the SSR profiles. Power Marker (ver 3.0) was used to analyze the data and calculate the Major allele frequency, Genetic diversity, and Polymorphic information content (PIC) indices, all of which were discrete variables. Additionally, the matrix was exported to GenAIEx version 6.41 (Peakall and Smouse 2010) to establish banding patterns and Principle coordinates. POPGENE version 1.13 (Francis and Rong 1999) was utilized to determine the proportion of polymorphic loci in the samples. According to Nei's genetic distances, a genetic link was demonstrated (Nei 1987). The dendrogram displays the results of a dissimilarity analysis conducted with Unweighted Neighbor-Joining and 1000 bootstrap samples.

Table 1. Assessed morphological characteristics in *S. nigrum* populations

Trait	Features
Nature of leaf surface	Hairy, smooth
Nature of leaf margin	Smooth, Sinuate
Fruit color	Purple, red
Leaf shape	Ovate, lanceolate, Diamond

Table 2. Primers used in the study

Primer	Sequence 5' 3'	Tm (°C)	Repeats
SB4-32	fw-ctcggcggttagcacagtcac rv-gcccatagacagacagcaaagcc	59	$(AG)_{15}$
SB6-36	fw-gcataatgacggcgtgct rv-cttccaagtgaaagaaaccatca	60	$(AG)_{19}$
SB6-57	fw-acagggctttagggaaatct rv-ccatcaccgtcggcatct	60	$(AG)_{18}$
SB6-84	fw-cgctctcgggatgaatga rv-taacggaccactaacaaatgatt	58	$(AG)_{14}$
TMS29	fw-ccttgcagttgaggtgaatt rv-tcaagcacctacaatcaatca	55	$(CT)_3$ $(C)_{14}$
TMS 37	fw-ccttgcagttgaggtgaatt rv-tcaagcacctacaatcaatca	55	$(GA)_{21}$ (TA)20
STWIN 12G	fw-tgtygattgtggtgataa rv-tgttggacgtgacttgta	46	$(TGAAA)_2$

Correlation of morphological and genetic data

The genetic profile of each plant sample was independently associated with each morphological character listed in Table 1. To estimate the Principle coordinates, a comparison between morphological characters and genetic diversity was performed using the gel scores of SSR profiles for each sample.

RESULTS AND DISCUSSION

Morphological characteristics

Different plants in the five farms (A, B, C, D, and E) exhibited different berry colors, from purple to red. However, the color of all unripe berries was green (Figure 2). In evaluated samples of black nightshade, leaf margins ranged from complete to sinuous. The leaf surface was either hairy or smooth, with leaf forms ranging from ovate to lanceolate to diamond (Figure 3).

According to Tukey's test (HSD), there was a significant difference between the means of features detected in *S. nigrum* samples from the four geographic locations. At α 0.05, the P-value ranged between <0.001 and 0.009 (Table 3). Significant variations in leaf surface, leaf edge, and fruit color were observed. However, whereas ovate and lanceolate leaf forms demonstrated significant variance, diamond leaf shape did not exhibit significant variation among the populations evaluated.

Figure 2. Mature fruits of *S. nigrum* samples collected from the three regions. A. Large purple fruits, B. Unripe fruits green in color, C. Small red ripe fruits, D. Small purple ripe fruits

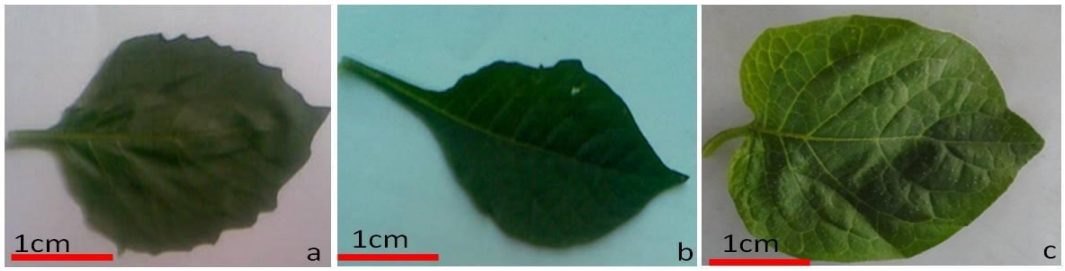

Figure 3. Mature leaves of *S. nigrum* sampled collected. A. Sinuate margin, B, Lanceolate with entire margin, C. Ovate with entire margin

