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MORPHOLOGICAL CHARACTERIZATION OF SOME WILD AND CULTIVATED WATERMELON (*Citrullus sp.*) ACCESSIONS IN KENYA

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ABSTRACT

Genetic diversity and relatedness were assessed among three most common commercial watermelon cultivars in Kenya; one newly introduced commercial cultivar from the U.S., one Kenyan landrace and one wild (*Citrullus colocynthis*) accession. The six accessions were grown in the field for two seasons under sub humid tropical conditions. Randomized Complete Block Design (RCBD) with three replications was used. Data was collected on morphological features of watermelon which include vine, leaf, flower, fruit and seed characteristics. A descriptor list with 21 morphological (qualitative and quantitative) characters was adopted from Diez *et al*., (2005) and Jarret and Griffin, (2007) and was refined and used in characterization. The data was used to calculate genetic similarity and to construct a dendrogram using the unweighted pair-group method with arithmetic average (UPGMA). Data on quantitative characters was subjected to Analysis of Variance (ANOVA) using SAS statistical package and effects declared significant at 5% level. The procedure PRINCOMP was then used to perform a principle component (PC) analysis using six quantitative variables and accessions plotted on two dimensions using the first two principle components (PC1 and PC2). The cluster analysis results demonstrated high morphological diversity (54-42%) between unimproved accessions (wild accession and landrace) and commercial cultivars and low morphological diversity (8-27%) among commercial cultivars. The ANOVA conducted on quantitative characters of cultivated accessions demonstrated highly significant variation between accessions. Results of the principle component analyses for the six quantitative traits indicated that the first two PCs explained 68% and 29% (a total of 97%) of the total variation. The low morphological diversity observed among commercial cultivars emphasizes the need to expand the genetic base of the cultivated watermelon in Kenya.

Keywords: watermelon, kenya, morphological diversity, cultivars, landrace, wild accession.

INTRODUCTION

Watermelon belongs to the family *Cucurbitaceae* and the genus *Citrullus* and it's the only cultivated species of this genus (Bisognin, 2002). It is believed to have originated in Africa (Simmonds, 1979) but is now widely spread throughout the tropics and the Mediterranean (Tindall, 1983). Wild watermelon (*Citrullus colocynthis*) is a native of arid soils in Africa. Watermelon is thought to have been domesticated in Africa at least 4000 years ago and now grown worldwide, particularly in regions with long, hot summers (Robertson, 2004). Watermelon is one of the most widely cultivated crops in the world (Huh *et al.*, 2008). Its global consumption is greater than that of any other cucurbit. It accounts for 6.8% of the world area devoted to vegetable production (Guner and Wehner, 2004; Goreta *et al.*, 2005). China is the leading country in production of watermelon followed by Turkey, United States, Iran and Republic of Korea (Huh *et al.*, 2008; Wehner and Maynard, 2003). Several varieties of watermelons can be grown in Kenya (Tindall, 1983). However, watermelon production in Kenya falls far below its demand making the fruit expensive and only affordable to rich people. With local demand unsatisfied, its potential for export can not be realized. To meet the market demand, production of watermelon in Kenya obviously needs to be increased (HCDA, 2006).

Cucurbits are very similar in above ground development, but they have high genetic diversity for fruit shape and other fruit characteristics, resulting in a variety of uses (Bisognin, 2002). It is vital for plant breeding programmes to have sufficient diversity available to allow for the production of new varieties that are aimed towards the improvement of crop productivity and able to withstand damage from biotic and abiotic factors (Querol, 1987). Watermelon contains a fruit part and a plant part. Each part contains different traits that are desired by consumers and/or growers, including such traits as flavour, texture, disease resistance and appearance traits such as shape and colour (Wehner *et al*., 2001). Identification of watermelon cultivars and determination of their genetic purity and relatedness relies mainly on fruit characteristics (Levi *et al*., 2001b). Morphological markers can be an effective means to determine genetic relatedness among cultivars and among selections used in watermelon breeding programs. Levi *et al*. (2001b) reported that extensive variation in morphological characteristics exists among watermelon cultivars. These characteristics include rind color and thickness, fruit shape and size, flesh texture and color, sugar content, seed shape and color, days to fruit maturity, and disease resistance. Most of these characteristics are qualitative traits affected by a single or a few gene mutations (Levi *et al*., 2001b).

