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Genetic differentiation of watermelon landraces in Mozambique using microsatellite markers

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Landraces of seed and dessert type watermelons from three provinces of Mozambique with distinct agro-ecological conditions, including humid and semi-dry areas from the north to the south of the country, were analysed to assess their genetic differentiation. Ninety-six accessions (269 plants) were tested with 24 microsatellite markers resulting in 110 alleles. Analysis of molecular variance showed that 63% of the total variation in the plant material could be explained among the accessions, while 37% of the variation was within accessions. Molecular variance between material used for seed extraction and dessert consumption explained 34% of the total variation whereas villages explained 27% of the molecular variation among the sampled locations. Structure analysis revealed that the material could be differentiated into three genetic groups. The seed types clustered in one genetic group, irrespective of provincial origin. Dessert type accessions from the semi-arid south and the central part were assigned to a second genetic group, while accessions from the northern more humid region of the country were assigned to a third genetic group. The observed genetic diversity may reflect farmer selection under different agro-ecological conditions or an introduction of material from different sources into the growing areas.

Key words: *Citrullus lanatus*, DNA fingerprinting, landraces, Mozambique, microsatellites, simple sequence repeats (SSR).

INTRODUCTION

Watermelon [*Citrullus lanatus* (Thunb.) Matsun and Nakai] is an annual species, including cultivated, semi-domesticated and wild forms, which are widely distributed in tropical and sub-tropical areas (Robinson and Decker-Walters, 1997; Jeffrey, 2001). Three subspecies of *C. lanatus* are recognised by Jeffrey (2001): subsp. *vulgaris* (Schrad. Ex Eckl. et Zeyh.) Fursa, subsp. *lanatus* including var. *citroides* (L. H. Bailey) Mansf. Ex Grebensc, and sub sp. *mucosospermus* (Fursa), encompassing the 'egusi' seed watermelons. However, in more recent literature, the species has been differentiated into two botanical varieties: *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides* (Jarret et al., 1997; Dane and Liu, 2007; GRIN, 2012). Using this classification, the dessert watermelon and the 'egusi watermelons', belong to var. *lanatus,* whereas the wild forms (*citroides* group, also called citron melons) from the Kalahari Desert ('tsama') whose rind may be used for preserves, jellies and conserves, are assigned to

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Abbreviations: SSR, Simple sequence repeats; **RAPD,** random amplified polymorphic DNA; **CTAB,** cetyl-trimethyl-ammonium-bromide; **PCR,** polymerase chain reaction; **PIC,** polymorphic information content; **MDS,** multi-dimensional scaling.

Table 1. Use type and regional origin of 96 water-melon landrace accessions from Mozambique included in the present SSR study.

Use	Province			Total
	Cabo Delgado	Manica	Gaza	
Dessert	25	27	38	90
Seed	3	0	3	6
Total	28	27	41	96

var. *citroides* (Dane and Liu, 2007). The seeds and flesh of *citroides* may also be used for food preparations (Maggs-Kölling and Christiansen, 2003; Dane and Liu, 2007), but are often maintained for animal feed (cow-melons) (Mujaju et al., 2011).

Watermelon is known for its use as a dessert 'fruit', but has other versatile uses in Africa. In Southern Africa, farmers cultivate dessert, seed and cooking type watermelons (Maggs-Kölling et al., 2000; Mujaju et al., 2011). The fruits are nutritionally important as a natural source of citrulline and carotenoids such as lycopene and β-carotene, a precursor of vitamin A (Setiawan et al., 2001; Edwards et al., 2003; Collins et al., 2007). As a potential drought tolerant plant (Kawasaki et al., 2000; Yokota et al., 2002), it is of particular interest in semi-arid areas of Africa. Under such conditions, it is mostly intercropped with cereals to reduce the risk of complete crop failure and thereby ensuring food security (Munisse et al., 2012).

