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CHARACTERIZATION AND EVALUATION OF PUMPKIN (*Cucurbita moschata* Duch.)

Kiramana, J.K., Isutsa, D.K and Nyende, A.B.

Chuka University, P. O. Box 109-60400, Chuka

Institute of Biotechnology Research, JKUAT, P. O. Box 6200-00200, Nairobi

Email: kirimijk@yahoo.com, dorcaki@yahoo.com ; abnyende@hotmail.com; jkiramana@chuka.ac.ke; disutsa@chuka.ac.ke;

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ABSTRACT

Pumpkin is an emerging important indigenous vegetable in Kenya. However, its potential remains unexploited. This study collected, characterized and evaluated pumpkin germplasm accessions in Kakamega and Nyeri Counties. The 155 accessions collected consisted of 70 and 85 from Kakamega and Nyeri Counties, respectively. Morphological characterization and evaluation was done on-farm in completely randomized design, replicated three times. Morphological characterization and evaluation data were subjected to analysis of variance using the SAS program. Means were separated at P=0.05. Molecular characterization was done by extracting DNA, and polymerase chain reaction was done on 139 accessions using SSR and ISSR primers. Molecular characterization data were scored and phylogenetic analyses conducted using DarWin software. Significant variation ($P<0.05$) resulted among 146 accessions morphologically. Fruit length to width ratio and fruit weight ranged from 0.7-2.1 cm and 0.5-19.25 kg/accession, respectively. Predominant mature fruit skin colour ranged from green to orange; secondary fruit skin colour from speckled to striped; fruit surface from smooth to warty; and internal flesh colour from white to yellow. Main colour of inner flesh and of outer flesh ranged from yellow to pink-red. Molecular characterization revealed that accessions were varied with amplifications on different loci ranging from 100-500bp for SSR with band ranges of 1 to 4. ISSR primers were more polymorphic with accessions amplified between 200-2000 bp with band ranges of 2 to 7. A total of 526 alleles were identified with 5 SSR primer pairs and 509 alleles with 7 ISSR primers. The Unweighted Pair

Group Method of Arithmetic Mean based on Euclidean genetic distance with 1000 bootstraps constructed the dendrograms. There is great variation in pumpkins in Kenya. Cultivars are interbreeding a lot. Conservation of naturalized germplasm needs to be expedited to save it from further distortion and extinction.

Key words: *Characterization, Dendrograms, Genetic erosion, Mother trials, Pumpkin genotype, Pumpkin phenotype, Plant Biotechnology*

INTRODUCTION

Pumpkin is an emerging important indigenous vegetable in Kenya; Fruit, leaves and the flowers are edible (Robinson & Decker-Walters, 1997). Seeds are used as a vermifuge, and contain fatty acids and phytosterols for treating benign prostatic hyperplasia (Dvorkin & Song, 2002). Crushed, fresh seeds inhibit helminthes, skin infections and inflammations (Guha & Sen, 1998). Pumpkins supply calcium, iron, vitamin A, oil, and protein (Oswell et al., 2007). Pumpkin fruits can be stored for up to 6 months (Mendlinger et al., 1991). Pumpkin diversity contributes to stability and sustainability of farming systems, and products of cultural and socioeconomic importance (Virchow, 2003). Diversity is the main source for current crop improvement and adaptation to changing environmental conditions. Loss of biodiversity presents serious threat to agriculture and livelihood of mankind (Mohamed & Zakri, 2001). Numerous germplasm has already disappeared or is threatened with extinction due to absence of concerted efforts to collect, characterize, evaluate, document and conserve (Mohammed & Zakri, 2001). Therefore, germplasm collection, characterization, conservation and improvement deserve priority (Grubben & Chigumira, 2004). The major task, however, is to search for unique genetic traits that will provide the future breakthroughs for agricultural and industrial use of plant germplasm resources (Thomas & Mathur, 1991).

The challenge in agriculture today is to develop seed production and delivery systems that encourage wider use of quality seed throughout the marketing chain (Ayieko & Tschirley, 2006). Currently the indigenous pumpkins are on high demand because consumers have become increasingly aware of the nutritional and medicinal values of pumpkins, and the demand has been on the rise especially in major urban centres in Kenya. This has created demand especially in both formal and informal markets in the urban centres (Ngugi et al., 2007). The major hindrance to the production of pumpkins is lack of quality seed (Abukutsa Onyango and Onyango, 2005), coupled with the inability of the formal seed system to meet the demand by farmers (Nyoro & Ariga, 2004). With increasing demand for local pumpkin particularly in urban and peri-urban, there is a need for increased production and this call for good quality seed for increased pumpkin yields (Abukutsa-Onyango, 2007).

Statement of the Problem

Agricultural production has lost about three-quarters of its genetic diversity in the past century (Ekesa, 2009). Conventional agriculture has, to a large extent, concentrated on conserving the genetic resources of exotic rather than indigenous vegetables (Keding et al., 2007). Consequently, the latter are threatened with extinction (Keding et al., 2007). Indigenous knowledge on production methods, preservation, use and nutritive value is no longer transmitted systematically from one generation to another (Keding et al., 2007). Limited grassroots surveys have been undertaken to collect the valuable indigenous knowledge with the farmers on productivity and use (Chweya, 1994; Onyango, 2002a), occasioning pumpkin landraces to remain under-exploited and poorly documented (Hamisy et al., 2002). In Kenya, improvement of pumpkin is constrained by lack of evaluation, characterization and selection for desirable traits. This is because pumpkins have been neglected by researchers, policymakers and funding agencies. Consequently, naturalized pumpkins are threatened with extinction and erosion through cross pollination with introduced exotic cultivars. Most of the naturalized pumpkins are not easily available as users now gather them with great drudgery and difficulty from the few stands left in the wild (Adebooye et al., 2003).