Although many watermelon cultivars have been developed throughout the world, there is little information regarding their ancestries. Some wild accessions and landraces of watermelon have been identified in different parts of Kenya. However, there is scant data available comparing the modern varieties with local landraces and

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the factors which result in farmers preferring local landraces over modern varieties are not very well understood. The available information suggests that modern varieties often lack additional characters which farmers consider important (Hardon and Boef, 1993). Significant genetic variation may exist among watermelon accessions. Some may be superior in certain traits but lacking in other aspects. Their morphological characteristics may also be different. There is therefore need for a detailed study of genetic variation in cultivated as well as wild watermelon accessions to generate data on local crop development. This data will be essential to validate suggested comparative advantages and may provide new options for plant breeding. The objective of this study was to determine the genetic variation in some wild and cultivated watermelon accessions available in Kenya.

MATERIALS AND METHODS

The study was carried out at Maseno University Research fields. The site lies along Kisumu-Busia highway in Maseno Division, Nyanza Province, Kenya within the upper Midland 1 agro-ecological zone (Jaetzold and Schimidt, 1982). Maseno lies at latitude 0^o 1'N-$0^0$12'S and longitude 34^{o}25'E-47'E and it is approximately 1500m above the sea level. The area receives a bimodal mean annual rainfall of 1750mm (Mwai *et al.*, 2001) with the first rainy season falling between March and July; and second season falling between September and early December. No month, however, is completely dry (Jaetzold and Schimidt, 1982). The mean annual temperature is 28.7^{o}C (Mwai *et al.*, 2001) with the hottest season occurring between January and April (Jaetzold and Schimidt, 1982). The soils are classified as dystric nitisols. They are well-drained, deep reddish brown, slightly friable clay with pH ranging between 4.5 and 5.4. Soil organic carbon and phosphorous content are 1.8% and 4.5mg/kg respectively (Mwai *et al*., 2001).

Six watermelon accessions that are available in Kenya were used in this study. These include four commercial watermelon cultivars, one landrace (GBK-043014) from Kakamega district (altitude 1250-1500m ASL) in Western Kenya and one wild accession (GBK-043457) that was collected from Kilifi district (altitude 30-60m ASL) in Coast Province, Kenya. The landrace and the wild accession were obtained from National Genebank of Kenya (Muguga) in March 2007 and were grown at Maseno University Horticultural Fields for seed bulking between April and August 2007, before proceeding to the main study in September 2007. The four commercial cultivars included three most common watermelon cultivars in Kenya namely; 'Sugarbaby', 'Charleston Grey', and 'Crimson Sweet' and one newly introduced cultivar from USA called 'Yellow Crimson'. The commercial cultivars were obtained from local shops except 'Yellow Crimson' which was obtained from Rispern Seeds, INC. Beecher, Illinois. 'Sugarbaby', 'Charleston Grey' and 'Crimson Sweet' were from East Africa Seeds, Kenya. 'Yellow Crimson' was included in the study because it has also been noted in some local supermarkets although the seed is not readily available in seed shops here in Kenya.

The seeds were directly sown in the field at a spacing of 1.5m x 1.5m. Since watermelon is reported to have poor germination, five to ten seeds were planted per hole but were thinned to one seedling three-four weeks after planting. Organic manure and NPK fertilizer were applied in the planting holes before sowing at the recommended rate of thirty (30) t/ha and 200 Kg/ha, respectively. Two rows of 'sugarbaby' were used as guard rows around the experimental field. Other agronomic practices including irrigation, weeding and top dressing were conducted uniformly and as required in all plots. The first season experiment was conducted between September and December, 2007 followed by the second season experiment between January and May, 2008. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications.

