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Full Length Research Paper

Local-level assessment of watermelon genetic diversity in a village in Masvingo Province, Zimbabwe: Structure and dynamics of landraces on farm

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Watermelon landraces provide valuable food for human consumption as well as animal feed in the drought-prone parts of Zimbabwe, especially in the Masvingo area where subsistence agriculture is predominant. Using random amplified polymorphic DNA (RAPD), this study investigated intra- and inter-landrace genetic variation at the village level. Seedling offspring from 29 landraces, collected at four recently established farms in the same village, were assessed; 20 landraces of sweet watermelon and 9 landraces of cow-melon. Analysis of Molecular Variance (AMOVA) and ordination revealed much variation across the landraces, and strong differentiation between the two main forms of sweet watermelons and cow-melons. Within each of these two forms, landraces from the same farm formed well-separated sub-clusters. The farmers' perceptions with regards to culture, cropping systems, seed systems and utilization were also documented. Obtained information about, e.g., farmers' use of own seed or seed acquired from close family members, traditional myths and different cultivation practices, are concordant with the results from the RAPD analysis. This study is relevant for the development of *in situ* management strategies for conservation of watermelon landraces at the village level.

Key words: *Citrullus lanatus*, genetic diversity, sweet watermelons, cow-melons, landraces, conservation.

INTRODUCTION

In traditional agroecosystems, crop species and landraces usually show high levels of genetic variability. Such landraces have been considered highly pertinent for studying evolutionary forces, because they are cultivated in a dynamic situation where human and environmental selection, gene flow, and genetic drift all interact to shape genetic diversity (Barnaud et al., 2007). Diversity and household food security are strongly linked in the traditional agroecosystems since farmers are dependent on a sustainable crop production, security against unpredictable weather conditions, and products for diverse uses. Different landraces can thus complement each other in fulfilling farmers' needs (Bellon, 1996). The resulting gene pool of landraces in farmers' fields constitutes an important source of germplasm with many specific ecological adaptations, useful in breeding programmes and/or crop improvement. However, to understand the dynamics of diversity in agroecosystems, genetic variability must be investigated at a very local scale.

Watermelon, *Citrullus lanatus* (Thunberg) Matsum & Nakai, is a diploid species (2n = 22), consisting of the domesticated watermelons known as sweet watermelon (*C. lanatus* var. *lanatus*) widely grown around the world, and the citron types (*C. lanatus* var. *citroides*) including the cow-melons found in southern Africa, both in the wild and in cultivation. In southern Africa, watermelon cultivation is especially important in drought-prone, semi-arid areas with an annual rainfall below 650 mm. Here,

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watermelon is grown as a staple food (edible seeds), a dessert (edible flesh), and for animal feed. The fruit flesh can be eaten fresh or cooked, the rind can be pickled or candied, and the seeds are baked or roasted for consumption. Watermelon exhibits polymorphism both in wild populations and in cultivated forms like sweet watermelon, cooking melon and seed melon landraces of the traditional agrosystems. In addition, intermediate types, generally regarded as agronomic weeds, appear to have resulted from hybridizations between sweet watermelons and cow-melons (Maggs-Kolling et al., 2000). These types have soft rind, very juicy flesh, insipid taste and reach the same size as cultivated watermelons.

In Zimbabwe, cultivated watermelons are broadly differentiated into sweet watermelons and cow-melons based on taste. While sweet watermelons are eaten fresh or sold for generation of household income, cow-melons are consumed as a meal called 'Nhopi' in the Shona language after cooking, or in some areas fed to animals. The wild and weedy watermelons are also used for animal feed.

The extent and pattern of genetic diversity within germplasm collections of watermelon has generally been studied at a wider scale, mostly country-level or worldwide, and may not reflect genetic diversity of landraces at the local level. Within southern Africa, a country-wide study on morphological variation in *C. lanatus* for the various morphotypes in Namibia supported the indigenous classification system used, with distinct groups (seed, cooking and fresh-eating types) based on gross morphology, ecology and usage (Maggs-Kolling et al., 2000). Wide variation was found within the local types whereas the genetic basis of the commercial type appeared to be narrow. At farmer or community level, seed exchange, pollen flow, farmers' practices, and environmental pressures all affect genetic diversity *in-situ.*

Molecular tools constitute an efficient means of assessing genetic diversity. In our previous diversity study across selected watermelon-growing districts in Zimbabwe, random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSRs) produced highly correlated similarity matrices, suggesting that the less demanding RAPD can be very useful, especially in developing countries where access to technical facilities may be limited (Mujaju et al., 2010). Our data from a medium-sampling scale across districts inhabited by two major cultural groups (Shona and Ndebele people) showed that levels of variability were substantial among the accessions belonging to the two watermelon forms (sweet watermelons and cow-melons). Comparing the two watermelon forms, there was however, no significant difference in the level of variability between them. RAPD markers have also been used for estimating genetic relatedness among U.S. Plant Introductions of watermelon (Levi et al., 2000, 2001a, b). The data suggested that diversity is higher in the wild taxa *C. colocynthis* and *C. lanatus* var. *citroides* than in *C. lanatus* var. *lanatus*.

In the present paper, we assess the pattern of genetic diversity of watermelon landraces in Masvingo province, Zimbabwe, using RAPD markers and accessions obtained from an in-depth sampling that covered most of the range of morphological diversity. The study will seek to elucidate the relationship between the organization of genetic variability and the local farming practices and socio-cultural differences.