Table 3. Mean number of *S. nigrum* with observed characteristics

Morphological characters		Location				
		Kipkaren	Makuyu	Matanya	Mauche	p-value
Lsurface	Smooth	4.8±0.2^{ab}	5.2±0.4^{ab}	4.2±0.4^{b}	6.0±0.0^{a}	0.009
	Hairy	1.2±0.2^{ab}	0.8±0.4^{ab}	1.8±0.5^{a}	0.0±0.0^{b}	0.009
Lmargin	Entire	4.4±0.2^{ab}	4.8±0.2^{a}	3.2±0.2^{b}	4.4±0.7^{ab}	0.05
	Serrate	1.6±0.2^{ab}	1.2±0.2^{b}	2.8±0.2^{a}	1.6±0.7^{ab}	0.05
Lshape	Ovate	5.0±0.3^{ab}	5.0±0.0^{ab}	6.0±0.0^{a}	4.4±0.4^{b}	0.004
	Lanceolate	1.0±0.3^{a}	1.0±0.0^{a}	0.0±0.0^{b}	1.2±0.2^{a}	0.001
	Diamond	0.0±0.0^{a}	0.0±0.0^{a}	0.0±0.0^{a}	0.4±0.2^{a}	0.083
Fcolour	Red	6.0±0.0^{a}	4.4±0.2^{b}	1.4±0.2^{b}	3.6±0.2^{c}	<0.001
	Psmall	0.0±0.0^{c}	0.4±0.2^{c}	4.6±0.2^{a}	2.4±0.2^{b}	<0.001
	Plarge	0.0±0.0^{b}	1.2±0.4^{a}	0.0±0.0^{b}	0.0±0.0^{b}	0.001

Note: Data of morphological traits assessed in the four regions (Mean ± SE). Mean values followed by the same small letter(s) within the same row do not differ significantly from one another (One-way ANOVA, Tukey's studentized range (HSD)-test, α= 0.05), L-leaf, F-fruit

Genetic characterization

Primer sets SB4-32 and TMS-29 amplified with an annealing temperature of 45°C following the screening of seven primers. When the annealing temperature was increased to 50°C, Primers SB6-84 and SB6-57 were amplified effectively. Only the four primer sets that demonstrated amplification among the analyzed samples were employed on all genomic DNA samples.

Using the four primer pairs Sb4-32, Sb6-57, Sb6-84, and Tms-29, PCR yielded a total of 63 alleles. Primer Tms-29 was the most polymorphic, yielding 17 alleles, while primer Sb6-57 produced 14 alleles. Fragment sizes ranged from 50bp to 3000bp (Table 4; Figure 4).

Genetic diversity estimates

Major allele frequency ranged from 0.8164 (primer Sb4-32) to 0.8783 (primer Tms-29), with an average of 0.8506. Between 14 and 17, a mean of 15.75 alleles was found per primer. The average genetic diversity was 0.2083, with a range of 0.1429 for primer Tms-29 to 0.2324 for primer Sb4-32. In terms of average information content, polymorphisms provided an average of 0.1704. POPGENE version 1.13 found that primer Sb6-57 had the greatest PIC value of 0.1855, and primer Tms-29 had the lowest PIC value of 0.1429 (Table 5).

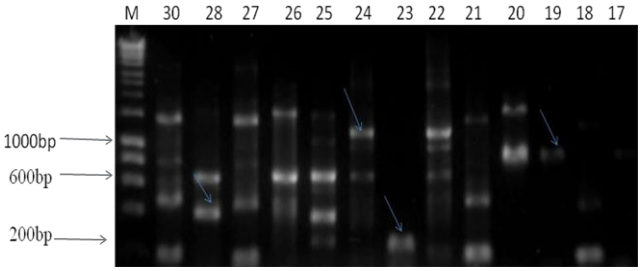

Figure 4. PCR products, amplified with primer Sb6-57 and visualized under UV light. Kipkaren population K17-K30, M: molecular marker, hyper ladder 1kb (Bioline). Polymorphic bands shown by arrows

Table 4. SSR markers used, number of alleles, and annealing temperature

Marker	Allele No.	Annealing temp
Sb4 32	16	45
Sb6 57	14	50
Sb6 84	16	50
Tms 29	17	45
Average	15.75	

Table 5. Allele frequency, allele number, genetic diversity, and PIC for SSR primers used in DNA amplification

Marker	Major allele frequency	Allele No.	Genetic diversity	PIC
Sb4 32	0.8164	16	0.2324	0.1827
Sb6 57	0.8513	14	0.2230	0.1855
Sb6 84	0.8619	16	0.2044	0.1703
Tms 29	0.8783	17	0.1429	0.1429
Average	0.8506	15.75	0.2083	0.1704

Note: PIC-polymorphic information content

All populations showed signs of having distinctive musical styles. Usually, there were between two and six distinct bands. The range of observed polymorphic loci was from 61.90 to 73.02%, with a mean of 68.25% and a standard error of 2.46 percentage points. Standard errors for the mean anticipated heterozygosity (He) varied from 0.015 to 0.019, between a value of 0.131 to 0.150. (Table 6, Figure 5). All primers had a size distribution with a minimum of 50 bp and a maximum of 3000 bp between their smallest and largest bands, respectively.