Data was collected on morphological (both qualitative and quantitative) characters of watermelon which include vine, leaf, flower, fruit and seed characteristics. A descriptor list with 21 morphological characters was adopted from Diez *et al*., (2005) and Jarret and Griffin, (2007) and was refined and used in characterization. Each qualitative descriptor was scored by observing three tagged plants per accession taking one plant from every block (replicate). Quantitative descriptors were taken as the mean value of three measurements made on three plants per replicate.

The data was organized into a matrix and subjected to cluster analysis using R Statistical Software (R Development Core Team, 2007) according to Grum (unpubl.) and Venables *et al*., (2006). Variables were segregated into discrete factors (e.g. Flesh colour- red, pink, yellow); rank-ordered factors (e.g. leaf blade- shallow, medium and deep); integers (e.g. number of days to first flower); and numerical variable (e.g. average vine length) and clustered using DAISY (dissimilarity matrix calculation) function and unweighted pair-group method with arithmetic average [UPGMA] (Grum, unpubl.; Venables *et al*., 2006). The statistical uncertainty of resulting hierarchical cluster groups was determined by calculating approximately unbiased p-values through multi-scale bootstrap re-sampling using the R package pvclust (R Development Core Team, 2007; Venables *et al*., 2006; Grum, unpubl.).

Statistical analyses were carried out on six quantitative characters namely; main vine length, number of branches on the main vine, fruit number, fruit weight, rind thickness and seed number, by employing SAS procedures using SAS version 9.1 (SAS Institute, 2005). First, descriptive statistics (mean, standard deviation, and coefficient of variation), were generated using UNIVARIATE procedure. The procedure PRINCOMP was then used to perform a principle component (PC) analysis using the six quantitative variables. In this procedure, first a similarity matrix was calculated and was used to calculate eigen values and scores for the accessions. The accessions were then plotted on two dimensions using the first two principle components (PC1 and PC2). The six quantitative characters were also subjected to analysis of variance (ANOVA) and Least Significant Difference ($LSD_{5\%}$) was used to separate the means.

RESULTS

The six watermelon accessions were evaluated for growth, leaf, vine, flower, fruit and seed characteristics. Variation in qualitative characters in the six watermelon accessions is summarized in Table-1. The landrace was morphologically very close to inbred cultivars except for its tan coloured seeds and white flesh which was also tasteless. In addition, its female flower had an unusually hairy ovary (Plate-1) unlike in commercial cultivars where the ovary was glabrous. Unlike the landrace, the wild accession was distantly related to cultivated accessions in most of qualitative characters (Table-1).

Plate-1. Hairy ovary of the Kakamega landrace (i) alongside glabrous ovary of ‘Sugarbaby’ (ii).