Knowledge on the genetic diversity and differentiation of watermelon resources is useful to develop and implement effective strategies for conservation and sustainable use. Information generated by genetic diversity and differentiation studies is a step towards useful identification of optimal parental combinations in plant breeding, assessment of the degree of variability for classification of accessions, the development of core collections as well as assessment of the extent of genetic erosion in genebank collections (Mohammadi and Prasanna, 2003). Since it is virtually impractical to characterise the whole genome, the assessment of crop diversity depends on the availability of suitable genetic markers. DNA based molecular markers are environmentally neutral and effective in providing useful information required for assessment of genetic variation (Kalia et al., 2011). One of the most useful and robust DNA based molecular marker systems are the microsatellites or simple sequence repeats (SSRs) which are short tandem repeats of one to six DNA nucleotides distributed throughout most plant genomes (Wang et al., 1994; Li et al., 2002). The difference in number of repeats in microsatellite regions can generate high levels of polymorphism, and are useful for a variety of genetic studies including the assessment of relationship between organisms; variety discrimination/genetic finger printing, genetic mapping and marker assisted breeding (Kalia et al., 2011). Several authors have evaluated SSRs for their potential use to assess relationships among *Citrullus* accessions or to discriminate varieties (Jarret et al., 1997; Guerra-Sanz, 2002; Joobeur et al., 2006; Levi et al., 2006). Based on SSR data, Jarret et al. (1997) differentiated *Citrullus* accessions into 4 major groups, namely *C. lanatus* var. *lanatus, C. lanatus* var. *citroides*, hybrid *C. lanatus* var. *lanatus* x *C. lanatus* var. *citroides* and *C. colocynthis*. While these studies have contributed to our understanding of the phenetic classification within the genus *Citrullus*, a low level of genetic diversity was found in material of cul-tivated watermelon. Nevertheless, 'egusi' seed type and dessert type watermelon accessions from Mali have been differentiated genetically (Nantoumé et al. 2013).

There is still little information on watermelon landrace diversity from Southern Africa. In Namibia, Maggs-Kölling and Christiansen (2003) validated the farmer classification of cultivated watermelon into dessert, seed and cooking types based on morphological cluster analysis. A diversity study using SSR and random amplified polymorphic DNA (RAPD) markers on landraces of cow-melons (*C. lanatus* var. *citroides*) and edible landraces from Botswana, Namibia, South Africa, Zambia and Zimbabwe revealed a genetic differentiation between the two types (Mujaju et al., 2011).

In view of developing national and regional strategies for conservation and sustainable use of watermelon genetic resources, it is of interest to broaden the knowledge related to the diversity of landraces in other countries of Southern Africa. This study aimed at assessing the differentiation and genetic structure of dessert and seed type watermelon landraces collected from farmers' fields in Mozambique.

MATERIALS AND METHODS

Plant material

Plant material for this study consisted of 96 landrace accessions collected from farmers providing a handful of seeds (100-200 seeds) in 12 villages in Cabo Delgado (Northern Mozambique), 8 villages in Manica (Central Mozambique) and 12 villages in Gaza (Southern Mozambique) in 2008. The accessions included seed and dessert types (Table 1) which are further described by Munisse et al. (2011). Among the dessert type watermelons, farmers recognized white, light red, and red fleshed types. The geographical coordinates for the accessions were recorded using a hand held global GPS positioning system (eTrex Venture HC, Garmin). Seeds of the commercial dessert cultivar type Sugar Baby, obtained from two different seed companies (Harris Moran Seed Company, Davis, USA, and Weibull Seed, Sweden), were included as controls.

DNA extraction

Seeds were germinated in plastic pots filled with sand in a greenhouse at 30-33°C. Total genomic DNA was extracted from young leaves of one to eight single watermelon plants randomly selected per accession (269 plants were sampled). From each plant, six leaf discs were sampled, using the lid of a 1.5 ml Eppendorf tube, and collected in 1.2 ml collection tubes (Qiagen). The leaf samples were freeze-dried for 24 h. The material in the tubes was subsequently crushed to a fine powder using glass beads on a mixer mill (Retsch, Type MM301) using a frequency of 30/s for 2 min, then the samples

Table 2. SSR's amplified from genomic DNA from 96 watermelon landraces from Mozambique, number of alleles and polymorphic information content values.