MATERIALS AND METHODS

Study site

The village of Chitanga (21°17´S, 30°45´E) in an area known as Limbugia, is located in Mwenezi district of Masvingo Province (Figure 1). Masvingo Province is in the southern part of Zimbabwe, most drought-prone and experiencing rainfall of usually less than 650 mm a year. As a result of the prevalent of droughts and natural disasters, the province is well-known for watermelon cultivation to guard against complete crop failure. The choice of the village surveyed was based on the co-habitation of the two socio-cultural groups, the Ndebele and the Shona people. This was done in order to allow genetic diversity assessment in the context of farming practices and cultural differences. Apart from being co-habited by two cultural groups, the choice of this village was also recommended by extension workers on the basis of higher production of watermelons in the year 2009 when compared with other areas in the country. The village is located 6 km from Rutenga Business Centre, through which the main highway connecting South Africa and Zimbabwe passes, and less than 100 km from Beitbridge on the border between the two countries. Chitanga was created through the land reform programme, and is presently inhabited by less than ten farmer households. The aim of the land reform in Zimbabwe was to redress past land imbalances through promoting equal access to land for the majority of the population. People who lived in densely populated communal areas, and owned less than 6 ha land, could apply to be relocated. Chitanga village was formally an animal ranch divided into paddocks, and cultivation only commenced in 2000, after the land redistribution exercise. The arriving farmer households were given 15 ha plots for cultivation.

Plant materials and farmers perceptions

Seeds from 29 watermelon landrace accessions (Table 1) were collected from the village. These were obtained during the harvesting period of April to May 2009 from four local farmer households who were the only occupants resettled in Chitanga village at the time. Each accession consisted of a batch of seed from a single plant grown on farm belonging to a distinct landrace as defined by local farmer. The accessions were collected from farmers belonging to two distinct groups of people, the Shona and the Ndebele to allow for socio-cultural comparisons. One survey questionnaire to capture farmer perceptions was implemented per farmer household. Each household was headed by a father practicing polygamy, with at least two wives. The respondents were females above 40 years or males above 59 years. In all, seventeen individuals (4 men and 11 women) were interviewed in local languages, each household forming a focused group. In order to guard against male dominance in focused group interviews, a

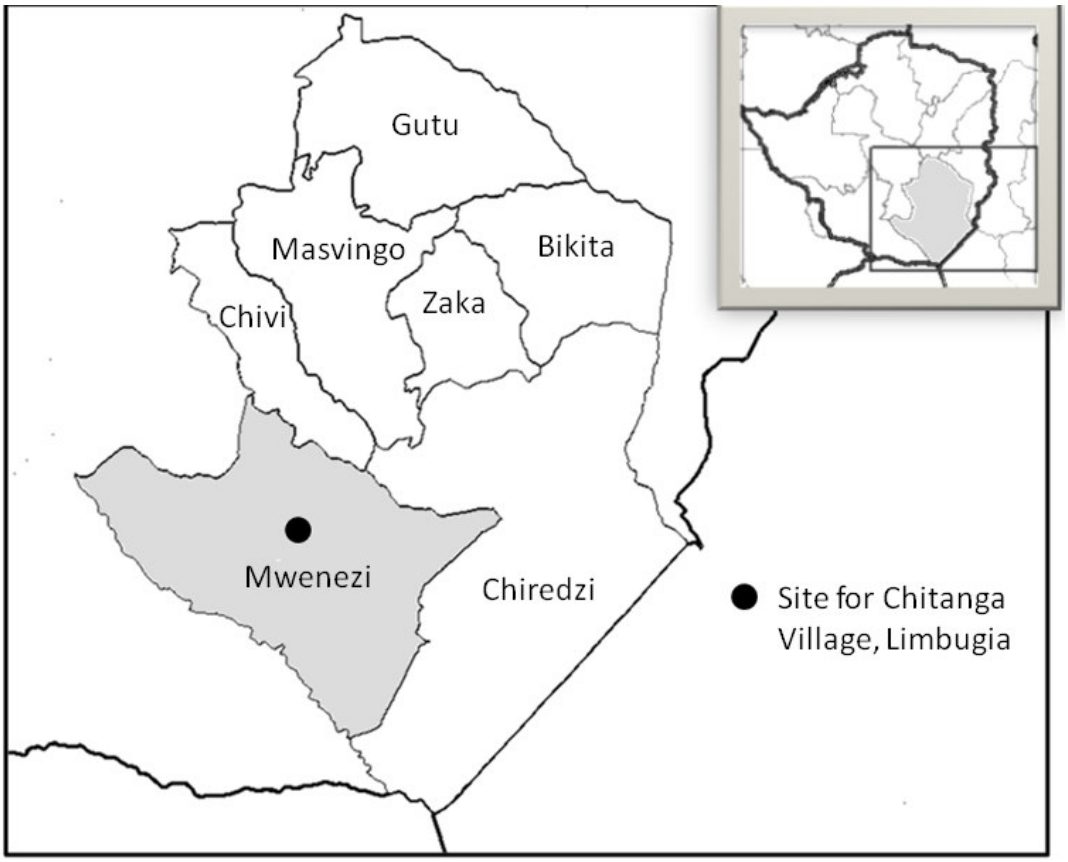

Figure 1. Map showing the site for Chitanga village in Mwenezi District relative to other districts within Masvingo Province. Small insert map shows location of Masvingo province in Zimbabwe.

Table 1. Origin and within-accession genetic variation of watermelon (CWM cow-melon, SWM sweet watermelon) collected in Zimbabwe, estimated as mean value for Jaccard's similarity coefficient (%JSC), percentage polymorphic bands/alleles, expected heterozygosity (H_E) and Shannon's index (I). Standard errors are indicated in parenthesis.

Accession code	Farmer	%JSC	%PL	H_E	I
		Cow-melons			
CWM-CM3	NM	96.71	6.72	0.022 (0.027)	0.033 (0.041)
CWM-CM4	NM	94.95	11.19	0.025 (0.025)	0.042 (0.040)
CWM-CM13	SZ	93.80	14.93	0.047 (0.041)	0.070 (0.059)
CWM-CM14	SZ	93.03	17.91	0.060 (0.045)	0.090 (0.066)
CWM-CM29	MC	84.83	35.82	0.118 (0.058)	0.177 (0.083)
CWM-CM30	MC	88.59	26.12	0.084 (0.052)	0.127 (0.075)
CWM-CM31	MC	92.92	13.43	0.047 (0.041)	0.070 (0.059)
CWM-CM32	MC	86.25	29.85	0.106 (0.057)	0.158 (0.082)
CWM-CM33	MC	85.11	32.84	0.111 (0.057)	0.167 (0.082)
Mean-CWM		90.69	20.98	0.069	0.104
		Sweet watermelons			
SWM-CM1	NM	91.82	19.40	0.046 (0.036)	0.073 (0.054)
SWM-CM2	NM	92.34	15.67	0.047 (0.039)	0.073 (0.058)
SWM-CM9	SZ	94.74	9.70	0.036 (0.036)	0.053 (0.053)
SWM-CM10	SZ	93.88	13.43	0.046 (0.040)	0.069 (0.059)
SWM-CM11	SZ	93.59	14.18	0.051 (0.043)	0.075 (0.062)

Table 1. Contd.