The informal seed sector lacks official interventions, oversight, research and sophisticated infrastructure for quality control. Seed scarcity contributes to low pumpkin productivity. Introduction of exotic pumpkins in mainstream agriculture has seen these naturalized ones only used minimally during famine and by the poor (Mnzava, 1997). Consequently, their potential remains unexploited, contributing to micronutrient malnutrition, especially among resource-poor families (Muthoni et al., 2010). The overall major constraints facing production of pumpkins in Kenya include low awareness of their potential, lack of quality seed, information on utilization and documentation of the priority landraces, among others (Maundu et al., 1999; Onyango, 2002b). These constraints notwithstanding, Kenya is endowed with great diversity of conditions suitable for pumpkin production. The study was aimed at contributing to solving some of these constraints by gathering pumpkin germplasm for characterization, evaluation, multiplication and conservation of seeds of preferred cultivars for commercial pumpkin production in future.

Research Justification

Pumpkins have an advantage over other vegetables in that the fruits can be stored for up to 6 months and can play an important role in poverty alleviation and maintenance of nutritional levels during the long dry seasons, when fresh vegetables are not available. In Kenya, primary agricultural production has neglected AIVs and is dominated by mixed farming of a few commodities (Nyangito, 1998). Pumpkins could contribute to reversing these narrow trends, because they have a considerable potential as income earners (Onyango, 2002a), particularly for the smallholders who account for over 65% of the total agricultural output. Enhanced knowledge of pumpkins could play a pivotal role in food and nutrition security of the underprivileged in both urban and rural settings (Schippers, 2000 & Onyango, 2002a).

Research indicates that pumpkins are nutrient-rich, grown as intercrops and less demanding in management since their short-growing periods are favourable to mineral nutrition intervention programmes (Onyango, 2003). Initial identification and characterization of collected accessions will provide breeders with considerable amount of information concerning their value in production of new improved cultivars. Evaluation of the indigenous cultivars will facilitate development of more productive and food-value cultivars. Conservation, utilization, improvement and cultivation will save these cultivars from erosion and/or extinction for the benefit of future generations (Chweya, 1997). Availing of production ethno-knowledge and quality seeds will empower small-scale Kenyan communities economically, and in food security (Habwe et al., 2008). A collective seed supply and husbandry is possible through participatory community seed banks that will further farmers' autonomy with regard to timely provision of seeds and conservation of agricultural biodiversity (Ekesa, 2009).

General Objective

The general objective was to identify, characterize and evaluate seeds of dual-purpose *Cucurbita moschata* accessions among smallholder farmers in Kenya.

Specific Objectives

1. Collect pumpkin germplasm in Kenya.

2. Evaluate pumpkin germplasm in Kenya.

3. Characterize pumpkin germplasm in Kenya

Expected Outputs

i. Preferred pumpkin cultivars among smallholder farmers in Kenya determined and selected.

ii. Dual-purpose pumpkin germplasm from two recommended regions in Kenya phenotypically and genotypically characterized.

iii. Dual-purpose pumpkin germplasm from two recommended regions in Kenya evaluated.

BENEFICIARIES OF THE OUTPUTS AND OUTCOMES

1. Small-scale and resource-poor farmers will gain preferred cultivars and quality seeds.

2. Extension officers capacity will be enhanced by availing to them pumpkin information for use in promotion of production and conservation.

3. Biotechnologists and future researchers will utilize the characterization and evaluation results to make, modify, improve or develop pumpkins for specific uses.

4. Seed companies will do business in seed production and sale.

5. Entrepreneurs will benefit through bulk-buying of pumpkins from growers to enjoy economies of scale.

METHODOLOGY

Germplasm was collected in two recommended region service units (RSUs) (Western and Central Kenya). The acquisitions were planted at the Chuka University College (CU) Ndagani Research Farm to provide material for morphological characterization based on Bioversity descriptor lists. Molecular characterization was done at the Jomo Kenyatta University of Agriculture and Technology, Institute Biotechnology Research (IBR) Field evaluation using baby trials was done on farmers' farms in the regions of collection, while mother trials were conducted at Kakamega and Embu, Kenya Agricultural & Livestock Research Organisation (KARLRO) research farms.

Germplasm Collection

Pumpkin germplasm collection was undertaken in Kakamega (26th -30th March, 2012) and Nyeri (16th - 20th April, 2012). Initial information on germplasm was obtained from Provincial Directors of Agriculture from each region. A diagnostic survey and formal surveys were conducted in the two regions with the help of ministry of Agriculture officials in both counties. Participatory Rural Appraisal (PRA) techniques were incorporated in the surveys, and included key informant interviews and checklists. Key informant interviews were based on IPGRI collection descriptors and were used to obtain specific information on practices, problems, ethno-botanic knowledge, constraints and opportunities in pumpkin production and utilization. The key informants were selected with the assistance of Field Extension Officers (FEOs) of the Ministry of Agriculture. Seventy five farmers identified through purposive sampling with were sampled in both counties on the basis of their interest and the constraints that needed to be addressed. Known pumpkin farmers were deliberately included and helped in the identification traditional pumpkins being grown and any other pumpkin introductions. The seeds and fruits of the landraces were collected and labeled with a date, place of collection, and number of accession. In cases where there were no seed stored, Fresh seeds, were extracted from pumpkin fruits washed and air-dried under room temperature. The seeds were placed in labeled packages for easy transportation.

Morphological Characterization

The collected accessions were planted in a 2 x 'n' factorial arrangement in a completely randomized design on-station at CU Research Farm, whereby 'n' refers to the number of accessions up to a maximum of 155. The land where the accessions were planted was prepared well to a fine tilth for easy and uniform germination. Five plants per accession were selected and tagged for morphological characterization and evaluation based on IPGRI descriptors for Cucurbits. Certain characters not in the IPGRI list were added (Fruit Flesh Thickness) and others in the IPGRI list were not be measured. Planting for morphological characterization was done on 23 may, 2012 and recording of vegetative characteristics, commenced 20 days after emergence. Plants for morphological characterization were selected randomly and each accession represented a plot. The characters measured included vegetative, stem, root, inflorescence, fruit and seed characters.