SSR	Number of allele	PIC[a]	SSR	Number of allele	PIC
ASUW13[b]	4	0.02	MCPI-25[c]	5	0.18
Cgb5009[b]	3	0.27	MCPI-26[c]	5	0.46
CLG8288[b]	4	0.38	MCPI-27[c]	4	0.39
MCPI-03[c]	5	0.43	MCPI-28[c]	5	0.53
MCPI-07[c]	2	0.31	MCPI-32[c]	3	0.50
MCPI-09[c]	7	0.63	MCPI-33[c]	7	0.28
MCPI-10[c]	3	0.34	MCPI-34[c]	3	0.49
MCPI-12[c]	7	0.49	MCPI-37[c]	5	0.16
MCPI-13[c]	10	0.74	MCPI-39[c]	4	0.42
MCPI-14[c]	6	0.12	MCpI-42[c]	6	0.40
MCPI-20[c]	5	0.18	MCPI-44[c]	1	0.00
MCPI-21[c]	4	0.24	MCPI-47[c]	2	0.14
Average				4.7	0.34

[a],According to Botstein et al., 1980; [b], Levi et al., 2006; [c], Joobeur et al., 2006.

were rotated and the milling was repeated. DNA was extracted using a modified cetyl-trimethyl-ammonium-bromide (CTAB) method (Saghai-Maroof et al., 1984). The modifications included isoamyl-alcohol substitution for octanol, the final rinsing step used 70% ethanol, and DNA was dissolved in 1 x TE buffer. The protocol was adjusted to enable extractions in collection microtubes (Qiagen). DNA concentrations were adjusted to 20 ng/µl based on spectro-photometer measurements (GeneQuant *pro,* Amersham Pharmacia Biotech).

Polymerase chain reaction (PCR) amplification and allele scoring

The PCR amplifications were performed in 96 well plates (96 Multiply, Sarsted) in a PCR GeneAmp 2700 thermal cycler (Applied Biosystems) using 24 selected SSR primers developed by Levi et al. (2006) and Joobeur et al. (2006), listed in Table 2. PCR for generation of SSR fragments used the M13-tail nested PCR approach with fluorescent labelling of the products according to Schuelke (2000). Forward primers were 5'-tailed with a 19-base pair M13 universal sequence: 5´- CACGACGTTGTAAAACGAC-X-3´, where X denotes the specific microsatellite primer sequence. Each PCR reaction of 7.5 µl contained 60 ng genomic template DNA, 1x PCR reaction buffer (Ammonium buffer, GenScript), 0.2 mM of each of the four dNTPs (Bioline Ltd.), 7.5 µg bovine serum albumin BSA (Calbiochem), 0.6 mM $MgCl_2$, 0.4 pmol forward primer with 5'-M13 tail, 1.6 pmol reverse primer, 1.6 pmol fluorescently labelled M13 primer (labelled with 6-FAM (blue), NED (green) or VIC (yellow) fluorescence (Applied Biosystems), and 0.2 U Taq DNA polymerase (GenScript).

The thermal cycler was programmed as follows: 1 initial cycle at 94°C for 1 min; 30 cycles of denaturation at 94°C for 1 min, primer specific annealing at 50-64°C for 1 min and extension at 72°C for 1 min; followed by 8 cycles of denaturation at 94°C for 1 min, annealing at 55°C and extension at 72°C for 1 min, and a final extension step at 72°C for 5 min; finally held at 4°C. Amplified PCR products labelled with the three different fluorescent dyes were pooled and a ROX labelled (6-carbon-X-rhodamine) molecular size standard (13 fragments, 58-508 bp) was added before denaturation at 95°C for 2 min. SSR fragments were separated by capillary electrophoresis on an AB 3130*xl*Genetic Analyzer (Applied Biosystems and Hitachi Ltd.). Fragments were sized relative to the internal ROX size standard and scored with manual bin setting using the software program GeneMarker, version 1.75 (SoftGenetics LLC & Bioké). Control samples of DNA from cultivar Sugar Baby were included in all 96 well plates.