SWM-CM12	SZ	91.90	17.16	0.055 (0.044)	0.083 (0.063)
SWM-CM15	SZ	95.13	11.19	0.031 (0.032)	0.048 (0.048)
SWM-CM16	SZ	95.42	8.96	0.029 (0.031)	0.044 (0.046)
SWM-CM17	SZ	95.38	8.96	0.035 (0.036)	0.052 (0.053)
SWM-CM18	SZ	94.92	11.19	0.036 (0.035)	0.055 (0.052)
SWM-CM19	SZ	90.35	25.37	0.080 (0.049)	0.122 (0.071)
SWM-CM20	SZ	88.97	26.12	0.089 (0.052)	0.133 (0.076)
SWM-CM21	SZ	91.72	17.16	0.066 (0.049)	0.097 (0.070)
SWM-CM22	SZ	95.88	9.70	0.036 (0.037)	0.053 (0.054)
SWM-CM38	MC	95.16	9.70	0.036 (0.038)	0.054 (0.054)
SWM-CM39	JN	91.28	19.40	0.063 (0.046)	0.095 (0.066)
SWM-CM40	JN	93.73	14.93	0.052 (0.043)	0.078 (0.062)
SWM-CM41	JN	94.34	11.94	0.049 (0.043)	0.071 (0.062)
SWM-CM42	JN	95.59	9.70	0.029 (0.032)	0.045 (0.047)
SWM-CM43	JN	85.14	31.34	0.100 (0.054)	0.152 (0.078)
Mean-SWM		93.06	15.26	0.051	0.076
Grand mean		92.33	17.04	0.056	0.085

Table 2. Farmer perceptions documented in four farmer households in Chitanga village.

Parameter assessed	Farmer households				*A*
	NM	**SZ**	**MC**	**JN**	
Socio-demographic information about respondents					
Socio-cultural group	Ndebele	Shona	Shona	Ndebele	
Respondents by gender					ns
Male	1	1	1	1	
Female(s)	3	4	4	2	
Respondent age by gender (years)					ns
Male	62	67	59	66	
Female (minimum)	41	46	43	45	
Status on land					
Owner or spouse owner	100%	100%	100%	100%	
Rent	0	0	0	0	
Main staple crops	Maize, millets, sorghum, beans, sweet potatoes and watermelons	Maize, millets, round nuts and watermelons	Maize, sorghum, and watermelons	Maize, millets and watermelons	
Seed source and cropping systems					
Source of seeds					ns
Owner	90%	95%	95%	95%	
Family/Relative	8%	5%	5%	5%	
Non-relative/Neighbor	0	0	0	0	
Markets (SA)	2%	0	0	0	
Seed storage					
Containers	Traditional baskets	Tins and bottles	Tins and bottles	Traditional baskets	
Storage place	kitchen	kitchen	kitchen	kitchen	
Cropping System					ns

Table 2. Contd.

Sole cropping	0	0	0	0	
Intercropping	100%	100%	100%	100%	
Intercrop	Maize, sorghum and millets	Maize and millets	Maize and sorghum	Maize and millets	
Watermelon types					ns
Sweet watermelons	2	12	2	5	
Cow-melons	2	2	5	0	
Planting of watermelon	Same furrow as intercrop	Same furrow as intercrop	Same furrow as intercrop	Same furrow as intercrop	
Sowing time	After rains	Before rains	Before rains	After rains	
Watermelon forms in field	Separated	Mixed	Mixed	Separated	
Field spacing in metres					ns
Between plants	1	1	1	1	
Between rows	1	25	25	1	
Fertilizer and plant protection use					
Organic manure	No	No	No	No	
Chemical fertilizer	No	No	No	No	
Herbicide	No	No	No	No	
Pesticide	No	No	No	No	
None	Yes	Yes	Yes	Yes	
Special attributes					
Drought tolerant	Yes	Yes	Yes	Yes	
No disease observed	Yes	Yes	Yes	Yes	
None	-	-	-	-	
Watermelon uses					
Sweet watermelons by proportion					ns
Food (dessert)	70%	50%	75%	60%	
Income	30%	50%	25%	40%	
Cow-melons by proportion					ns
Food (cooked)	0	70%	65%	0	
Fodder	100%	30%	35%	100%	
Traditional myths	Fear of witchcraft leading to a field failing to produce crop – no seed exchange between farmers				

A: Analysis of variance; ns: not significant at 5% level between the Shona and Ndebele people.

government female extension worker was used to moderate. Furthermore, the extension worker would also provide interpretation from English to local languages and vice versa to create the same level of understanding. The average ratio of adult females to males interviewed in each focused group was 3 to 1. Questions were asked as open-ended to allow farmers to discuss widely on their landrace perceptions. Interactions between females and males were moderated during discussions also by directing certain questions to individuals in order to promote gender balance. The survey findings were generally descriptive, and where statistics were involved respondents would provide estimates in terms of percentages (%) or numbers. The data were recorded during the focus group discussions. The documentation included socio-demographic information about respondents, seed source and cropping systems, and watermelon uses (Table 2). Statistical data collected were analyzed using analysis of variance (ANOVA) in Minitab 16 (product licensed to Swedish University of Agricultural Sciences) to test for significant differences between the two socio-cultural groups.