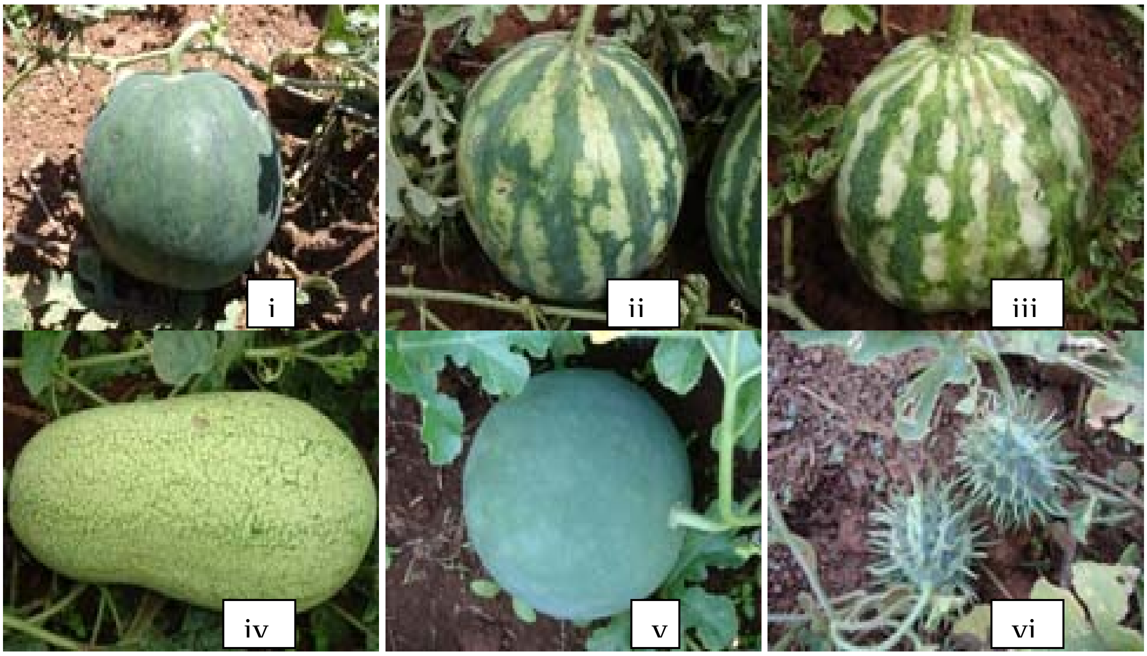

Plate-2. External fruit features of the six accessions; I = Sugarbaby, ii = yellow crimson, iii = crimson sweet, IV = charleston gray, v = landrace and vi = wild accession.

Quantitative characters that were evaluated include vine length, number of branches on the main vine, fruit number, fruit weight, rind thickness and seed number. The wild accession could not compare with cultivated accessions in all these quantitative characters and was therefore excluded during statistical analysis. For example, it had no main vine as all its vines were found to emerge from one point at the base of the plant, just above the soil surface. The wild accession was also very prolific but the fruits were very small compared to those of cultivated accessions. This observation had also been reported by Yaniv *et al.* (1999). Highly significant ($p<0.0001$) variation was observed between cultivated accessions (commercial cultivars and the landrace) in all the six quantitative characters that were measured (Table-2).

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Table-1. Variation in qualitative characters.

Accession	**Growth habit**	**Leaf blade** (depth of incisions)	**Leaf shape**	**Petal colour**	**Fruit shape**	**Main rind colour**	**2^0 rind colour pattern**	**2^0 rind (Stripe) colour**	**Flesh colour**	**Grooves**	**Seed shape**	**Seed colour**
Sugarbaby	Runner	Medium	Pentalobate	Deep yellow	Round	Dark green	Stripped	Medium Green	Red	Absent	Roundish	Brown
Yellow crimson	Runner	Medium	Pentalobate	Deep yellow	Round	Light green	Stripped	Dark green	Yellow	Absent	Roundish	Dark-Brown
Crimson sweet	Runner	Medium	Pentalobate	Deep yellow	Round	Light green	Stripped	Dark green	Red	Absent	Roundish	Brown
Charleston gray	Runner	Medium	Pentalobate	Deep yellow	Elliptic	Gray	Mixed	Light green	Pink	Absent	Roundish	Brown
Landrace	Runner	Medium	Pentalobate	Deep yellow	Round	Green	Solid	No stripes	White	Absent	Elliptical	Tan
Wild	Runner	Shallow	Trilobate	Light yellow	Elliptic	Green	Stripped	Light green	Cream-white	Absent	Elliptical	White

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Table-2. Variation in quantitative characters.