Data analyses

Genetic analyses were performed with macros in Excel (Microsoft Excel v. 2007, Redmond, USA). Polymorphic information content (PIC) values were calculated as in Botstein et al. (1980), and genetic distances were calculated using modified Rogers's distance (Wright, 1978). The matrix of genetic distances was used in non-parametric multi-dimensional scaling (MDS) with the R Statistics Package (R Development Core Team, 2008) with the MASS package (Venables and Ripley, 2002). The GenAlEx vers. 6.4 software program (Peakall and Smouse, 2006) was used as an add-in in Excel to determine the proportions of the molecular variance (AMOVA) among and within the accessions, and to estimate partitioning of variation among accessions, use groups and villages, using 9999 permutations. A matrix of geographical distances was calculated on the basis of longitude and latitude GPS positions from sampling sites to perform a Mantel test (Mantel, 1967) in GenAlEx, to study correlation between this matrix and the genetic distance matrix using 9999 permutations.

A model-based approach using a Bayesian algorithm, implemented in the software package STRUCTURE version 2.3.3 (Pritchard et al., 2000) was used to explore and identify population clusters. This analysis included samples of the commercial cultivar Sugar Baby. Overall, the model assigns individual genotypes to unknown clusters (groups) by considering the presence of Hardy-Weinberg equilibrium, so that population groups as far as possible are not in disequilibrium (Pritchard et al., 2000). STRUCTURE was run with number of clusters (K) from 2 to 11. Each K was run 20 times with a 'burn-in period' of 10.000 rounds, assuming an admixture model. The most likely number of genetic groups was estimated by the statistical ad hoc criterion (ΔK) described by Evanno et al. (2005). For an assessment of a likely assignment of landrace types to a proposed grouping in 3 clusters, the average proportion of the individuals, belonging to seed and dessert types at the village level, was calculated (q-value).

Table 3. Analysis and partitioning of molecular variance for SSRs among watermelons from Mozambique for accession, use group, province and village level.

Source of variation	df	MS	% Var.	PhiPT[a]	P-value
Among accessions	95		63	0.626	<0.001
Within accessions	265		37		
Total[b]	360				
Between use groups	1		34	0.383	<0.001
Within use groups	267		66		
Total	268				
Among provinces	2		8	0.076	<0.001
Within provinces	266		92		
Total	268				
Among villages	31		27	0.268	<0.001
Within villages	241		73		
Total[c]	271				

AMOVA, 96 accessions included a total sample number of 269; [a]PhiPT, test statistics. P-value based on 9999 permutations; [b] 24 accessions were only represented by one plant, and 22 accessions were only represented by two plants. For the analysis, the data from these accessions were duplicated. [c]One village was represented by only one accession, and one by only two accessions. For the analysis, data from these were duplicated.

RESULTS

Marker statistics

The 24 primer pairs produced an array of 110 different alleles in the tested landraces, with an average of 4.7 alleles per SSR marker locus and a maximum number of 10 alleles recorded for MPCI-13 (Table 2). The PIC varied from 0.0 (SSR marker MCPI-44 was monomorphic) to 0.74 (SSR marker MPCI-13) with a mean of 0.34 (Table 2). Markers MPCI-13, MPCI-9, and MPCI-28 were the most informative with PIC values of 0.74, 0.63 and 0.53, respectively.

Analyses of molecular variation

Analysis of molecular variance based on SSRs revealed that 63% of the variation could be explained among accessions and 37% of the variation within accessions (Table 3). A separate AMOVA showed that 34% of the variation could be explained by usage (dessert or seed). Variation among provinces accounted for only 8% of total variation while the variation among districts explained 13% of the total variation (results not shown). When analysing the variation at the village level, 27% of the variation was accounted for by the villages. The Mantel test for collection distances of sample material up to 1573 km showed a low positive significant correlation between genetic and geographical distance ($R^2 = 0.035$, $P<0.001$).