Accession Evaluation

The collected accessions were planted in a 2 x 'n' factorial arrangement in a completely randomized design with three replications, both on-station and on farmers' farms, whereby 'n' referred to the number of accessions. Exotic cultivars were used as controls. Evaluations were done for 2 seasons. All accessions were planted at a standard spacing of 2 m x 2 m. Land was ploughed and pulverized into fine tilth. Farmers' practices were followed during plant management. Data recording was undertaken 20 days after planting up to maturity. At maturity different types of plants observed were harvested individually. Each plant selected and harvested in the previous season was planted again. Data on plant characteristics, leaf and fruit yields was recorded again to obtain plant characteristics, leaf and fruit yields stability. Mother trials were conducted to evaluate growth and yield. The Mother trials had all the cultivars planted in 2 locations, with 3 replicates per location. The locations were the Kakamega and Embu KALRO research stations. The entries were compared using matrix ranking through focus group discussions.

DNA Extraction and Quantification

Leaf samples from the tips of young and healthy actively growing pumpkin plants, 5 per accession up to a total of 139 accessions (65 and 74 Kakamega and Nyeri counties respectively), were plucked and used to extract DNA. Seven day old leaf samples were picked, labeled and placed in cool box with ice bags in it immediately and there after transported to the Jomo Kenyatta University of Agriculture and Technology, Institute Biotechnology Research (IBR) laboratory and stored in a refrigerator at -25°C. The samples were arranged in split plot factorial with counties and accessions representing main plots and subplots, respectively. Prior to DNA extraction the leaf samples were stored at -85^{0}c refrigerator to facilitate crushing of the leaf samples to powder form instead of using liquid nitrogen. The mortar and pestles to be used for crushing were soaked in a Jik detergent for 30 minutes and thoroughly washed and first rinsed in cold water and then warm water. The mortar and pestles were then wrapped with foil

paper and autoclaved for 30 minutes to sterilize them to eliminate any possible contamination which could interfere with the results validity. The leaf samples were crushed into powder form and placed in sample tubes and then stored at -85^{0}c again to avoid any enzymatic reaction which could interfere with DNA extraction process. A new mortar and pestle was used for every sample then Soaked in bleach water for at least ½ hour before rinsing and autoclaving. DNA was extracted using the method described by Doyle & Doyle, (1987) and quantified using the NanoDrop-1000 spectrophotometer (Beauman, 2007). CTAB DNA Extraction Protocol was used for DNA extraction.

The Agarose Gel Electrophoresis

To confirm the presence of DNA strands from the sample extract, horizontal gel electrophoresis was conducted. Gels were prepared such that it was 0.8-1% agarose by weighing out approximately 0.8-1g of the agarose powder. The amount of agarose depended on the size of the gel rig. Gels were fairly thin, approximately 1/4 to 1/2 inch. 100 mL 1X TAE or 1X TBE buffer was added to the agarose powder. Ethidium bromide 1 ug/ul (1/10th ul 10 mg/ml etBr per ml of agarose) was used. A 5 µl drop of bromophenol blue or glycerol loading dye was used to stain the DNA samples. A maximum 20 and a minimum of 12 wells were used to load the stained DNA samples. Samples were loaded and ran at constant voltage towards the anode (red) end. For a small gel, the power pack was set to about 100 volts/ ma and a large gel, about 120 volts/ma. Milliamperage/ voltage increased during the run, so it was checked periodically and stopped the run before the bromophenol blue or glycerol loading dye front exited the gel. The power pack was turned off, the gel removed and visualized with UV light (taking proper precautions!) to visualize the presence of DNA in the sample extracts and photographed with a Photo documentation camera. The gel was then disposed off properly into carcinogenic disposal tanks for further proper disposal of ethidium bromide safely. The samples which had no DNA visualized the DNA extraction and electrophoresis procedure were once repeated until DNA was visualized and then samples stored at 4^{0}c for the Polymerase chain reaction (PCR) amplification using the simple sequence repeats (SSR) and inter-simple sequence repeats (ISSR) primers

Molecular Characterization

Plants of 139 accessions were subjected to PCR using 5 pumpkin SSRs primer pairs and 7 ISSR for the polymorphism survey (Table 1).

Table 1: SSR Primer Pairs

Primer Name	Primer sequence (5' to 3') Forward	Length	Primer sequence (5' to 3') Reverse	Length
PKCT 47	GGTCCCAATAATAGCAACCAA	21	GTGGGACACATCTTGAGCA	19
PKCT 62	GAAGTTCGTGGTCTGTGCAAGTC	23	CCTGAGTAACCTCCGTGCTTCC	22
PKCT 111	GTTGCAGCGACCGTTCTTCTTC	22	GCATCTGAAGACGATGCGTCGT	22
PKCT 122	CTAAACAGGATGCCTCTGACAC	22	CGGGATTTCCGAAACAACGT	20
PKCT 133	TCGGAATCGTCTTCAGCAATAGTC	24	TCCTCTTCCATTCCACTTTCTCCT	24

Length variation was the source of polymorphisms in SSR loci and could be identified by the PCR using primer pairs specific to the flanking SSR regions. The SSRs were distributed throughout a genome, and were proven to be useful as genetic markers and for cultivar identification (Watcharawongpaiboon and Chunwongse, 2007). Seven ISSR primers based on dinucleotide, tetranucleotide or pentanucleotide repeats was used in ISSR analysis (Table 2).