Accession	Main vine length (cm)	Branch number	Fruit number	Fruit weight (kg)	Rind thickness (mm)	Seed number
Sugarbaby	227.860^{c}	6.8333^{c}	2.3883^{c}	2.0492^{b}	8.6100^{c}	275.722^{b}
Yellow Crimson	243.555^{b}	9.3867^{b}	3.4450^{b}	3.0133^{a}	7.5833^{d}	126.388^{d}
Crimson Sweet	200.555^{d}	5.1133^{d}	0.8900^{e}	1.4375^{d}	9.0833^{bc}	191.945^{c}
Charleston Gray	232.833^{bc}	6.5567^{cd}	1.5000^{d}	1.7708^{c}	9.6100^{b}	190.917^{c}
Landrace	447.665^{a}	11.3317^{a}	5.6667^{a}	1.9867^{bc}	13.2217^{a}	372.332^{a}
$LSD_{5\%}$	15.616	1.646	0.578	0.235	0.590	15.534
F Test	***	***	***	***	***	***
CV (%)	34.19	37.14	27.89	29.41	20.81	37.89
SD	92.495	2.913	1.797	0.603	2.002	87.711

NB: Means followed by the same letter are not significantly different *** = Highly Significant

The landrace consistently recorded significant ($p<0.05$) differences from commercial cultivars in all quantitative characters except in fruit weight. 'Yellow Crimson' was also significantly ($p<0.05$) different from other commercial cultivars in all the six quantitative characters. This cultivar recorded significantly ($p<0.05$) high branch number, high fruit number, high fruit weight, thinner rind and low seed number as compared to other commercial cultivars (Table-2).

Results of the principle component analyses for the six quantitative traits indicated that the first two PCs explained 68% and 29% (a total of 97%) of the total variation (Table-3). All quantitative characters contributed almost equally to PC1 except fruit weight which contributed the least. On the other hand, fruit weight had the biggest contribution to PC2. The two dimensional presentation of all accessions grouped by seasons (1 and 2) is presented in Figure-1. The landrace (LR) separated clearly from the commercial cultivars and was located on the upper part of the PCA graph (Figure-1) while the commercial cultivars were on the lower part. 'Yellow Crimson'(YC) also separated from the rest of commercial cultivars and was located on the right hand side of the PCA graph (Figure-1) while other commercial cultivars were on the left hand side. 'Sugarbaby' (SB), 'Crimson Sweet' (CS), and 'Charleston Gray' (CG) were grouped together in the PCA graph (Figure-1). Seasonal variations were observed within accessions as all accessions in the PCA graph (Figure-1) separated into two groups according to seasons (1 and 2).

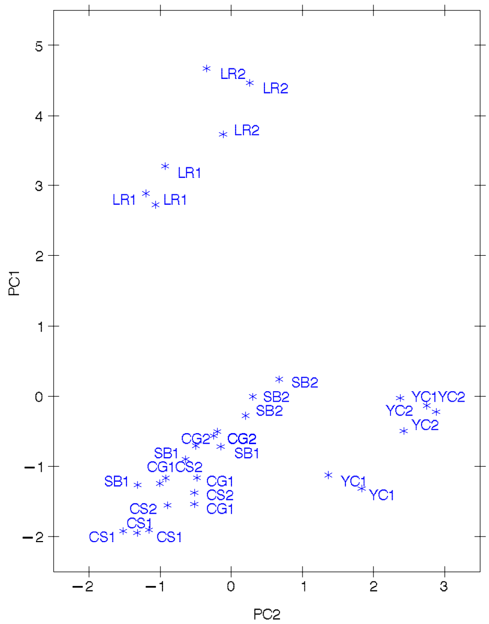

Figure-1. Principle component (PC) analysis plot of first two principle components, depicting relationship among watermelon accessions.