Genetic structure

STRUCTURE analysis showed the highest likelihood for differentiation of the tested material into 3 genetic groups (Δ-K = 3.82) followed with lower likelihood for structuring into 10 groups (Δ-K = 1.10) (Figure 1). The three genetic groups are shown in Figure 2 using multiple dimensional scaling based on Rogers's modified distance. One of the groups (group 1) is separated from the other two, whereas genetic group 2 and 3 are close to each other and show some overlap (Figure 2). Further analyses were performed with special interest on the three group structure.

In Mozambique, the dessert landrace accessions generally have names associated with flesh colour: white, light red and red. However, colour categories did not associate with the genetic grouping of accessions (data not shown). The analysis of molecular variance indicated that use group (seed or dessert type) and village origin explained a considerable amount of the variation in the material (34 and 27%, respectively). Therefore, we attempted to explain the three genetic groups based on these two factors. Average q-values for the three genetic groups are presented for the use groups at the village level in Table 4.

Among the seed types in the study, one village from Cabo Delgado (Milamba) and two villages from Gaza (Nwamalanzdele and Muzamane) cultivated accessions, which exclusively belonged to genetic group 1. Another two villages (Nhanupo and Maputo) from Cabo Delgado cultivated seed types which were assigned partly to genetic group 1 and group 2 (Table 4). Thus overall, the seed type watermelons from Mozambique belong to genetic group 1 (5 out of 6 accessions), irrespective of province of origin, with genetic admixture to group 2 in the humid province of Cabo Delgado.

For the dessert type accessions, there are indications that genetic grouping relates to the province origin (Table

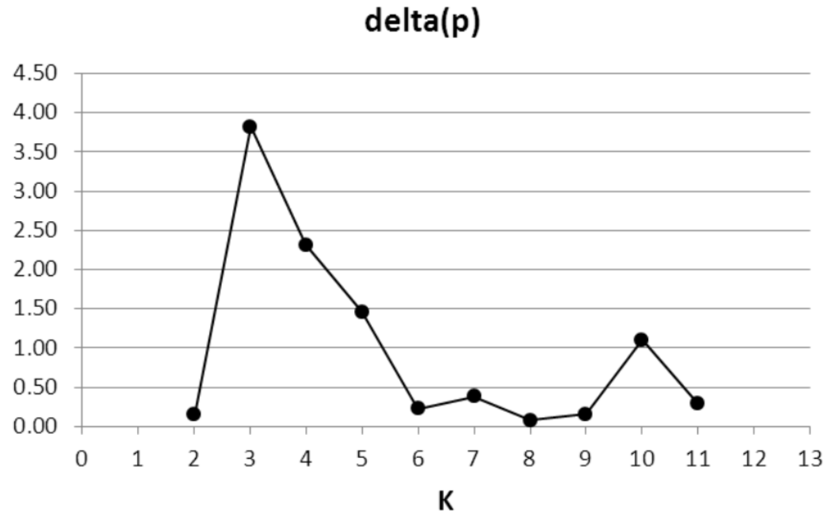

Figure 1. Delta(p) for number of groups in the watermelon material suggested by STRUCTURE analysis.