Table 2: ISSR Primers

Primer Name	Primer sequence (5' to 3')	Length
ISSR 814A	CTC TCT CTC TCT CTC TTG	18
ISSR 844A	CTC TCT CTC TCT CTC TAC	18
ISSR 844B	CTC TCT CTC TCT CTC TGC	18
ISSR 17898A	CAC ACA CAC ACA AC	14
ISSR 17898B	CAC ACA CAC ACA GT	14
ISSR 17899A	CAC ACA CAC ACA AG	14
ISSR 17899B	CAC ACA CAC ACA GG	14

ISSR, which depend on PCR, rapidly differentiated closely related individuals. The method called ISSR was based on the amplification of DNA region located between two microsatellites locus. When the primers successfully located two microsatellite regions within an amplifiable distance away on the DNA strands, the PCR reaction generated a band of a particular size for that locus (Heikal, et al., 2008). PCR amplifications was performed with a Gene-Amp PCR system 9700 in a 10 µL final volume containing a PCR mixtures Primers were tested and optimized with 2 randomly selected accessions (KAPAP/CU/JKK/NY-135 for SSR and KAPAP/CU/JKK/KK-95) by PCR amplification. PCR reactions were performed with a stock solution containing 30ng/µl of DNA, 10X PCR buffer, 2.5 mM dNTP, 10 mM MgCl2, 5.0 pmoles /µl each of forward and reverse primers and 5 U/µl of *Taq* polymerase. The PCR per reaction was 0.5µl of DNA, 0.5 µl PCR buffer, 0.4 µl dNTP, 0.4 µl MgCl2, and 0.5µl each of forward and reverse primers, 4.2 µl of *Taq* polymerase making a total volume of 10 µl, 3.0µl H2O for SSR and 3.5µl H2O for ISSR. The micro tubes were placed in a thermal cycler (a Gene-Amp PCR system 9700 and the thermo cycling reactions done in the following scheme: Initial denaturing at 94°C for 3 minutes, 30 cycles of 30 seconds at 94°C, 1minute for 55°C, and 2 minutes at 72°C, elongation at 72°C for 20 minutes and a final hold at 4°C for SSR and Initial denaturing at 94°C for 3 minutes, 35 cycles of 30 seconds at 94°C, 1 minute for 47°C, and 2 minutes at 72°C, elongation at 72°C for 20 minutes and a final hold at 4°C for ISSR.

Data Collection and Analysis

Photographs were taken during all stages of collection, characterization and evaluation. The collected data was arranged in nominal, ordinal and continuous categories. Nominal and ordinal data was expressed as modes, whereas the mean was used for continuous data. Measures of dispersion were used to express diversity within accessions for each character. Standard deviation and the range were used to express the accession diversity and variability. Frequency of the mode was used as an indication of variation within accessions. Data was scored as the presence (1) or absence (0) of SSR and ISSR bands. Only sharp and precise bands were scored to generate a data matrix.

Bands present in all accessions were scored as 1 for present and 0 for absent of bands. Distance matrices for all pairs of genotypes were constructed from the SSR and ISSR data matrix using the Euclidean distance method (Rousseeuw & Kaufman, 1990). Cluster analysis was performed using UPGMA and the genetic distance matrices generated by the Euclidean distance method with 1000 bootstraps to reveal the patterns of genetic relationships among genotypes. Cluster analysis results were presented in dendrograms to infer relationships among genotypes, with 1000 bootstrap replicates (Felsenstein, 1985). Morphological characterization and evaluation data were subjected to analysis of variance using the SAS program. Means were separated at P=0.05. Molecular characterization data were scored and phylogenetic analyses conducted using DarWin software (Perrier and Jacquemoud-Collet, 2006).

RESULTS AND DISCUSSION

Germplasm Collection: Kakamega and Nyeri Counties.

Collection was conducted from 26th to 30th March and 16th to 20th April, 2012 in Kakamega and Nyeri counties respectively. Purposive sampling was used and first collected germplasm was labeled: KAPAP/CU/KK/JKK/1 and up to last collected accession KAPAP/CU/NY/JKK/155. A total 155 accessions were collected 70 from Kakamega and 85 Nyeri counties. The collection itinerary was as listed in Table 5 in that order.

Table 3: Germplasm Collections Target Areas Description per Sub County

No.	Sub county	Altitude M)	Agroecological Zones (AEZs)	Accessions Codes	No. of Samples
1	Kakamega Central	1441- 1547	UM 0, UM 1, LM 1, LM 2	KAPAP/CU/JKK/KK-1-14	14
2	Kakamega East	1522 -1562	UM 0, LM 1, LM 2	KAPAP/CU/JKK/KK-15-30	16
3	Kakamega South	1478-1534	UM 1, LM 1, LM 2, LM 3	KAPAP/CU/JKK/KK-31-53	23
4	Butere	1383-1417	LM 1	KAPAP/CU/JKK/KK-54-57	4
5	Khwisero	1466-1556	LM 1	KAPAP/CU/JKK/KK-58-70	13
6	Mathira East	1687-1843	LH 1-3, UM 1-3, UH 0, UH 1	KAPAP/CU/JKK/NY-71-97	27
7	Mathira West	1649-1817	UM 2-4, LH 2-3,	KAPAP/CU/JKK/NY-98-108	11
8	Nyeri Central	1767-1858	UM 2-4, LH 1-3	KAPAP/CU/JKK/NY-109-129	21
9	Tetu	1772-2128	UH 1, UM 1-3, LH 1-2	KAPAP/CU/JKK/NY-130-143	14
10	Nyeri South	1870-1950	UH 1, LH 1, UM 1-2	KAPAP/CU/JKK/NY-144-155	12