Partitioning genetic variation

An AMOVA study was carried out in GenAIEx, and the results showed that 86% of the total genetic diversity was present within populations, whereas only 14% was present between populations (Table 7). Furthermore, each and every alteration at the molecular level was statistically significant ($p<0.001$).

Table 6. Total band patterns, % polymorphism, and Mean heterozygosity of *S. nigrum* collected from four study areas

Population	Unique bands	% Polymorphic loci	Mean heterozygosity
Kipkaren	2	61.90	0.135
Makuyu	6	73.02	0.144
Matanya	2	71,43	0.131
Mauche	3	68.25	0.150
Mean	3.25	68.25	0.137

Table 7. Analysis of molecular variance (AMOVA) within and among the *S. nigrum* population

Source	df	Ss	ms	Est var.	%	P value
Among regions	2	72.245	36.122	0.000	0%	0.001
Among populations	3	37.518	37.518	1.061	14%	0.001
Within pops	109	754.098	6.794	6.794	86%	0.001
Total	114	863.861		7.864	100%	

Note: df. – degree of freedom, SS – the sum of squares, MS – mean squares, *p* – level of significance

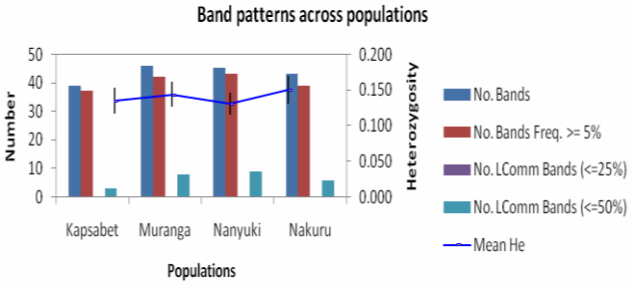

Figure 5. Band patterns of the populations showing the number of bands, bands with frequency >=5%, and mean heterozygosity in each population

Population cluster analysis

According to the findings of the PCoA run with GenAIEx, PC1 was responsible for 23.35% of the overall population, while PC 2 was responsible for 21.15% (as shown in Figure 6). On the other hand, based on the percentage of patterns seen on the axis, PCoA was only moderately effective. The Makuyu samples tended to congregate along the 1st axis, whereas the Kipkaren and Matanya samples tended to congregate along the 2nd axis.

Binary data was used to generate a similarity matrix. By calculating genetic distances between the four populations, Nei could determine their ancestry (Nei 1987). The readings were between 0.010 to 0.020, with lower numbers indicating a stronger connection. The Kipkaren and Matanya people shared the most DNA (0.010). Makuyu and Mauche, two *S. nigrum* populations located in the Central area and the Rift valley, respectively, showed the lowest similarity (highest diversity) of any studied populations. Based on the dissimilarity matrix cluster analysis, a phylogenetic tree was displayed via Dice Unweighted Neighbor-Joining (Figure 7).

Black nightshade samples from different populations were closely related, as measured by their small pairwise distances. It ranged from a minimum of 0.09 dissimilarity to a maximum of 1 dissimilarity. Between the initial tree and the bootstrapped tree, the distance between the edges was 0.8345.

Correlation of morphological characters and genetic data

Neither sinuate nor whole leaf margin samples clustered together. However, PCoA did not show any clear clumping (Figure 8). Axis 1 contributed 23.35 %, whereas axis 2 contributed 21.15 %.

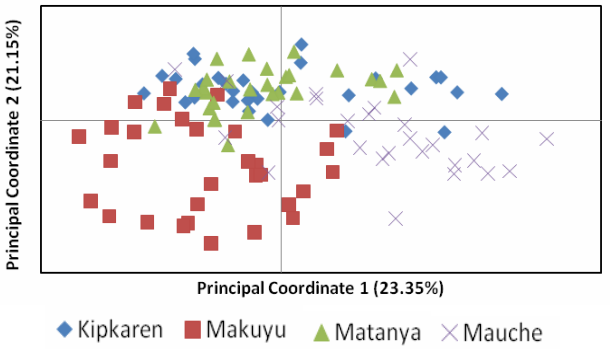

Figure 6. Clustering of *S. nigrum* population as revealed by Principal Coordinate Analysis. Axis 1 and 2 account for 44.65% of the variance in the population distribution