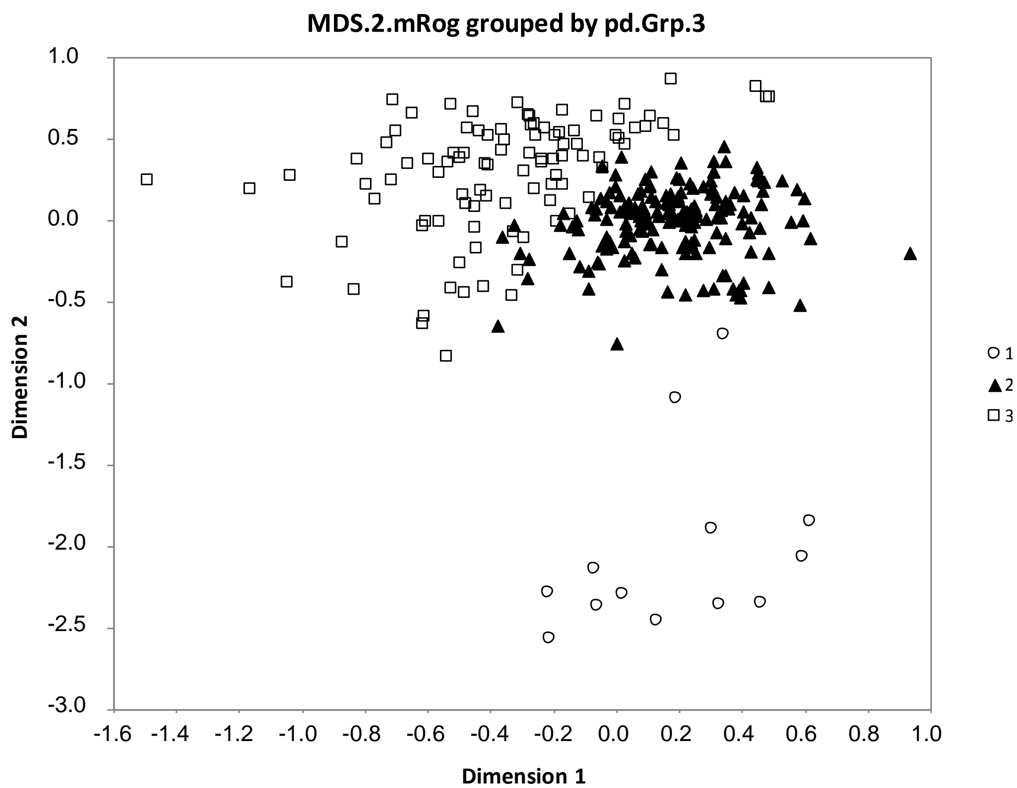

Figure 2. Plot of first two dimensions from multi-dimensional scaling based on Rogers's modified distance of 24 SSR loci with the three proposed structure groups.

4), although it was only explained by 8% of the variation in AMOVA. Villages from the northern Cabo Delgado province tended to grow dessert types with a genetic group 2 and 3 background. Five of the 10 villages in Cabo Delgado (Lianda, Macapa, Mpeme, Nhanupo and Nhanupo Bloco Ncunama) had material exclusively assigned to group 3, while another three villages (Mucojo, Lago Chico and Koko) had accessions assigned to both group 2 and group 3. The dessert accessions from the remaining two villages of Cabo Delgado (Cipembe

and Miula) were not assigned clearly to any of the three groups. Overall, the majority of dessert water-melon accessions from the north (16 out of 25 accessions) had a genetic background most similar to group 3.

Only dessert types were collected from villages in the central Manica province. Accessions from four of the eight villages sampled (16 de Junho, Guezane, Magagodza and Muda Serracao), were clearly assigned to group 2. Another two villages in Manica (Nhangary and Massimino) had dessert accessions assigned to group 3 and two villages (Benhenhe and Bunga) grew material which shared a genetic background with both group 2 and 3 (Table 4). Overall, the majority of dessert type accessions in the central province revealed a genetic background mainly belonging to group 2 (22 out of 27 accessions).

In the southern province of Gaza, dessert types mainly belonged to group 2, irrespective of the village. Thus, among the 11 villages of Gaza sampled during this study, seven villages (Machaila, Mapai, Mapunganhana Mavue, Namandjio, Nwamalandzele and Xifumana) grew dessert type watermelons, which were exclusively assigned to group 2 (24 accessions). However, accessions from four of the villages (Chituri, Madlambuti, Munginge and Mutchele) showed genetic admixture to group 1 and group 3 (q-value>0.2).