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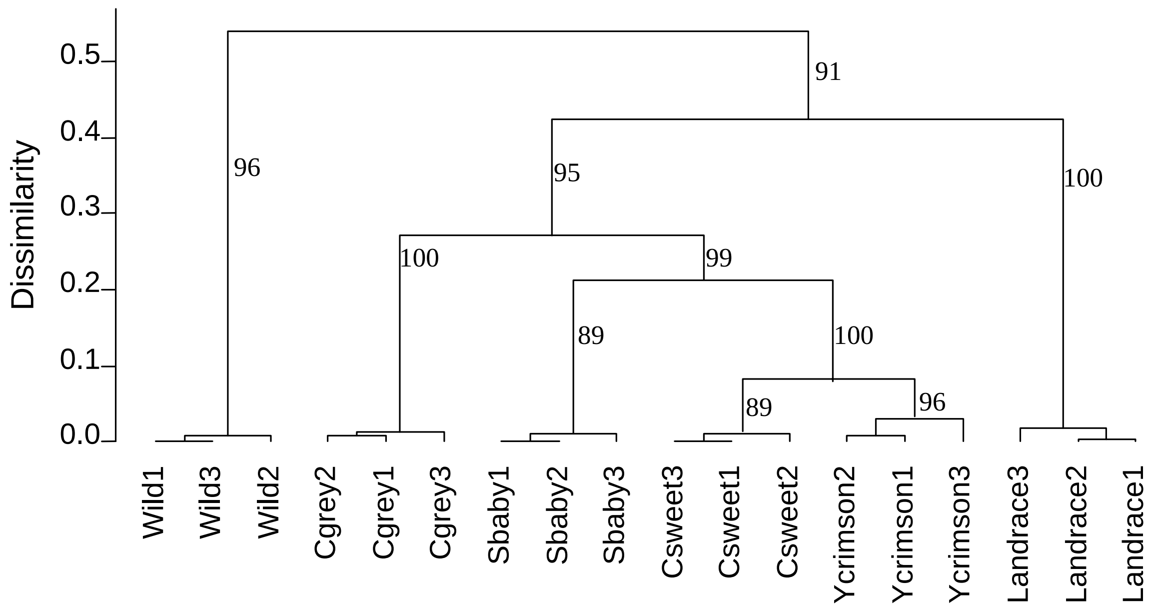
Table-3. The first two principle components (PC) of the six quantitative characters.

Variable	PC1	PC2
Main vine length	0.49	-0.04
No. of branches on the main vine	0.44	0.35
Fruit Number	0.47	0.25
Fruit Weight	0.07	0.75
Rind Thickness	0.43	-0.36
Seed Number	0.40	-0.36
Eigen Value	4.07	1.74
Proportion	0.68	0.29
Cumulative	0.68	0.97

Results of the cluster analysis using morphological data are illustrated in Figure-2. High morphological diversity (53-54%) was detected between wild and cultivated watermelon accessions and between the landrace and commercial cultivars (42-44%). However, low morphological diversity (8-27%) was detected between commercial cultivars. 'Charleston Gray' was 27% morphologically different from other commercial cultivars while 'Sugarbaby' portrayed 21% diversity from the two 'Crimsons' which were the most closely related accessions out of all the six studied recording only 8% diversity. All clusters had high bootstrap probability support (p values) ranging from 83-100 (Figure 2a and 2b).