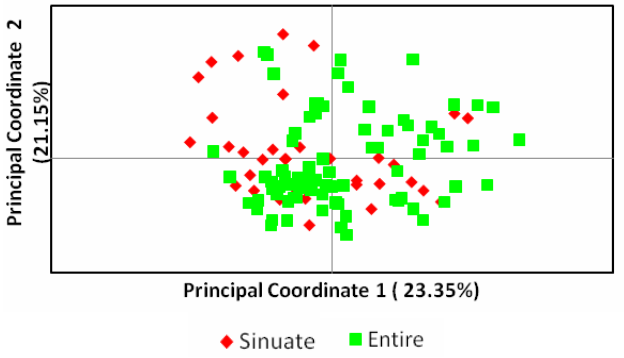

Figure 8. Principle coordinates analysis showing the relationship between the leaf margin and genetic data. PC 1 and 2 account for 44.50% of the variance in *S. nigrum* samples

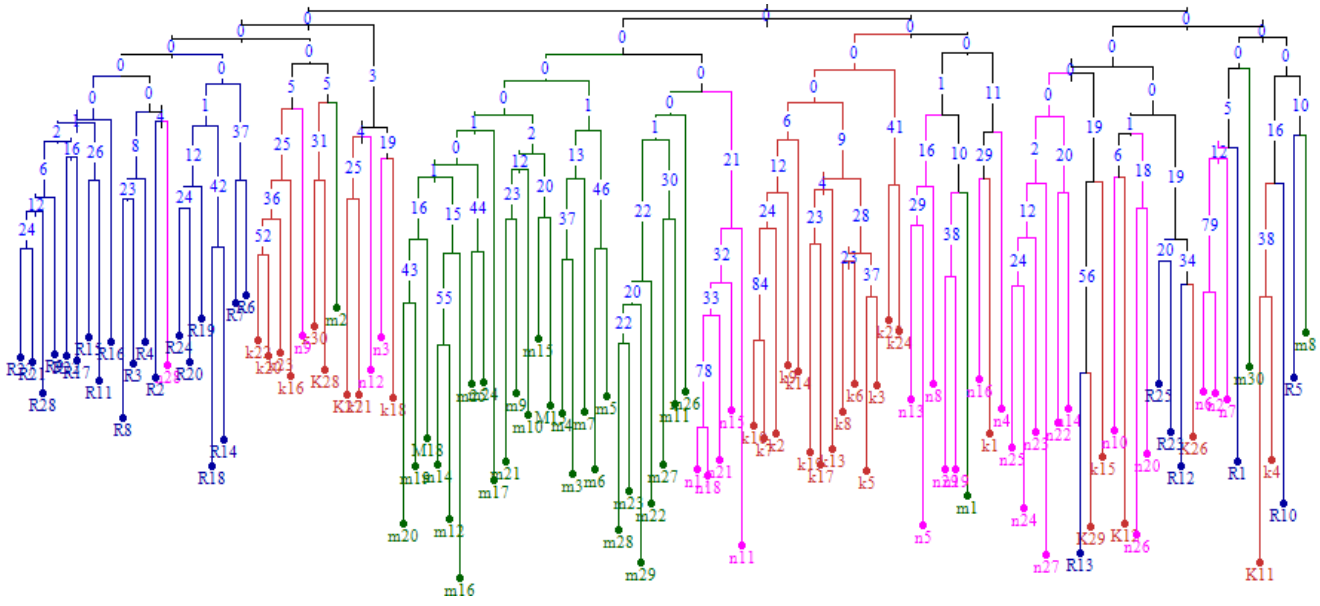

Figure 7. Phylogenetic relationship among *S. nigrum* populations. Unweighted Neighbor Joining with 1000 bootstrap values in DARwin 6.0.5 (Perrier and Jacquemoud 2006)

Some grouping was observed between the character of the leaf surface and genetic data (Figure 9). Samples with a hairy leaf surface cluster closely with a few outliers, indicating a tight genetic relationship. Axis 1 exhibited 23,35% variation in the examined samples, whereas axis 2 revealed 21,15%.

In contrast, leaf shapes did not exhibit different grouping tendencies. Axis 1 explained 23.47% of the variance, while axis 2 explained 20.83% (Figure 10).

Small purple fruits clustered along one axis, whereas the remainder lacked any distinct grouping (Figure 11). Axis 1 accounted for 23.15 % of the variation, while axis 2 accounted for 19.67%.