DNA extraction and RAPD analysis

The seeds were germinated at 25°C in a greenhouse at Balsgård in Sweden, and a total of 290 plants (10 plants from each accession) were chosen for this study (Table 1). DNA was extracted from young leaf tissue using the Qiagen DneasyTM Plant Mini Kit following the manufacturer's protocol. DNA concentration was estimated visually using DNA low mass ladder (InvitrogenTM Life

Table 3. Nucleotide sequences of RAPD primers used in the present study, number of polymorphic (PM) and monomorphic (MM) bands produced by each primer, PIC values and RAPD marker index values (RMI).

Primer	Nucleotide sequence (5'—>3')	PM	MM	PIC	RMI
OPB-11	GTAGACCCGT	16	0	0.689	11.02
OPC-05	GATGACCGCC	10	5	0.609	6.09
OPD-20	ACCCGGTCAC	17	4	0.740	12.59
OPE-04	GTGACATGCC	8	4	0.621	4.96
OPJ-06	TCGTTCCGCA	14	1	0.640	8.95
OPJ-13	CCACACTACC	17	1	0.564	9.59
OPK-14	CCCGCTACAC	17	1	0.735	12.50
OPK-20	GTGTCGCGAG	15	1	0.607	10.31
OPT-01	GGGCCACTCA	10	4	0.638	6.38
OPT-05	GGGTTTGGCA	10	5	0.609	6.09
Total		134	26		

Technologies (Carlsbad, CA, USA)) and electrophoresis in a 2% agarose gel. A total volume of 25 µl was used for the RAPD PCR protocol, containing 0.2 µl of 5 U/µl Taq DNA polymerase (Amersham Biosciences, Uppsala), 3 µl of DNA template (10 ng/µl), 0.5 µl of 10 mM dNTPs, 1.0 µl of primer (5 µM), 16.2 µl dH_2O, 1.6 µl of 25 µM $MgCl_2$ and 2.5 µl of reaction buffer (Thermo Fisher Scientific, Surrey). PCR was performed with a VWR Unocycler (VWR, Stockholm) programmed for 45 cycles of 94°C for 15 s, 36°C for 45 s (with a ramp rate of 0.4°C/s), 72°C for 1.5 min. Separation of the amplified products was by electrophoresis in a 1.8% agarose gel, stained with ethidium bromide and photographed under UV illumination. Only clearly visible DNA fragments with a length between 150 and 2200 bp were used as markers. Scoring for the presence or absence of DNA fragments was aided by the use of a 1 kb DNA ladder, and 5 control samples (1 sample with water as negative control, 2 sweet watermelon samples and 2 cow melon samples), to check for reproducibility. Ten primers, initially screened from a total of twenty-seven RAPD primers, were used on the entire material (Table 3).

Statistical analysis

Each RAPD band was considered as an independent locus, and polymorphic bands were scored as absent (0) or present (1) for all the 290 plants. A polymorphic index content (PIC) was calculated to evaluate the informativeness of each RAPD primer, according to Smith et al. (1997), as follows: $PIC = 1 - \sum P_i^2$, where P_i is the band frequency of the i-th allele. A marker index for each of the RAPD primers was obtained by multiplying the PIC-value by number of polymorphic loci. A pairwise genetic similarity matrix was generated using the Jaccard similarity coefficient (Weising et al., 2005). Four different parameters were used to estimate variation within accessions: (1) mean percentage polymorphic bands, (2) mean Jaccard similarity, (3) the expected heterozygosity which is equivalent to Nei's unbiased gene diversity HS (Nei, 1978) when calculations are based on polymorphic and biallelic loci, and when sample sizes are equal among populations, and the Shannon diversity index (Weising et al., 2005).

Variation among accessions was calculated as the coefficient of genetic differentiation GST (equivalent to the fixation index F_{ST} for biallelic loci) according to the formula $G_{ST} = (H_T-H_S)/H_T$ where H_T is the total genetic diversity and H_S is the mean within-accession diversity (Nei, 1977). Gene diversity parameters were obtained using POPGENE version 1.32 (Yeh et al., 1997), assuming Hardy-Weinberg equilibrium since watermelon plants have mainly unisexual flowers and are expected to be outcrossing to a high degree. Analysis of molecular variance (AMOVA) using Arlequin version 3.0 (Excoffier et al., 2005) was calculated to partition genetic variation at different levels; between sweet watermelons and cow-melons, between and within accessions, between and within two cultural groups of farmer households, and between and within individual farmer households.

To evaluate relatedness among different accessions, genetic distances (Rogers' distance as modified by (Wright, 1978), here forth referred to as Rogers'-W) were calculated and quantified with an UPGMA (unweighted pair-group method using arithmetic averages) cluster analysis using NTSYS-pc, version 1.80 (Rohlf, 1993). Distortion was estimated with a cophenetic correlation analysis between the two triangular matrices (Jaccard similarity matrix and Rogers'-W distance matrix) and their respective similarity matrices generated from the dendrograms (Rohlf, 2000). An ordination method, multidimensional scaling (MDS), was used as a complement since it is more appropriate under a non-hierarchical model of infraspecific variation (Swofford and Berlocher, 1987).

RESULTS

Spatial patterns of planting and farmers' perceptions

Among the respondents, all were mature people with a minimum age of at least 41 years for women and 59 years for men owning farms. No young-aged people between 18 and 40 years were available as many were reported to be working in towns. Apart from watermelons, other main staple crops grown were maize, millet, sorghum, beans, sweet potatoes and roundnuts. Maize and watermelon crops are the predominant crops across all the farmers. The watermelon accessions sampled from each farmer were viewed as different landrace varieties. However, no specific local name was given to any particular form of either cow-melons or sweet watermelons. The naming only distinguishes groups of