DISCUSSION

The average PIC value of 0.34 obtained in this study of 96 accessions from Mozambique and two commercial cultivars of Sugar Baby is somewhat lower than that reported in other watermelon diversity studies with material from southern Africa. Joobeur et al. (2006) reported a PIC value of 0.53 using 36 polymorphic SSR markers on watermelon material including 12 genotypes of *C. lanatus var lanatus* and *C. lanatus* var *citroides.* Studying 8 accessions belonging to the same two *C. lanatus* varieties, a PIC-value of 0.79 was obtained by Mujaju et al. (2010). Although such PIC estimates depend on both the diversity of the sampled material as well as the polymorphism of the SSR markers used for genetic analysis, there are some indications that these, mainly dessert type landraces grown in Mozambique, are not highly diverse. The two most informative markers in this study (MPCI-13 and MPCI-28) were also highly informative in the studies by Joobeur et al. (2006) and Mujaju et al. (2010). Average number of alleles per SSRs locus was 4.7 in this study, which is comparable to the average value reported by Mujaju et al. (2010) using SSR markers.

Several previous studies showed a difference in the genetic background between dessert type watermelons (*C. lanatus* var. *lanatus*) and semi cultivated seed types (*C. lanatus* var. *citroides*) (Jarret et al., 1997; Joobeur et al., 2006; Mujaju et al., 2010). In this study, AMOVA showed that 34% of the genetic variation was attributed to difference between the two types (seed and dessert). The seed types mainly belonged to one genetic group (group 1: 5 out of 6 seed type accessions) with some admixture to group 2 and the dessert types differentiated in two groups (group 2 and 3). Genetic admixture between the two main types (seed and dessert) has also been reported by Mujaju et al. (2010) for cow-melons in Zimbabwe. The morphology of the seed types is remarkably distinct from the sweet dessert types. For instance, the leaves of the seed types tend to have less lobes and a lower leaf blade ratio (length/width ratio) closer to 1 than the dessert types. Most of them also have firm flesh. Cross-compatibility between seed and dessert type water melons is expected, leading to introgression of genes from group 2 of the dessert type when insect pollination can take place and plants are not isolated by distance. Nevertheless, the present study supports the differentiation of seed and dessert type watermelons in Mozambique.

Regarding dessert landraces from Mozambique, it is generally difficult to assign them to distinct landraces as farmers generally give them different names associated with the colour of the flesh. In our study, flesh colour alone is not sufficient to differentiate landraces and this supports previous studies (Dane and Liu, 2007). Instead, there are some genetic distinctness between dessert type accessions from different regions of Mozambique. In the humid northern Cabo Delgado province, the majority of dessert watermelon accessions have a genetic background belonging to group 3. Interestingly, the two commercial Sugar Baby cultivars also belonged to this group, although most of the commercial production of watermelon takes place in the southern province of Gaza.

The majority of the dessert accessions in the central region of Manica belonged to the genetic group 2, and this is the case for all accessions from the southern dry province of Gaza. This substantiates the AMOVA result, showing that 8% of the variation in the material could be explained by the province. Interestingly, there seems to be a plausible explanation for the dessert group structure related to geography. Cabo Delgado in the north is isolated from the central province of Manica and southern province of Gaza. Also, Manica and Gaza are neighboring provinces with more similar agro-ecological zones, so trade and exchange of material more likely takes place here than between these two provinces and Cabo Delgado. This may explain why the dessert types in these two provinces are genetically similar (group 2) with some admixture to group 3. This admixture may be related to the introduced material from other regions with a genetic background similar to that of Sugar Baby.

Also, contributing to the provincial differences is the fact that external cultural influence has been quite different in the north and the central and southern part of Mozambique. Cabo Delgado in the north has substantial contact with ecological communities in Tanzania, while the southern region of Gaza is influenced more from the surrounding countries Zimbabwe and South Africa.

Cultivation practices for watermelon in semi-arid areas of Gaza are different from those in the humid Cabo

Table 4. Proportion of the genome (q-value) allocated to watermelon types and collection sites at the village level, in three genetic STRUCTURE groups.