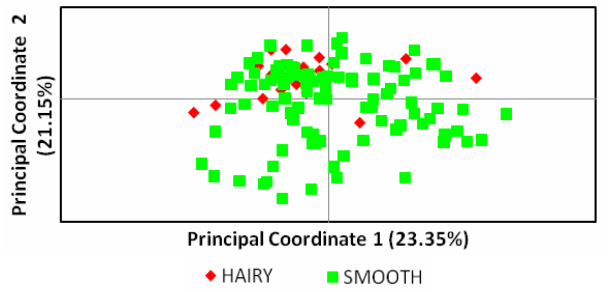

Figure 9. Principle coordinate analysis of leaf surface against genetic data. Axis 1 and 2 account for 44.50% of the variance in *S. nigrum* samples

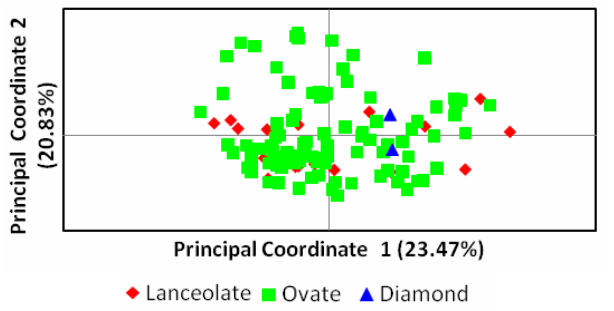

Figure 10. Principle coordinates analysis of leaf shape against genetic data. Axis 1 and 2 account for 44.30% of the variance in *S. nigrum* samples collected

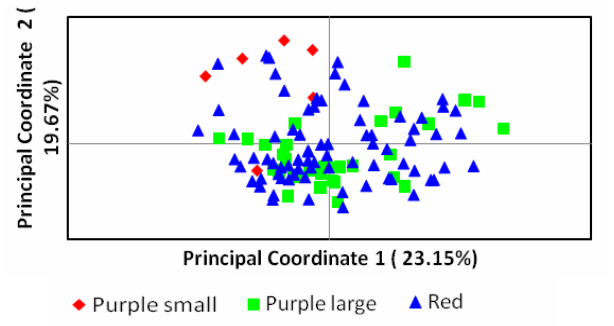

Figure 11. Principle coordinates analysis of fruit color against genetic data. Axis 1 and 2 account for 42.52% of the variation in *S. nigrum* samples

Discussion

It was found that the evaluated *S. nigrum* samples displayed substantial morphological variation. Per Nandhini et al. (2014) morphological characters showed changes in the nature of the leaf surface, leaf border, and fruit color at p = 0.009, 0.005, and 0.001, respectively. While there was significant variance in ovate and lanceolate leaf shapes, Manoko et al.'s (2008) hypothesis that diamond leaves would show little variation was not supported by the data (p = 0.083). Such heterogeneity may have arisen due to the widespread distribution of seed stock and the subsequent adaptation of plant breeds to local environmental conditions. Qualitative features can be used more accurately than quantitative characters to infer the genetic relationship between nightshades (Olet 2004).

Sixty-three polymorphic bands, ranging from 14 to 17, were seen per primer. In addition, 232 bands ranging from 1 to 13 bands were obtained using 7 SSR primers in a 2012 study conducted in South Africa. Using RAPDs, we found 65 polymorphic bands, with band sizes ranging from 600 bp to 3000 bp (6-13 bands per primer) (Stracke and Hammer 2008). At least 90 SSR bands were discovered by Elena et al. (2010), with primers yielding an average of 6.0 bands. Allozymes employed in the same study showed a mean of 2.8 bands across all loci. There were issues with spurious high-molecular-weight bands (up to 3000 bp), as reported by Angeline et al. (2010). These bands likely originated from either substantial insertion in the flanking sequence or non-specific priming. Because the primers were developed for species outside the *S. nigrum* complex, the inserts would have been to blame for the high yields (Angeline et al. 2010). The SSR markers found 13 distinct bands. The presence of these bands, which are unique to certain populations, was found in abundance in Makuyu samples. In this study, SSR markers performed better than other methods at identifying distinct subpopulations among the *S. nigrum* cultivars produced in Kenya.

The average major allele frequency was 0.8506, ranging from 0.8164 (Marker Sb4 32) to 0.8783 (Marker Tms 29). Allele frequency measures genetic variety at the individual, population, and species levels. Allele frequency describes how frequently one particular gene's allele occurs. Estimates for genetic diversity have a wide range, from H = 0.1429 (Marker Tms 29) to H = 0.2324 (Marker Sb4 32), with an average of H = 0.2083. They were low but consistent with Péter and Hyvönen's (2011) research. Farmers may reduce genetic diversity by selectively breeding for desired features while ignoring negative ones. It results in plants that are essentially similar genetically. Crops with little to no genetic diversity cannot respond to shifting climates and are highly vulnerable to epidemic illnesses (Stracke and Hammer 2008). For marker loci in linkage analysis, polymorphism is measured by the polymorphism information content (PIC). Markers' PIC values, which can be interpreted in light of their allele, ranged from 0.1429 (Marker Tms 29) to 0.1855. (Marker Sb6 57). In genetic diversity studies, a genetic marker's value is quantified by its PIC. The number of details obtained from a given experiment is proportional to the number of primers utilized and the number of plants tested.