watermelons. For the parameters with statistical data, there were no significant differences when comparison was done between the two socio-cultural groups. Differences were however observed across individual farmers. Number of landrace varieties grown by the four farmer households varied (Table 2); SZ had the highest number of sweet watermelon landraces (12), followed by JN with 5, and finally NM and MC with 2 each. With regard to cultivated cow-melon landraces, MC had the highest number (5), NM and SZ had two each, whereas JN had none. All farmers grow watermelons mixed with other crops in the same field. Generally, Ndebele farmers mostly cultivate sweet watermelons, and they do so after rains. Shona farmers practice dry planting before the onset of rains. In addition, Shona farmers who grow cow-melons, use them as both food and animal feed, whereas Ndebele farmers restrict them to animal feed. Ndebele farmers planted sweet watermelons and cow-melons in separate parts of the same field (approximately 100 m apart, personal observation). Closely knit rows of millets and sorghum were grown between the sweet watermelons and the cow-melons. Each landrace of sweet watermelon was grown in a row, with a spacing of 1 m between plants and separated from the next landrace by a space of 1 metre. In the Shona farmers' fields, plants of the same landrace were also grown one metre apart, but distances between different landraces were at least 25 m. Apparently, the likelihood of pollination between landraces was therefore much lower than in the fields of the Ndebele people. No effort was made to further separate sweet watermelon and cow-melon landraces.

Farmers used their own varieties of landraces, obtained through seed selection from their own fields and/or sourced from close family relatives. In addition to seed from within Zimbabwe, one Ndebele farmer (NM) obtained some seed through informal trade with relatives in South Africa. The variety originating in South Africa was sweeter than the local varieties. In support of using their own seed, farmers reported of a common traditional myth related to fear of witchcraft that was said to be linked to a field failing to produce any crop. This negatively affects seed exchange across tribal groupings and between different families. Seeds used are generally stored in kitchens, in tins and bottles for the Shona farmers and in traditional baskets for the Ndebele farmers. All farmers, in spite of varying ratios of consumption versus sale, used sweet watermelons mostly for human consumption. Generation of household income, although critical to meet other goods and services, usually came second. Due to the drought tolerance, absence of diseases and valuable contribution to livelihood needs, all farmers alluded to continued watermelon cultivation as mitigation measure against unpredictable weather patterns. In addition, the farmers concurred that there is no requirement of fertilizer and pesticide use in watermelon cultivation.

RAPD analyses

The 10 RAPD primers used in this study produced 160 scorable RAPD markers of which 134 (63.75%) were polymorphic (Table 3). PIC values for these RAPD primers ranged from 0.56 (OPJ-13) to 0.74 (OPD-20), while RAPD marker index ranged from 4.96 (OPE-04) to 12.59 (OPD-20).

Four different estimators of within-accession variation were calculated (Table 1), ranging from 84.83 to 96.71% for mean Jaccard similarity (JS), from 6.72 to 35.82 for percentage polymorphic bands (%PL), from 0.022 to 0.118 for expected heterozygosity (HE), and from 0.033 to 0.177 for Shannon's index (I). The five most diverse accessions according to all of these estimators were CWM-CM29, CWM-CM33, SWM-CM43, CWM-CM32 and SWM-CM20, whereas CWM-CM3, CWM-CM4, SWM-CM16 and SWM-CM42 were the four least variable. When calculated across all of the plant material, variability was only slightly lower for sweet watermelons (20 accessions) compared to cow-melons (9 accessions). Calculation of accession means separately for the two forms show that accessions of cow-melons are somewhat more variable than accessions of sweet watermelons (90.69 vs. 93.06 for %JS, 20.98 vs. 15.26 for %PL, 0.069 vs. 0.051 for H_E, and 0.104 vs. 0.076 for I).

Analysis of molecular variance (AMOVA) within and among the 29 accessions, divided into cow-melons and sweet watermelons, revealed that 72.3% of the total variation resides between these two main forms, 16.1% between accessions within forms and 11.6% within accessions (Table 4). The overall G_{ST} for estimating between-accession differentiation regardless of main form was 0.774, that is, very similar to the AMOVA Φ_{ST} value of 0.807. G_{ST} and AMOVA Φ_{ST} values obtained in calculations carried out separately for the two main forms, showed less differentiation among cow melons (G_{ST} = 0.567 and Φ_{ST} = 0.547) than among sweet watermelon accessions (G_{ST} = 0.649 and Φ_{ST} = 0.615). Partitioning variation with respect to individual farmer households and the two socio-cultural groups, apportioned 32.0% of the variation between farmer households, and only 2.6% between cultural groups.

Results of the cluster analysis were illustrated in a dendrogram (Figure 2). The cophenetic correlation between the Rogers'-W distance matrix and the dendrogram was 0.986, suggesting a very high goodness of fit (Rohlf, 2000). Two major clusters were differentiated at 29% genetic similarity: one larger cluster containing the 20 sweet watermelon accessions and one smaller cluster with the nine cow-melon accessions. Within the two major clusters, distinct subclusters contained all (for cow-melons) or at least most samples (for sweet watermelons) collected from a single farmer household. Multidimensional scaling similarly revealed the two major clusters of sweet watermelons and cow-melons as well

Table 4. Partitioning of genetic variation using G_{ST} and AMOVA on RAPD data taking into account (a, c) grouping accessions into two main forms (cow-melons and sweet watermelons) (b) no prior grouping of accessions, (d) grouping of accessions into individual farmers and (e) grouping of accessions into two cultural groups: Shona and Ndebele.

Source of variation	Value
(a) Partitioning (AMOVA) with two main forms, cow-melons and sweet watermelons	
Between-form diversity	72.30%
Between accessions within forms	16.14%
Within-accession diversity	11.55%
(b) Partitioning all accessions	
G_{ST}	0.774
Φ_{ST}	0.807
(c) Partitioning among accessions within each main form	
Cow-melons	
G_{ST}	0.567
Φ_{ST}	0.547
Sweet watermelons	
G_{ST}	0.649
Φ_{ST}	0.615
(d) Partitioning (AMOVA) with four individual farmer households	
Between farmer household diversity	31.95%
Between accessions within farmer households	50.57%
Within-accession diversity	17.48%
(e) Partitioning (AMOVA) with two cultural groups: Shona and Ndebele	
Between cultural group diversity	2.55%
Between accessions within groups	78.39%
Within-accession diversity	19.06%

Significant at 0.1%, *P < 0.001*.

as a clear tendency for grouping of landraces belonging to the same farmer household (Figure 3).