***UM**-Upper Midland, **LM**-Lower Midland, **LH** Lower Highland, **UH**-Upper Highland and **0**-Perhumid, **1**-Humid, **2**-Sub humid, **3**-Semi humid, **4**-Transitional. Agro-Ecological Zones LH 1-4, UM 1-5 and LM 3-4 occurs at descending altitudes, the zones UH 2, 3 and LH 2, 3, 4, 5 indicate decreasing rainfall already in higher altitudes.*

The traditional landraces collected were 105 and exotic 50. The traditional (105) were always associated with the place and the community, 45 exotic were introduced but the date not known, 2 were borrowed from the neighbours around the county and 3 were introduced by a Non-Governmental Organization (NGO). Ninety collections were from seed, 65 from fruits, and seeds extracted, processed, air dried and packaged. The collections had varied characters for the fruits and seeds. The fruit shapes were ovate (12), Globular/round (18), Elliptical (11), Pyriform /pear-like (6), Flattened (6), Acorn (9) and 3 elongate in shape. The predominant fruit colour was: cream 4, 8 pale green, 16 green, 24 dark green, 5 blackish green, 2 Light yellow, 2 orange and 4 grey in colour. The seeds of local landraces were yellow-white, cream yellow and those from exotic cultivars, light brown or tan to brown colour. The farmers had indigenous technical knowledge which they used to encourage yielding and fruiting of pumpkins. Sixty two percent of the farmers interviewed sold pumpkin fruits and leaves, 37% didn't sell any pumpkin produce.

Morphological and Molecular Characterization

Morphological data was subjected to analysis of variance using the SAS program. Means were separated at P=0.05. Out of 155 accessions collected, 8 failed to germinate and one died prematurely. The remaining 146 accessions data on vegetative, stem, root and Inflorescence characters was recorded. Only 126 accessions were able to form fruits. There was significant variation (P<0.05) among 146 accessions (Table 6).

Table 4: Summary of Morphological Traits

	Leaf Ratio	Days to 1st Flowering	Pendicle Length	Fruit Flesh Thickness (mm)	Fruit L/W Ratio	Days1st Fruit Maturity	Maturation period	Total Fruit wt /Plant (kg)	Stem Thickness (mm)
Min	0.58	49	4.0	10.5	0.6	107	39	0.25	7.9
Max	0.97	87	16.5	42.6	3.0	141	89	19.25	14.9

Sum	112.03	10083	1044.6	3148.4	144.6	16073	7171	493.75	1554.3
Mean	0.7673	69.06	8.290	24.987	1.148	127.56	56.91	3.919	10.65
Var	0.004	102.444	5.550	41.101	0.156	48.760	145.65	8.263	2.152

Morphological variation of fruits was great. Fruit length to width ratio and fruit weight ranged from 0.6-3 cm and 0.25-19.25 kg/accession, respectively. Variation of peduncle length and total fruit weight was 5.55 cm and 145.65 kg/accession, respectively. All the accessions characterized had leaf veins, their leaf size was large, leaf pubescence density-adaxial was dense while leaf pubescence density-abaxial intermediate, leaf lobes were shallow, leaf base shape was cordate. Leaf shapes were pentalobate and all accessions had roots at their internodes. Leaf outline of 108 accessions was broadly ovate compared to 38 accessions with very broadly ovate leaf outline. Most accessions (101) had variable leaf colour i.e. green with silvery strips. 45 accessions had one colour either green or dark green with no silvery strips. Accessions (88) showed moderate senescence when fruits matured with 44 accessions portraying Conspicuous concurrent senescence. In all the accessions the sex type was monoecious male and female, with most flowers being mostly male. Most male flowers were early compared to female flowers. Only 9 accessions had female flowers appearing before the male flowers. Flower colour was variable with most accessions (101) having orange colour flowers. Predominant qualitative fruit characteristics were fruit shape with 38 accessions exhibiting globular shape. Most accessions (42) had an average fruit size averaging 1.2 kg. One accession matured within the range of 91-110 days, 125 their fruit matured above 110 days. The delay in fruit maturity could have been attributed to the long dry spell experienced during the growing period. Accessions (99) showed the ability to regenerate second fruit cycle as they continued to produce leaves and male flowers even after harvest. Accessions (27) had no signs of second fruit cycle as most of the vegetative part had dried up and showed no signs of regeneration even after watering. Predominant fruit skin colour at maturity, ranged from green to orange, secondary fruit skin colour pattern varied from speckled to striped, fruit surface ranged smooth to skin surface with warts, and internal flesh colour from white to yellow. Main colour of flesh and flesh colour of outer layer ranged from yellow to salmon (pink-red). All the accessions had fruit vein tracks and the peduncle abscised when overripe. Deep fruit ribbing was only in 40 accessions and 69 had small blossom scars. The minimum and maximum total fruit weight 0.25 and 19.25kgs respectively. The mean fruit weight was 3-9 kg.

Molecular Characterization

The 5 SSR markers (loci) were able to detect a total of 437 alleles for all the accessions. A total of 258 bands were not amplified by the SSR markers. The Number of bands amplified by the ISSR primers were 510 with 464 not amplified respectively. DNA band sizes were measured with 1.5% gel agarose 5µL per

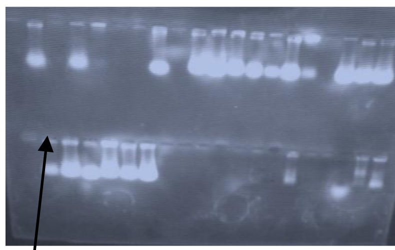
lane bioline DNA ladder [50-2000 base pairs-(bp)]. The SSR amplified band size range was between 100 to 500 bp. Primer PKCT-62 and PKCT-111 had the lowest amplified band sizes. The band sizes for the ISSR were more polymorphic with the lowest band size ranging between 200-500 bp and the highest range between 1200 to 2000 bp. ISSR 17898B and ISSR 17899A had the highest band range. ISSR 814A had band sizes within the range of 500-1200bp with the rest of the primers with a lower range of 200-500bp.