The Makuyu sample has the highest proportion of polymorphic SSR loci (73.02%) and the smallest proportion (61.90%). (Kipkaren). In the Atacama Desert of Peru and Chile, *S nigrum* populations revealed 80% polymorphic loci when analyzed with SSR. Researchers found that *S. nigrum* populations have 91.7% polymorphic bands using AFLP analysis (Manoko et al. 2008). The high polymorphism levels seen in this study may be linked to the polyploidy of *S. nigrum* (Patricia et al. 2011). Due to the strong reproductivity of SSR markers, a single person can exhibit many alleles at a given location. Success in their new environment can be attributed to a high genetic polymorphism, the outcome of recombinations between ancient polyploids and diploids (Péter and Hyvönen 2011). The widespread reports of polyploidy and hybridization in *S. nigrum* suggest that these processes contribute significantly to the species' genetic diversity.

As a better indicator of variety, average heterozygosity is the better statistic. He=0.1370 was the mean heterozygosity. These findings are consistent with Elena et al.'s (2010) prior determination of He=0.1450. Low levels of heterozygosity were detected by genetic markers; this may be due to inbreeding, which is actively encouraged by farmers to preserve certain features with agronomic value.

It was determined if there was a hierarchy of variation among individuals using AMOVA to partition the genetic distance between populations. There was more genetic variation between people inside a community than between other populations or geographical areas. An analysis of molecular variance (AMOVA) using an SSR marker revealed substantial variation (86%) among individuals within a community. For example, the SSR studies conducted on pepper by Ganapathy et al. (2012) showed a variance of 59.51% across individuals but only 6.55% within areas. Poczai et al. (2010) hypothesized that reproductive and genetic isolation across *S. nigrum* populations would lead to high levels of variation within individuals. Cross-pollination is a widespread occurrence in *S. nigrum*, and it may be the cause of intrapopulation variation (Sarah 2009).

Separate cluster analysis was conducted on SSR markers, and the resulting principal coordinates analysis (PCoA) revealed unique patterns of variation among SSR markers. Almost half (44.50%) of the variance in SSR could be accounted for by just two main axes of differentiation (PCoA 1 and PCoA 2). The separation of samples reflects the level of genetic diversity present in the population. People who were near together were linked more closely, while those who were further apart were assumed to be unrelated or unrelated (Ojiewo et al. 2013). Populations may not have clustered because of gene flow or because DNA markers were chosen randomly.

Consequently, when two gene pools are combined, there is less genetic diversity among the resulting populations. That's what research by Manoko et al. (2008) found, too. Materials from Uganda, Indonesia, and Europe cluster similarly (Olet 2004). The lack of genetic diversity is reflected in a lack of regional grouping. It has been noticed that the plants belonging to the *S. nigrum* complex (Angeline et al. 2010).

Approximately 0.010–0.020 genetic-distance units were observed. The genetic distance between Makuyu and Mauche is the greatest, with Kipkaren and Matanya being the closest. Recent findings by Angeline et al. (2010), showing a genetic distance between 0.33 and 0.55, corroborated this finding of a low genetic distance. It is indicative of the population density and density of the surrounding areas. However, Schippers (2002) found a fairly large gap between *S. nigrum*, while Mwai et al. (2007) found a rather small one. There may be little genetic diversity because of inbreeding. However, several samples from the same area clustered together, suggesting they have a high degree of genetic similarity.

Disparate grouping patterns were seen for morphological traits and genetic information. There were no obvious correlations between leaf margin variation and genetic information. The coordinate axes of the sinuous and smooth leaf margins were nearly identical. Samples' modest genetic distance may explain why they don't cluster. Samples with a hairy leaf surface concentrated along one axis, which is a strong indicator of their genetic similarity. The lack of a clear grouping pattern in the leaf shapes indicates that the sample under study was of high genetic similarity. Still, some clustering was evident in the fruit color, with small purple fruits tending to group together. While there was no link between leaf margin and leaf shape and genetic data, there was a strong correlation between leaf surface and fruit color. It indicates that samples with small purple fruits are genetically connected.