Cluster analysis

a) Cluster dendrogram for first season experiment



b) Cluster dendrogram for second season experiment

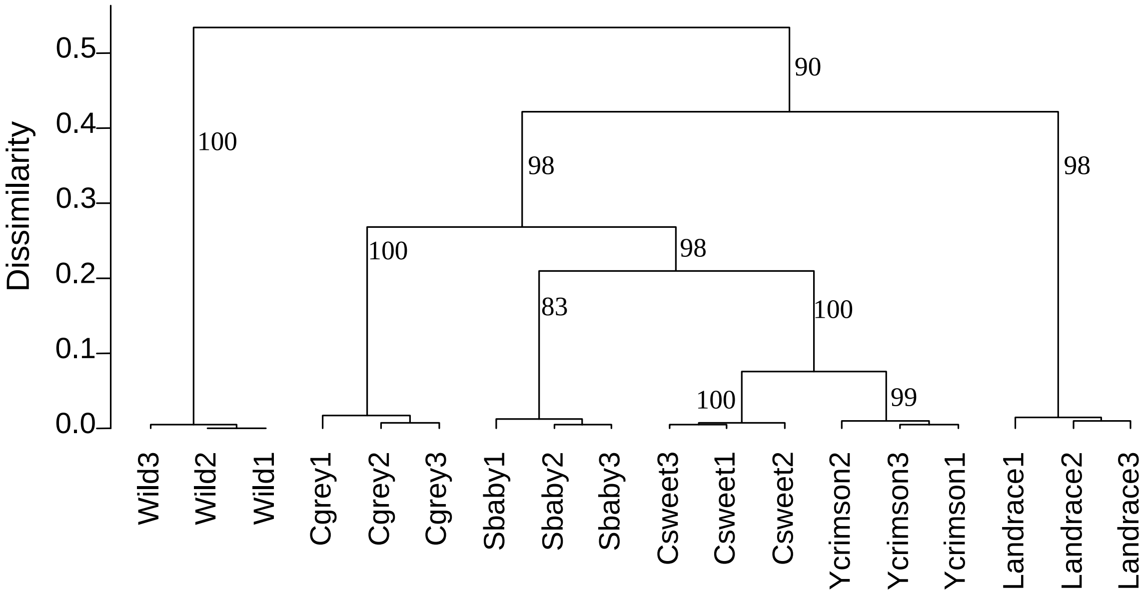


Figure-2. Cluster dendrogram illustrating morphological diversity between the six watermelon accessions characterized using 21 morphological descriptors. The figures show bootstrap probability support values for respective cluster groups.

DISCUSSIONS

Cultivated accessions (inbred cultivars and landrace) portrayed a wide range of diversity in qualitative characters including fruit shape, rind colour, flesh colour, seed shape and seed colour. However plant growth habit, leaf shape, flower type and flower colour were constant for all cultivated accessions. This was consistent with the results of Huh *et al.*, (2008) on Korean and Turkish watermelon populations. The greatest diversity was in fruit characters especially fruit shape, rind colour (plate 2) and flesh colour. This concurs to the findings of Bisognin (2002) who reported that cucurbits are very similar in above ground development but have high genetic diversity for fruit shape and other fruit characteristics. The landrace was morphologically very close to inbred cultivars especially 'Sugarbaby'. The two could easily be confused by an inexperienced observer. It was, however, different from all commercial cultivars in all quantitative characters except fruit weight (Table-2). It was found to contain some desirable traits that can be exploited in breeding programs. These include long and highly branching main vine which directly translated to high yields. However, it was found to have a relatively thick rind and high seed number. Being a landrace, chances that it has undergone any degree of selection for are very low. Wehner *et al.* (2001) reported that breeding for high yielding, low seeded or seedless watermelon fruits with thin but tough rind have been one of the primary goals in watermelon breeding. This also explains why the cultivar 'Yellow Crimson' which has been bred recently had significantly ($p<0.05$) high branch number, high fruit number, high fruit weight, thinner rind and low seed number compared to the other three inbred cultivars which are relatively older. Selection for these traits may have been employed during its development.

Morphologically, the wild accession could be separated easily from cultivated accessions. It was very different in many qualitative characters especially fruit and leaf characters (Table-1). Its leaf was smaller with shallow incisions from the margin compared to the leaves of cultivated accessions which were bigger with medium incisions from the leaf margin. Although all accessions had lobed leaves, the wild accession was trilobate while cultivated accessions were pentalobate. It was also quite prolific producing an average of 16 fruits per plant but the fruits were relatively very small in size recording an average weight of 8.8g (data not presented). According to Yaniv *et al.* (1999), each plant of colocynthis produces 15–30 round fruits, about 7-10 cm in diameter. The rind was dark-green in colour with light green stripes and covered with spike-like features giving the fruit a strange appearance (plate 2). Yaniv *et al.* (1999) indicated that the rind of *C.* colocynthis is green with undulate yellow stripes, becoming yellow all over when dry. The flesh was extremely bitter, creamish-white, with a slimy juice. The seeds were white in colour and elliptical in shape, measuring approximately 4mm long and 2mm wide unlike what was reported by Yaniv *et al.* (1999) that the seeds are brown and they measure approximately 6mm in length.