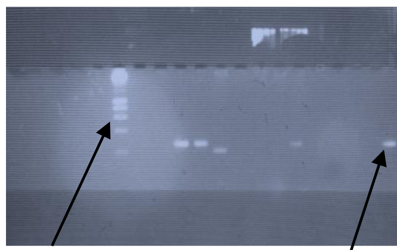
Primer Polymorphism

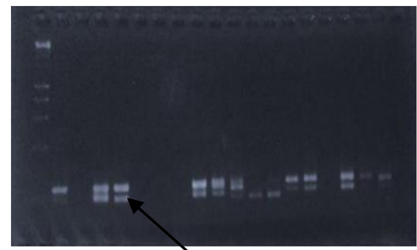
The number of alleles per locus ranged from 1 for markers PKCT-47, PKCT-62, PKCT-111 and PKCT-133 to 2 for marker PKCT-111 and PKCT-122, 2 and 3 for PKCT-62, 3 to 4 for PKCT-47. There was observed heterozygozity for marker PKCT-47, PKCT-62, PKCT-111 and PCKT-122, and homozygozity for marker PKCT-47, PKCT-62, PKCT-111 and PKCT-133 for the accessions in both counties. SSR marker PKCT-122 had the most polymorphic bands in all the accessions. All the ISSR bands were polymorphic ranging from 1 to 7 bands. ISSR 17898A all the bands were polymorphic. ISSR 814A had the most Monomorphic bands, with ISSR 17899A having the most polymorphic bands.

Figure 1: DNA Confirmation **Figure 2**: PCR Monomorphic **Figure 3**: PCR Polymorphic

Presence Gel Electrophoresis DNA Bands DNA Bands







Detected DNA Bands **DNA Ladder** **Monomorphic Band** **Polymorphic Bands**

Polymorphic Information Content (PIC)

Polymorphic information content (PIC) is the value of a marker for detecting polymorphism within a population. It measures the usefulness of a marker and Informativeness in specific families. Polymorphic information content (PIC) depends on the number of detectable alleles and the distribution of their frequency (El-Awady et al., 2012). Marker PKCT-47 revealed the highest polymorphic information content (PIC). The PIC value for SSR primers was 0.62 (PKCT-47) and 0.38 (PKCT-62), with a PIC mean value of 0.5. A total of 526 polymorphic alleles were generated using the SSR primers with a

polymorphism rate of 21.3%. ISSR analysis generated a total of 509 alleles, and the polymorphism rate was 74.01%. The PIC value for ISSR primers was 0.50 (ISSR 844B and ISSR 17899B), 0.53 (ISSR 814A), 0.66 (ISSR 17898A), 0.60 (ISSR 17898B), 0.67 (ISSR 17899A) and 0.72 (ISSR 844A), with a PIC mean of 0.597.

Dendogram based on Morphological and Molecular Characters

Morphological variations constructed dendograms based on quantitative and qualititative characters. Accessions with missing data were not included. Clustering of quantitative and qualitative characters was done using the Unweighted Pairs Groups Method of Arithmetic Averages (UPGMA) based on morphological similarity. The dendrograms revealed 3 main cluster groups from the cultivars evaluated based on either quantitative or qualitative characters. Combining the quantitative and qualitative characters 2 main clusters formed (Figure 4).