This fuzzy connection suggests that leaf margin and leaf shape are not particularly useful for species identification (Dehmer and Hammer 2004). Similarly, *Solanum villosum* Mill yielded the same outcomes (Manoko et al. 2008).

These findings sometimes support the morphological classification, but in other instances, they reveal striking discrepancies between the two. SSRs are randomly chosen, and environmental factors influence the shape of their offspring (Manoko et al. 2008). Evidence from Katarina (2003) suggests that a given trait may be genetically determined in some species while remaining phenotypically malleable in others. The *S. nigrum* may not be primarily self-pollinating, which could explain why there is variance even among members of the same species.

The data suggests no statistically significant variations in physical and genetic traits across the samples. Farmers' selection for varying plant varieties may explain the discrepancy between morphological and genetic variances.

In conclusion, the *S. nigrum* samples used in the analysis displayed a wide range of morphological diversity. The observed levels of variation may help characterize the genetic traits of *S. nigrum*. (i) The genetic diversity of *S. nigrum* species gathered from the designated regions of Kenya is low. (ii) Variance within *S. nigrum* populations was significantly higher than the variation between *S. nigrum* populations and between areas. (iii) The observable physical traits of the *S. nigrum* complex are unrelated to the genetic variation in this species. (iv) The findings of this study indicate that disparities in the agro-ecological zone do not automatically imply disparities in the *S. nigrum*

species cultivated. (v) SSR Marker systems may benefit breeding by allowing for easier discrimination between closely related individuals. (vi) Samples from the Mauche population are genetically distinct from any other community. (vii) The *S. nigrum* complex consists of a collection of plants that are closely linked to one another.

REFERENCES

Anas J, Yoshinda L. 2004. Genetic diversity among Japanese cultivated sorghum assessed with simple sequence repeats markers. Plant Prod Sci 5: 242-247.

Angeline V, Maryke L, Elizma K. 2010. Microsatellite-based assessment of five *Solanum nigrum* complex species and their progeny. Soil Plant Sci 60: 494-499. DOI: 10.1080/09064710903233839.

Barkley N, Roose M, Krueger R, Federic C. 2005. Assessing genetic diversity and population structure of citrus germplasm collection utillising SSR. Theor Appl Gen 112: 519-1531. DOI: 10.1007/s00122-006-0255-9.

Chweya S. 1997. Identification and nutritional importance of indigenous vegetables in Kenya. Afr SympHortic Crops 2: 142-169.

Dehmer J, Hammer K. 2004. Taxonomic status and geographical provenance of germplasm accessions in the *Solanum nigrum* L complex, AFLP data. Genet Resour Crop Evol 5: 551-558. DOI: 10.1023/B:GRES.0000024163.86762.fc.

Edward C, Charles B. 1990. Crossing relationships among diploid species of *Solanum nigrum* complex in North America. Am J Bot 66: 709-716. DOI: 10.1002/j.1537-2197.1979.tb06275.x.

Elena A, Miguel E, Roger T. 2010. Genetic diversity and population structure in the tomato like nightshades – *Solanum lycopersicoides* and *Solanum sitiens*. Ann Bot 105: 535-554. DOI: 10.1093/aob/mcq009.

Falck ZJS. 2010. Weed-An Environmental History of Metropolitan America. American Historical Review, University of Pittsburgh, Pennsylvania.

Francis C, Rong C. 1999. Population Genetic Analysis (POPGENE ver. 1.31).

Ganapathy K, Gomashe S, Rakshit S, Ambelkar S, Biradar B. 2012. genetic diversity revealed utility of SSR markers in classifying parental lines and elite genotypes of sorghum. Austral J Crop Sci 6: 1486-1493.

Grieve M. 2012. A modern herbal. Botanical.com, 2.

Heather M, Matthew R. 2012. Genotyping with molecular markers: Scoring a molecular marker on an agarose gel. Plant Breed Genom 3: 25-34.

Jagatheeswari T, Bharathi H, Jahabar A. 2013. Black nightshades (*Solanum nigrum* L.) An updated overview. Intl J Pharmac Biol Arch 4: 288-295.

Jennifer M, James A. 1977. Black nightshades-*Solanum nigrum* L. and related species. Promoting Conservation and Use of Underutilized and Neglected Crops. International Plant Genetic Resources Institute, Rome, Italy.

Juma C. 1989. Biological Diversity and Innovation: Conserving and Utilizing Genetic Resources in Kenya. Africa Centre for Technology Studies, Reseaech Series, Nairobi, Kenya.