DISCUSSION

Genetic diversity in watermelons

Although concerns about reproducibility, primer competition and the inability to distinguish heterozygotes from homozygotes are regarded as limiting factors for using RAPD in estimating genetic diversity (Nybom, 2004; Weising et al., 2005), RAPD markers have proved reliable and informative for assessing genetic diversity in numerous studies, including watermelon landrace accessions obtained from farmers' fields (Mujaju et al., 2010). In the present study, number of polymorphic marker bands per primer averaged 13, a relatively high figure but comparable to our previous study (Mujaju et al., 2010).

Differentiation between the two main forms, that is, sweet watermelons and cow-melons, was strongly supported by both cluster analysis and multidimensional scaling. In addition, AMOVA partitioning of variation exhibited significant variation (72%, $P < 0.001$) between these forms. Considerable differentiation between sweet watermelons and cow-melons has been reported also in a number of previous studies (Jarret et al., 1997; Levi et al., 2000, 2001a, b, 2005; Navot and Zamir, 1987) as well as in our recent study on watermelon accessions from Zimbabwe (Mujaju et al., 2010).

There was significant differentiation between accessions in our study, both when calculated across all accessions and when calculated within each of the two main forms, cow-melons and sweet watermelons. When calculated across all watermelon accessions, the

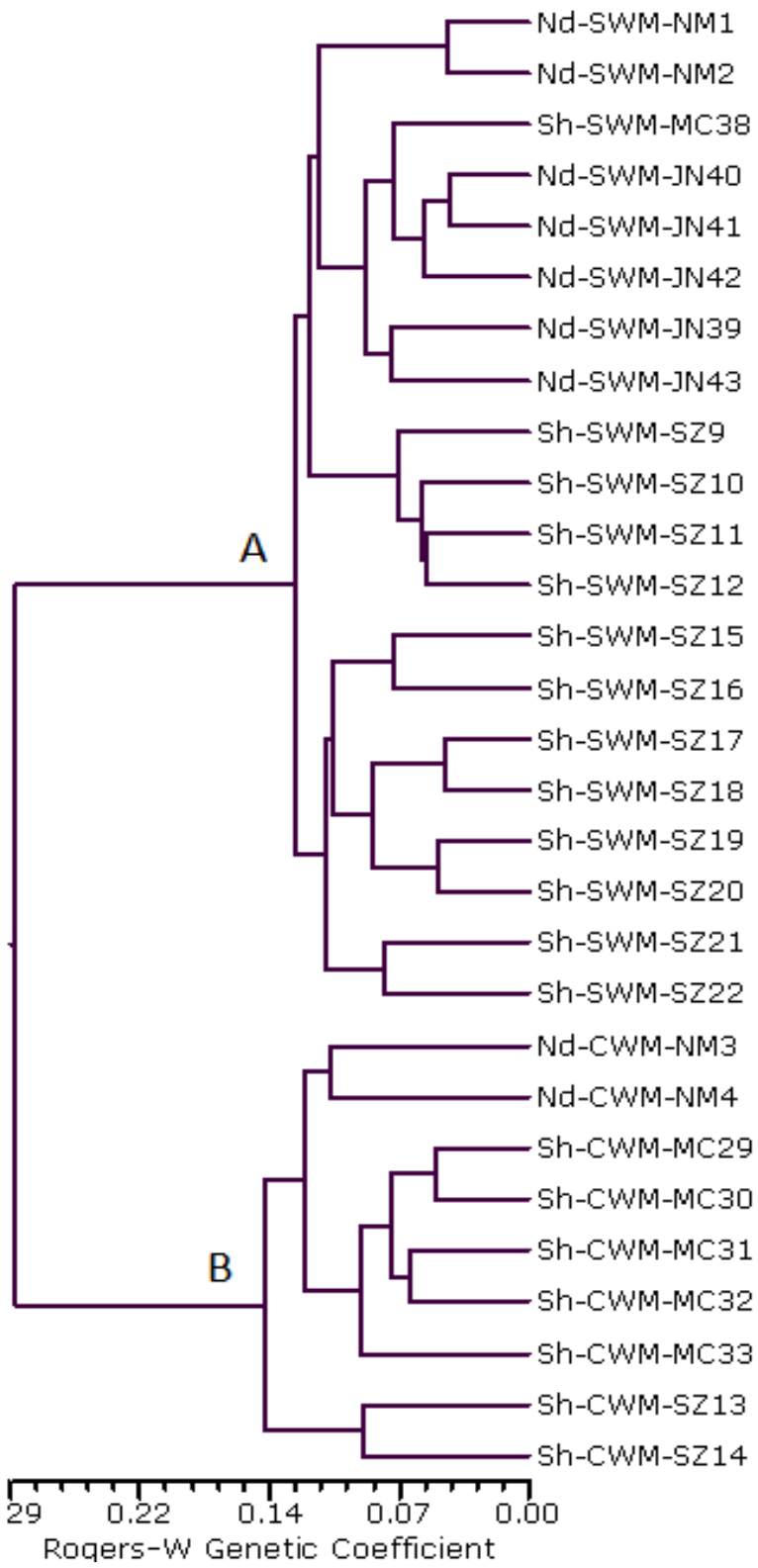

Figure 2. RAPD-based UPGMA dendrogram of watermelon landraces from Chitanga village, Zimbabwe, collected from four farmers, belonging to two cultural groups, **Nd** Ndebele and **Sh** Shona. The two major clusters represent **A** cow-melons (CWM) and **B** sweet watermelon (SWM). The farmers names are represented by the initials (NM, MC, JN and SZ), numbers following correspond to accession codes.