Yellow Crimson' and the landrace separated clearly from other cultivated accessions in the PCA graph (Figure-1). These separations could be explained by significant ($p<0.05$) differences that were observed between the two accessions and the rest of the accessions in all the six quantitative characters that were measured (Table-2). However, 'Yellow Crimson' was more closely related to other commercial cultivars than to the landrace.

'Sugarbaby', 'Crimson Sweet', and 'Charleston Gray' were grouped together in the PCA graph (Figure-1) indicating that the three could not be separated clearly using the six quantitative characters that were measured. The three, relatively old, cultivars recorded close values in the six quantitative characters (Table-2) which in most cases were not significantly different (p>0.05). Seasonal variations that were observed within accessions in the PCA graph (Figure-1) may be attributed to genotype by environment (GXE) interactions as reported by Simmonds (1979).

The cluster dendrogram combined both qualitative and quantitative characters and was used to estimate morphological diversity among the six species giving an idea of how closely related different accessions were. The two dendrograms (for the two seasons) were very similar indicating that morphological markers can be an effective means of determining genetic relatedness among cultivars and among selections used in watermelon breeding programs. High morphological diversity (42% - 54%) was detected between unimproved watermelon accessions and commercial cultivars but low morphological diversity (8%-27%) was detected between commercial cultivars. This was in contrast to Vavilov (1992) who reported that there is astonishing variation among cultivated watermelon. However, some genetic differences might have gone unnoticed because morphological markers are usually limited in determining genetic diversity. In addition, the samples used were also limited. The results were, however, consistent with the findings of Levi *et al*. (2001a) and Levi *et al.* (2001b). The commercial cultivars were most closely related to the landrace and more distantly related to the wild type. This observation concurred to the results of Maggs-Kolling *et al.* (2004) on morphological variation between wild and cultivated watermelon in Namibia.

Overall, this study demonstrated low genetic diversity among cultivated watermelon which may be attributed to their narrow genetic background as reported by Bisognin (2002), Levi *et al*. (2001a) and Levi *et al.* (2001b). Bisognin (2002) attributed the narrow genetic diversity among inbred cultivars to continued inbreeding which has occurred in quest for uniformity and selection for earliness, fruit size, color, shape, less bitter flesh, larger and fewer seeds. This selection has resulted in high homozygous and true breeding cultivars, which are more uniform and homogeneous than previous open pollinated cultivars (Bisognin, 2002). It is commonly assumed that intensive selection within a population leads to reduction in genetic variability and ultimately to erosion of the basis for further selection responses (Berg, 1993). As a consequence, there is a risk of intensive damage to the inbred cultivars by pests and diseases (Querol, 1987). This study emphasized the need for broadening the genetic base of the cultivated watermelon to reduce their vulnerability to diseases and pests as earlier reported by Levi *et al.* (2001b).

CONCLUSIONS

The study was the first report comparing the Kenyan commercial watermelon cultivars with unimproved accessions. Although few samples of unimproved watermelon accessions were used, the study demonstrated that high diversity for morphological traits (qualitative and quantitative) exists between improved and unimproved watermelon accessions. Selection of desirable traits in unimproved accessions especially in the landrace has priority for the future breeding programs. The study also demonstrated low genetic diversity among commercial watermelon cultivars in Kenya and emphasized the need to broaden the genetic base of these accessions.

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