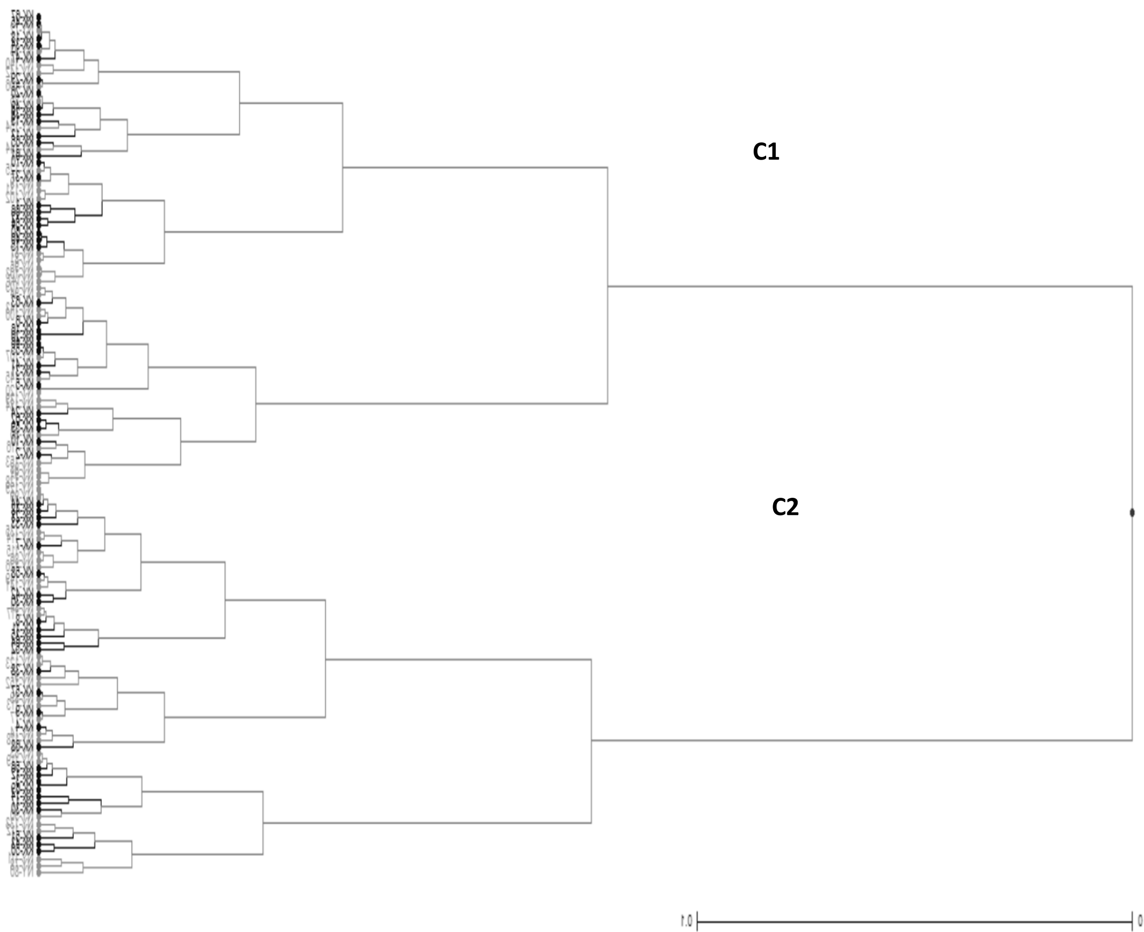


Figure 4: Dendrogram of Hierarchial Clustering for Quantitative and Qualitative Morphological Characters Based on Euclidian Distance Method by UPGMA With 1000 Bootstraps.

The presence (1) and absence (0) of PCR DNA amplicons were used to construct a dendogram. The Dendrogram resulting from UPGMA hierarchial cluster analysis revealed 3 clusters based on SSR or ISSR primers. SSR and ISSR primers combined also revealed 3 main clusters (Figure 5).

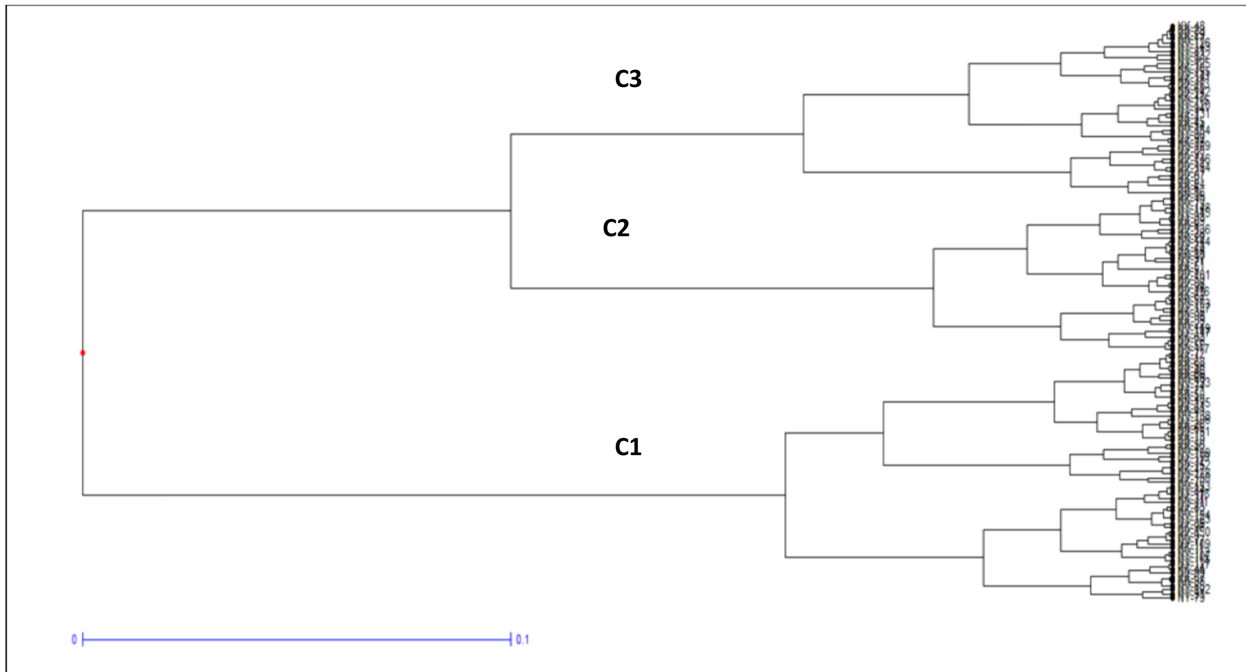


Figure 5: Dendogram of Hierarchial clustering for SSR and ISSR based on Euclidean Distance Method by UPGMA with 1000 Bootstraps.

Yield evaluation

Pumpkin accessions were planted for 2 seasons in three replications at Kakamega and Embu KARI Research stations for yield evaluations. The Plants were rain fed and when there were no rains irrigation was applied at 2 litres per plant. First season was planted on 21st June and 29th September 2012, and second season on 8th April and 13th April 2013 at Embu and Kakamega KARI respectively. Comparing both seasons it took longer for germination and fruit maturity to take place in season two 7 and 89 days compared to season one 6 and 86 days with a mean of 6.94 and 88.23 days in both seasons respectively (Table 5).

Table 5: Summary of Yield Evaluation for Seasons 1 and 2 at Embu and Kakamega KALRO Stations

	Days to Germ	Days to Flowerg	Days to fruit Maturity	Average fruit Wt	Total Fruit wt / plant	Number of fruits/ plant	100 seed wt	Fruit Length	Fruit Width
Min	5	26	29	.2	.2	1	5.56	7.0	7.0
Max	15	122	120	4.2	15.9	9	32.86	35.1	24.5
Sum	5675	53427	51790	791.5	1857.5	1369	7737.9	10848	8559
Mean	6.94	66.70	88.23	1.353	3.175	2.34	13.227	18.54	14.63
Var	2.318	290.363	176.320	.330	5.699	2.184	24.569	44.06	8.627