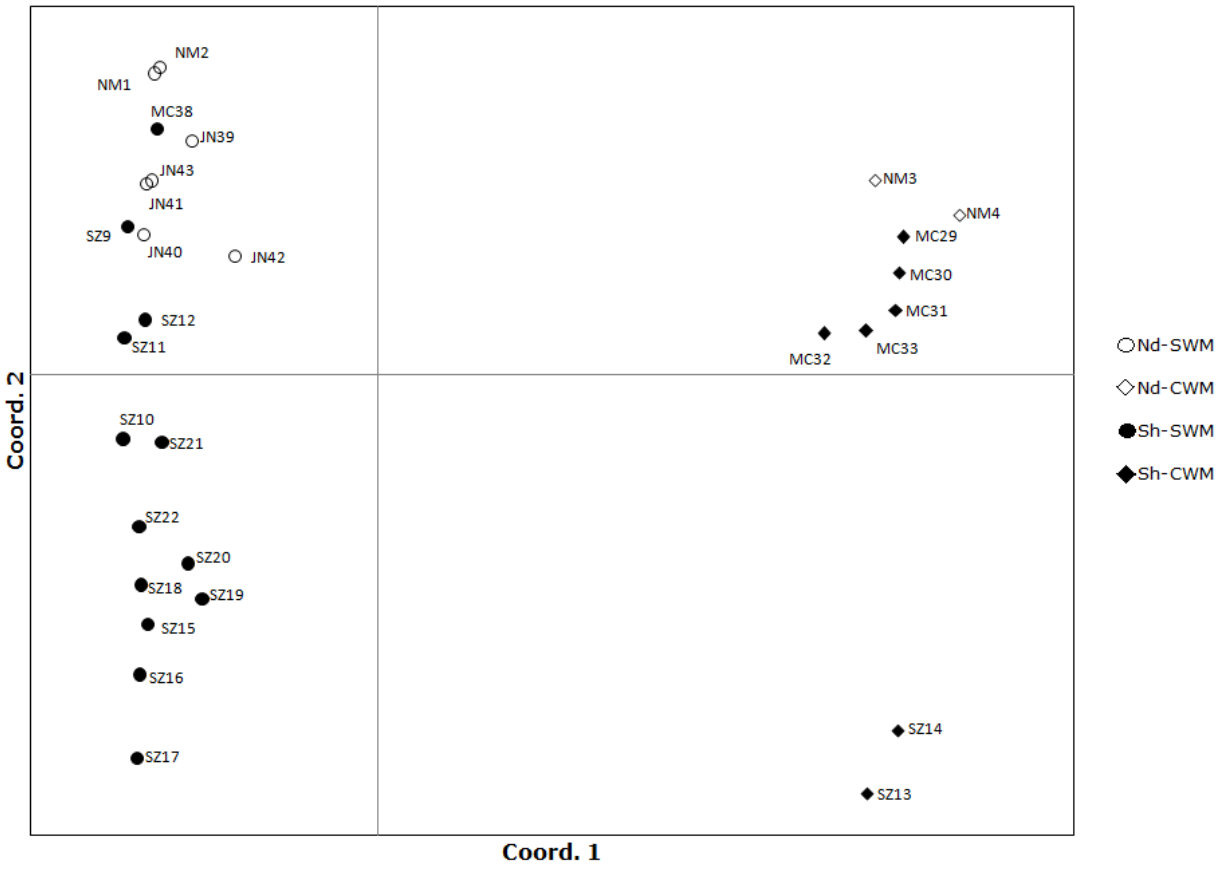

Figure 3. RAPD-based two-dimensional plot of MDS analysis of watermelon landraces from Chitanga village, Zimbabwe, collected from four farmers, belonging to two cultural groups, **Nd** Ndebele and **Sh** Shona, growing SWM (sweet watermelon) and CWM (cow-melon). The farmer's names are represented by the initials (NM, MC, JN and SZ), followed by accession number.

estimates of among-accession differentiation (Φ_{ST} = 0.81, G_{ST} = 0.77) were higher than values obtained for wild annual (Φ_{ST} = 0.62, G_{ST} = 0.47) or short-lived perennial species (Φ_{ST} = 0.41, G_{ST} = 0.32), or for mixed breeding (Φ_{ST} = 0.40, G_{ST} = 0.20) or outcrossing species (Φ_{ST} = 0.27, G_{ST} = 0.22) in a large data compilation reported by Nybom (2004). This discrepancy was expected since our material contained the two strongly differentiated forms mentioned above. In addition, we did not analyze wild populations but instead groups of either full siblings or half-sibs since the seed batches were collected from fruits of a single plant. Grouping of accessions within each of the two forms, in the dendrogram and MDS, was strongly farmer-related. The AMOVA similarly demonstrated a significant differentiation between farmer households (32%, $P < 0.001$). On the contrary, socio-cultural differences (Shona versus Ndebele) had no effect on the distribution of genetic variability.

Values for expected heterozygosity within watermelon accessions ranged between 0.022 and 0.118. A larger sample size for each accession could possibly have detected more overall variation and produced somewhat higher values for expected heterozygosity values as previously described (Nybom, 2004). Mean value for within-accession expected heterozygosity was slightly higher (0.069) for cow-melons than for sweet watermelons (0.051). In previous studies, higher levels of genetic diversity have similarly been reported within *C. lanatus* var. *citroides* compared to *C. lanatus* var. *lanatus* (Navot and Zamir, 1987; Jarret et al., 1997). Our H_E values were considerably lower than the mean values for within-population expected heterozygosity reported for annuals (0.13), short-lived perennials (0.20), selfing (0.12), mixed breeding (0.18) and outcrossing species (0.27) (Nybom, 2004). Again, this could be expected since our accessions consisted of closely related seedlings. Our values are, however, also lower than RAPD-based values for accessions consisting of single-fruit offspring in obligately outcrossing species like *Hippophae rhamnoides* (Bartish et al., 2000b), H_E = 0.069 to 0.134) and *Chaenomeles* spp. (Bartish et al., 2000a), H_E = 0.139–0.258). Possibly, our watermelon seedlings derived mainly from a mixture of selfing and pollination between closely related genotypes (plants of the same

landrace), and only to a minor extent from pollination between more distantly related genotypes (plants of different landraces).