Type	Village	Number of accession	Group 1 (q-value[a])	Group 2 (q-value)	Group 3 (q-value)	Group[b]
Province Cabo Delgado (North) with high rainfall						
Dessert						
	Lianda	1	0.118	0.007	**0.874**	**3**
	Macapa	2	0.002	0.021	**0.976**	**3**
	Mpeme	2	0.137	0.013	**0.851**	**3**
	Nhanupo[c]	1	0.002	0.015	**0.983**	**3**
	Nhanupo, Bloco Ncunama	2	0.027	0.014	**0.959**	**3**
	Mucojo	5	0.016	**0.674**	***0.311***	**2**, 3
	Lago Chico	1	0.003	**0.608**	***0.389***	**2**, 3
	Koko	8	0.045	***0.247***	**0.708**	2, **3**
	Chipembe	2	***0.333***	***0.409***	***0.258***	1, 2, 3
	Miula	1	***0.329***	***0.351***	***0.320***	1, 2, 3
Seed						
	Milamba	1	**0.981**	0.003	0.017	**1**
	Nhanupo[c]	1	**0.615**	***0.369***	0.017	**1**, 2
	Maputo	1	***0.222***	**0.777**	0.009	1, **2**
Province Manica (Central) with medium rainfall						
Dessert						
	16 de Junho	3	0.003	**0.921**	0.076	**2**
	Guezane	4	0.016	**0.889**	0.095	**2**
	Magagodza	2	0.003	**0.926**	0.072	**2**
	Muda Serracao	1	0.005	**0.914**	0.082	**2**
	Nhangary	1	0.002	0.020	**0.977**	**3**
	Massimino	4	0.004	0.110	**0.886**	**3**
	Benhenhe	2	0.006	**0.662**	***0.333***	**2**, 3
	Bunga	10	0.006	**0.615**	***0.379***	**2**, 3
Province Gaza (South), semi-arid						
Dessert						
	Machaila	3	0.002	**0.825**	0.172	**2**
	Mapai	4	0.004	**0.970**	0.026	**2**
	Mapunganhana	1	0.003	**0.991**	0.007	**2**
	Mavue	7	0.033	**0.851**	0.116	**2**
	Namandjio	4	0.010	**0.853**	0.138	**2**
	Nwamalandzele[c]	1	0.004	**0.960**	0.035	**2**
	Xifumana	4	0.016	**0.945**	0.039	**2**
	Chituri	3	***0.333***	**0.653**	0.014	1, **2**
	Madlambuti	5	0.105	**0.626**	***0.269***	**2**, 3
	Munginge	2	0.004	**0.797**	***0.200***	**2**, 3
	Mutchele	4	0.058	**0.609**	***0.333***	**2**, 3
Seed						
	Nwamalanzdele[c]	1	**0.989**	0.007	0.003	**1**
	Muzamane	2	**0.995**	0.003	0.002	**1**
	32 villages	**96**				
Dessert	Cv. Sugar Baby	1	0.003	0.025	**0.972**	**3**

[a],q-values: Boldface: q>0.6; *Boldface italic:* 0.2<q<0.6. [b]Group, Groups mentioned are those represented with q-values>0.2. Bold face is the dominating group. [c]From the villages Nhanupo and Nwamalanzdele was collected both a seed and a dessert type accession.

Delgado region. In contrast to areas with relatively high precipitation, the semi-arid areas like Gaza have a limited choice of crops that are drought tolerant. Watermelon production is one of few choices. Farmers in Gaza pay more attention to their watermelons. They sow their seeds in separate holes in the field and select for sweetness of the flesh, thinner rind and larger seeds. In contrast, in the more humid Cabo Delgado, most farmers sow watermelons by simple scattering of seeds on the soil, and they are not actively performing seed selection (Munisse et al., 2011).

The present study reveals a genetic structure in the cultivated watermelon types in Mozambique. The genetic differences associate to some extent with geographical separation, perhaps caused by different cultural influence and cultivation practices between the north and the central/south. The result is the development and perhaps introduction, of watermelons with distinct genetic backgrounds in the two regions of Mozambique. Further knowledge on the genetic diversity of the cultivated African watermelons may lead to higher awareness locally and globally of the considerable genetic resource available for the crop on the continent.

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