Days to first flowering were shorter in season two. There was no significant difference in female flowers in both seasons. Total fruit weight (2.9), number of fruits per plant and average fruit weight (1.5) was higher in season two on average compared to season one. Season one had higher number of leaves per plant (182), weight on 100 seed weight (14.9 gms), Biological yield (344.8 gms), longer period of vegetative period (217 days) and a higher germination percentage. In season two there was higher number of seeds per plant (261), Main vine length (642.5 cm), fruit length (15.5 cm) and fruit width (14.9 cm) and a lower germination percentage of 87%. Fruit weight in grams and yields in kilograms per hectare were also high in season two. Kakamega KARI accessions had higher number of female and male flowers. Average fruit weight (1.5 kgs), Total fruit weight per plant (3.99 kgs) and the average number of fruits per plant (3), number of seeds per fruit per plant (291) with low 100 seed weight (12.45 Gms), Fruit length (21.5 cm), fruit width (15.2 cm), main vine length (597.17) cm in both seasons. Kakamega accessions had a larger number of leaves (216) and high biological yield (377.58 Gms). Embu KARI accessions had longer vegetative period (184 days).

DISCUSSION

In Kenya pumpkins are referred to as “orphaned”. Orphaned crops are those crops which have great prospective for food production and development, but whose potential is not adequately appreciated and fully exploited. Consequently, these crops remain “underutilized” in national development (Naluwairo, 2011). Introducing exotic cultivars in the main stream agriculture has replaced/reduced the population of the local varieties (Ngugi, et al., 2007). However, producing more exotic crops is not a panacea to food and economic problems faced by the farmers; instead they have resulted to loss in pumpkin diversity. The loss of pumpkin species during the last decade was derived by asking farmers directly about the history of pumpkin cultivation in each village, if there was any difference between their own landraces with those from the neighboring villages etc. In addition farmers were asked to

identify the most important uses for specific pumpkins such as for selling, domestic consumption, provision of seeds, medicinal and/or folklores. These questions were important because it was thought that if a crop had a wide range of uses then its cultivation may have been sustained for longer (Davari, et al., 2013).

From this point of view the threat of genetic erosion was not a serious problem in target areas. It was determined that most pumpkin species in both counties were cultivated for selling and that there was a negligible number of species cultivated for other reasons such as domestic consumption or for seed provision. In terms of medicinal use, there were no results for researched species (Davari, et al., 2013), but farmers believed the local landraces could cure stomach worms. Thus, economic objectives were clearly identified as the main reason for cultivating specific pumpkins in some of the target areas. This highlighted the threat of existence to the local pumpkins because it means that only those that could fetch high market prices were selected for cultivation. Some farmers used cultural values and indigenous knowledge to discourage leaf harvesting and disturbance of pumpkin plants to encourage yielding and fruiting. These and other social cultural values attached to pumpkins tended to safeguard the growth and development of pumpkin fruits (Ondigi, et al., 2008). These helped in preserving the genetic diversity in some of target areas.

One hundred and forty six pumpkin accessions from Kakamega and Nyeri counties were characterized for morphological and yield attributes. The range of first flowering was 49-87 days. The range of variability was distinct for leaf ratio, days to first flowering, fruit flesh thickness, Fruit length/width ratio, days first fruit maturity, Maturation period and Total fruit weigh per plant. The qualitative characteristics of these pumpkin accessions showed variation in fruit color and flesh color. The variation in traits of the various accessions shows the diversity in the accessions. This diversity can be used to improve, modify and create new cultivars which are high yielding and more adapted to the changing climatic conditions.

The genetic diversity among the accessions was evaluated using SSR and ISSR markers. SSR PKCT-47 primer pair identified more fragments (4 alleles /primer) than any of the other primer pairs. The second most polymorphic SSR was PKCT-122, with 2 alleles /primer. The mean number of total alleles amplified per SSR primer pair was 105.2. PKCT-111 and 122 were biallelic and PKCT-133 monoallelic hence they had zero PIC. The number of alleles amplified per locus ranged from 3 to 4 for PKCT-47, 2 and 3 for PKCT-62. The number of fragments produced by each ISSR primer varied from 2 to 7 (ISSR 844B and ISSR 17898A), with an average mean of 72.7 of amplified alleles. ISSR 17899A was more polymorphic (89.02%) with ISSR 814A being lowest in polymorphism (50.59%). The number of amplified fragments was dependent on individual Accessions.

Morphological and molecular characters generated dendrograms showing similirarity in the number of clusters. The quantitative, qualitative characters, SSR and ISSR scores all produced 3 main clusters separately. The fact that 3 clusters can be observed is an indication of a high diversity, but it should be noted that the accessions are not all from the same Genera. Genera that cluster together may be a basis of possible introgressions between the genera in the field, as these are normally grown side by side (Xolisa, 2002).

Kakamega accessions were high yielders in both seasons, and could be attributed to the large number of average fruits produced per accession. Embu accessions had longer vegetative period (184 days) these was due to the prolonged growing period facilitated by the short rains in October, 2012. In both seasons the local accessions yielded more than the exotic cultivars. Introducing exotic cultivars to the main agriculture farming systems is not a panacea to economic and food security. The Embu accessions were collected from Nyeri County which had the highest number of exotic introductions. There could be some introgression and hybridization in these accessions than those from Kakamega.

CONCLUSION

The diversity of pumpkins is under serious threat due to exotic introductions brought in for economic reasons. Farmers who are the custodians of genetic diversity should be encouraged and supported to preserve the local landraces. The local landraces not only provide the required genetic material for crop improvement but also serve as food security crops during drought, maintain good health of the farmers and notwithstanding they have medicinal value important to the mankind. Despite the local landraces being important in social economic values they have a significant role in food security for the poor and under privileged. Collection, characterization and preservation of the local landraces therefore should be given uttermost priority and be expedited to capture maximum variation useful for crop improvement before there is too much loss of diversity and/ or extinction.

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