At village level, sweet watermelons (Φ_{ST} = 0.62, G_{ST} = 0.64) are slightly more differentiated than cow melons (Φ_{ST} = 0.55, G_{ST} = 0.57). This could be a result of the previously reported negative correlation between within-population and between-population variation (Nybom and Bartish, 2000), Nybom, 2004). Furthermore, differentiation in the absence of seed exchanges among farmers could have resulted in localized unique varieties of sweet watermelons, if not related to historical sources which have to do with the geographic distances among these groups.

Factors shaping watermelon genetic diversity patterns

According to Brocke et al. (2003), different farmer management strategies as well as the seed source and soil conditions contribute to the differentiation of plant populations within a village. Carefully collected information on farmers' practices and perceptions therefore has the potential to explain some of the patterns of genetic diversity on individual farms (Brush, 1991).

The accuracy with which farmers discriminate the diversity of their crop population has important evolutionary implications since it is closely related to the level of conscious selection that farmers can apply (Barnaud et al., 2007). Farmers in Chitanga village discriminate between all of their landraces based on taste, size of the fruit and softness of flesh. The cultivated cow-melons are normally of the same size as sweet watermelons, and differ mainly by their insipid taste and hard rind. These two groups of watermelons are distinguished from the wild weedy types based on fruit size, hardness of rind and seed size. The fruits and seeds of the cultivated cow-melons and sweet watermelons are larger compared to the wild weedy types. In addition, the rind of the wild weedy forms is much harder compared to the cultivated landraces of watermelons.

Among sweet watermelons, farmers distinguish landraces according to fruit color, seed color, flesh color and sweetness, while cow-melons are distinguished mainly by seed color and flesh color. However, because sweet watermelons are preferred for eating fresh and for income generation, they are sown in abundance in the fields compared to the cow-melons. Differences in social valuation of landraces can affect abundance in the fields and therefore also genetic drift (Barnaud et al., 2007). Farmers select fruits of each landrace for the next sowing according to size, taste and flesh color; their selection may preserve unique genotypes of landraces. Even though farmer selection is focused on improving similar traits, the differentiation of landraces observed may imply that specific farmers incorporate various and different concerns unique to them (Bellon, 1996).

Interestingly, genetic variation within the two Ndebele-grown cow-melon landraces was very low (H_E = 0.024) compared to variation within the Shona-grown landraces (H_E = 0.082). Cultivation of this crop is not very common among the Ndebele people who often regard, cow-melons mainly as wild weedy forms and may therefore have sown only a few seeds each year thus depriving these landraces of genetic variability. The spatial separation of sweet watermelons from cow-melons is further evidence of the attempt to keep cow-melons apart from the more favored sweet watermelons. By contrast, within-landrace variation was slightly higher in Ndebele-grown sweet watermelons (H_E = 0.055) compared to Shona-grown (H_E = 0.048) which may reflect the larger distance between rows with different landraces in fields of the Shona farmers.

The village of Chitanga was chosen for our study because it was recently populated by people from two different socio-cultural groups. In addition, the four farmer households investigated could be expected to have brought a wide variety of different watermelon landraces. Interestingly, no seed exchange is practiced among these farmers even after almost 10 years of co-habitation; each farmer uses his/her own seed or sometimes seed obtained from close family relatives. The severe draught requiring well-adapted landraces together with the existence of cultural myths among the farmers apparently act as deterrents to seed exchange practices. Provided that sufficient research funding can be secured, we intend to repeat sampling and analyses of watermelon landraces in Chitanga every five years in order to investigate effects of possible changes in both socio-cultural and biological factors that affect amount and distribution of genetic variability.

Implications for genetic conservation

Diversity within and among landraces can confer long-term adaptation of crop populations to fluctuating and heterogeneous environments. Farmers' management of landraces on farm has the potential to ensure a continuing high degree of heterogeneity and adaptation (Brocke et al., 2003). Key to conserving this important crop diversity is understanding how the diversity is perceived and valued by farmers (Elias et al., 2001).

Our molecular marker study demonstrated the existence of highly differentiated accessions within each of the two forms of watermelons, thus supporting the farmers' claims that they maintain individual watermelon landrace types. Our study also indicates that levels of genetic variability within landraces can be associated with both valuation of the crop in question, and planting distances. Consequently, landraces managed on farm could form the basis of *in-situ* maintenance units at

village level; particularly those grown in isolated patches or where increased planting distances is practiced. Since uniqueness of landrace types was linked to specific farmers, regardless of their cultural affiliations, the identification of germplasm for conservation should be done in collaboration with individual farmers for the maintenance of specific landrace genotypes. Specific landraces could be propagated by farmers following their traditional seed system. Ultimately, conservation strategies should be concentrated on as many landraces as possible, but most importantly those that might be at a risk of being lost in spite of their unique characteristics. The role of conservationists according to Maxted et al. (2002) should be relatively passive; monitoring farming practices or genetic diversity of the target taxa and intervening only if the farming system is threatened or if there is a significant deleterious change in genetic diversity. One such potential threat is the fact that selection for large fruit size, fleshy color and sweetness is more likely to increase in the future in order to satisfy the needs of buyers when income generation within farmer household becomes more important. Among the possible consequences is loss of diversity, especially of varieties with smaller-to-medium sized fruits and/or less desirable flesh color and taste; and thus of long-term adaptive potential of crop populations.

Conclusion

This study addressed the pattern of genetic diversity of watermelon landraces at the local scale and the results affirm the role of farmers' practices in the maintenance of unique genotypes of landraces. It demonstrates the usefulness of combining molecular genetics with participatory socio-economic data in order to elucidate the observed genetic diversity patterns at local level. The results of the study can help to identify the main forces that determine genetic diversity at local village level, as well as the active role of farmers in creating and using biological diversity. Further studies targeting a number of villages might reveal other patterns of genetic diversity, in particular taking into consideration original villages, which are not a result of the Government's Land Reform Programme. This approach is envisaged to provide holistic and additional social issues for investigating diversity of watermelons in marginal environments.

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