Katarina B. 2003. Phenology and Shadow Response of *Solanum physalifolium* var *nitidi-baccatum* and *Solanum nigrum* ssp. *nigrum*. [Dissertation]. Uppsala University, Uppsala. Sweden]

Manoko M, Feron R, Van der Weerden M. 2008. Genetic diversity of the African hexaploid species *Solanum scabrum* Mill and *Solanum nigrum* L Solanaceae. Genet Resour Crop Evol 55: 409-418. DOI: 10.1007/s10722-007-9248-z.

Maundu J, Kiminywe M, Ngugi G. 2004. Nutritional and Indigenous Vegetables in Urban and Peri-Urban Agriculture in Kenya. Bioversity International, Rome.

Mutegi E, Sagnard F, Semagn K, Due M, Muraya B, Kanyenji D. 2011. Genetic structure and relationship within and between cultivated and wild sorghum (*Sorghum bicolor*) in Kenya as revealed by microsatellites. Theor Appl Genet 122: 989-1004. DOI: 10.1007/s00122-010-1504-5.

Mwai N, Onyango J, Abukusta, M. 2007. Taxonomic identification and characterization of African nightshades (*Solanum nigrum* L section *Solanum*). Afr J food Nutr Dev 7: 216-232. DOI: 10.18697/ajfand.15.IPGRI2-1.

Nandhini T, Paramaguru P, Vajayakumar A. 2014. Morphological characterization and genetic variability study on Makoi (*Solanum nigrum* L) genotypes. Plant Arch 14: 35-49.

Nei M. 1987. Molecular Evolutionary Genetics. Columbia University Press, New York. DOI: 10.7312/nei-92038.

Ojiewo C, Mwai G, Abukutsa M, Stephen R. 2013. Exploiting the genetic diversity of vegetable African nightshades. Bioremed Biodivers Bioavail 7: 6-13.

Olet E, Kare A, Manfred H. 2011. Amplified fragment length polymorphism analysis of species of *Solanum* section Solanaceae from Uganda. Afr J Biotechnol 10: 6387-6395.

Olet M. 2004. Taxonomy of *Solanum* L Section *Solanum* in Uganda. [Dissertation]. Agricultural University of Norway, Klepp, Jæren. [Norway]

Onyango M. 1993. Effects of plant diversity and harvesting frequency on the yield of vegetable quality of four variant of black nightshades-*Solanum nigrum*. Afr Crop Sci Conf Proc 1237-1240.

Patricia F, Elizabeth A, Paulo C. 2011. Molecular characterization of potato cultivars using SSR markers. Horticultura Brasileira 29: 542-547. DOI: 10.1590/S0102-05362011000400017.

Peakall R, Smouse P. 2010. GenAIEX 6.41: Genetic analysis in Excel. Population genetic software for teaching and research. Mol Ecol Notes 6: 288-295. DOI: 10.1111/j.1471-8286.2005.01155.x.

Perrier X, Jacquemoud-Collet JP. 2006. DARwin Software. http://darwin.cirad.fr/darwin.

Péter P, Hyvönen J. 2011. On the origin of *Solanum nigrum*: Can networks help? Mol Biol Rep 38: 1171-1185. DOI: 10.1007/s11033-010-0215-y.

Poczai P, Matyas K, Taller J, Szobo L. 2010. Study of the origin of the rarely cultivated edible *Solanum* species: Morphological and molecular data. Biologia Plantarum 54: 543-546. DOI: 10.1007/s10535-010-0096-x.

Sarah D. 2009. The systematic and Genetics of Tomatoes on the Galapagos Islands (*Solanum*, Solanaceae). [Dissertation]. University College London, London. [UK]

Sayed A, Fereshteh M, Mehdi R. 2012. Phenolic compounds and antioxidant activity from saffron (*Crocus sativus* L) petal. J Agric Sci 4: 175-181. DOI: 10.5539/jas.v4n10p175.

Schippers R. 2002. African indigenous vegetables, an overview of the cultivated species. Revised version on Natural Resource International Limited, 252.

Stracke G, Hammer K. 2008. Genetic Diversity in the Collection of *Solanum nigrum* L. in the Gatersleben Genebank. Institute of Plant Genetics and Crop Plant Research (IPK) Genebank Corrensstr. 306466 Gatersleben.

Sue P, Grant L, Bernard R, Baun M. 1997. Mordification of a CTAB DNA extraction protocol for plants containing high polysaccharide and polyphenol components. Plant Mol Biol 15: 8-15. DOI: 10.1007/BF02772108.

Tindall H. 1977. African leafy vegetables in South Africa. Agric Res Counc 1: 317-332.

Wang M, Barkley N, Jenkins T. 2009. Microsatellite markers in plants and insects. Part I: Applications of biotechnology. Genes Genomes Genet 3: 54-67.