

## Molecular and farmer-based comparison of a wild-weed and landrace complex of watermelon in Zimbabwe

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### Abstract

Traditional farming systems have been associated with the occurrence of intervarietal and interspecific natural crosses in many crop plants, thus contributing to the genetic diversity of the crop through genetic exchange. In this study, a combination of farmer-preferred morphological traits and RAPD markers were used to evaluate the dynamics of genetic diversity in 43 watermelon accessions collected at a single village level with a traditional farming system. The molecular variability assessed with RAPD markers and analyzed with multidimensional scaling and cluster analysis, demonstrated a substantial differentiation among the accessions. Population structure analysis also demonstrated the existence of three major forms of watermelon, identified by a set of alleles predominant within each form. Dendrograms based on RAPD data and on farmer-preferred traits data were positively correlated according to a Mantel test. Although cultivated cow-melons were genetically most similar to wild-weedy plants at molecular level, they grouped more similar to sweet watermelons based on farmer-preferred traits. The present study revealed limited gene flow between three forms of watermelon and provides insight into how the genetic differentiation corresponds to farmers' classification of watermelons.

**Keywords:** watermelon; genetic diversity; genetic admixture; folk varieties; landraces; conservation.

**Abbreviations:** CWM - cow-melon; RAPD - random amplified polymorphic DNA; SWM - sweet watermelon; VAR - hybridogenous variant; WWM - wild-weedy melon; SS - seed size; DSC - dominant seed colour; SSC - secondary seed colour; TAF - taste when eaten fresh; SMF - size of mature fruit; FCP - fruit colour pattern; SML - shape of mature leaves; TFS - toughness of fruit skin.

### Introduction

Watermelon (*Citrullus lanatus*), an annual monoecious diploid species ( $2n=22$ ), belongs to the family Cucurbitaceae. It is characterized by sprawling hairy vines with pinnately lobed leaves and is generally treated as two botanical varieties: *C. lanatus* var. *lanatus* the cultivated sweet watermelons, and *C. lanatus* var. *citroides*, the citron melons or cow-melons found in Southern Africa in the wild and also in cultivation. Southern Africa is usually regarded as the centre of origin of watermelon (Dane and Liu, 2007). There is tremendous variation between and within forms of watermelon in the drought-prone agro-ecosystems of Zimbabwe and parts of Southern Africa within the periphery of the Kalahari and Namib Deserts. The watermelon complex spans from wild-weedy forms to farmer-selected landraces or folk varieties, and is integral to local farming practices aiming to meet specific purposes for human use (dessert, oil, seed and porridge) and animal feed. In general, weedy crop relatives are dependent on land-use practices, and mostly emerge after land tilling (Brush, 1991). In an outcrossing crop such as watermelon, interbreeding between cultivated and wild and/or weedy relatives, give rise to intervarietal or interspecific forms. Natural hybridization and introgression are considered to be vital components of the evolutionary

forces that shape and maintain crop diversity on-farm (Barnaud et al., 2009), and interbreeding complexes of wild, weedy and cultivated species are important for generating genetic variability in landraces of for example beans (Beebe et al., 1997). Understanding the wild-weed-crop relationships is key to the development of an effective on-farm conservation strategy. This requires a coherent approach integrating elements of population genetics, environmental and social aspects, local knowledge and seed systems (Brown, 2000). Extensive variation in morphological traits, from wild and weedy watermelons over to cultivated landraces, was observed during a plant collection expedition to Chitanga village where these forms were found in the same field. In watermelon, weedy forms cannot be distinguished from wild types, possibly because both (hereafter called wild-weedy melons), are dispersed mostly by livestock and wild animals, and often emerge in farmers' fields. Farmers distinguish and identify wild-weedy melons using local names: *Guna* in Shona and *Kiriwani* in Ndebele. The same local names also apply to putative hybrids between cultivated watermelons and wild-weedy melons. A putative hybrid between sweet watermelon and cultivated cow-melon is instead locally known as *Ganganwiwa* in Shona, and often as

*Kiriwani* in Ndebele. In Namibia, introgressed types are classified as agronomic weeds when growing together with the melon crops (sweet watermelon, cooking melon and seed melon landraces) in traditional agro-ecosystems (Maggs-Kolling et al., 2000). Due to a series of droughts, most farmers in Zimbabwe however prefer to keep those wild-weedy melons that emerge at the outskirts of the fields, for ultimate use as animal feed. The existence of wild-weedy melons in farmers' fields, close to the cultivated landraces, thus provides a niche for development of hybridogenous variants. Ultimately, the presence of a wild-weed-crop complex in farmer's fields could be indicative of ongoing domestication (Beebe et al., 1997). The present study sought to compare the degree of genetic differentiation of a wild-weed and landrace complex of watermelon at molecular and farmer-preferred trait levels. In addition, population structure analysis was used to investigate the possible existence of gene flow. Information about the structure and pattern of genetic diversity within and among different forms of watermelons has implications for conservation efforts.

## Results

### *Genetic structure of watermelon varieties*

Results of the RAPD-based cluster analysis were illustrated in a dendrogram (Fig. 1). The cophenetic correlation between the Nei's genetic distance matrix and the dendrogram was 0.969, suggesting a very high goodness of fit (Rohlf, 2000). Two major clusters were differentiated at 49% genetic similarity: one cluster containing the sweet watermelon accessions and another cluster with all cow-melons but separated into two main sub-clusters of cultivated cow-melons and wild weedy melons at 83.6% genetic similarity. Inside the wild-weedy melon cluster, one cultivated cow-melon accession (CWM04) formed a small subcluster with a wild-weedy melon (WWM05). These two accessions may be products of gene flow between cultivated cow-melon and wild-weedy melon. The sweet watermelon group was separated into two subclusters at 86.6% genetic similarity. The putatively hybridogenous accession VAR23 occurred as an outlier to the sweet watermelon cluster with which it shared 86% genetic identity. Multidimensional scaling (not shown) produced a similar grouping, confirming the cluster analysis. STRUCTURE-based analysis of population structure and gene flow generally confirmed the occurrence of three major watermelon forms (Fig. 2). At  $K = 3$ , sweet watermelons, cultivated cow-melons and wild-weedy melons were clearly resolved. Only a few admixtures were found; accession VAR23 was similar to sweet watermelon but also showed some influence from wild-weedy melon, whereas accessions WWM05 and WWM28 were most similar to wild-weedy melons but with influence from cultivated cow-melon and from sweet watermelon, respectively. At  $K = 5$ , STRUCTURE-based grouping resolved two cultivated cow-melon groups with accessions 13 and 14 deviating from the larger group (Fig. 2). Finding two allele patterns in cultivated cow-melon suggests the existence of two subgroups; one with accessions CWM13 and CWM14, and the other with the remaining accessions. In this latter subgroup, accession CWM04 showed a small amount of admixture with wild-weedy melons. This accession was the only one grouping with the wild-weedy melons in the dendrogram, where it was close to the admixed accession WWM05. STRUCTURE analysis found only one sweet watermelon group and, mainly, also only one group for wild-weedy melons. For two of the above-mentioned admixture accessions, VAR23 and

WWM05, the same combinations of sweet watermelon plus wild-weedy melon, and wild-weedy melon plus cultivated cow-melon, respectively, were suggested just as in the analysis with  $k = 3$ . For the third accession, WWM28, considerable influence of a unique fifth group was, however, indicated.

### *Farmer-preferred morphological traits*

The two matrices based on RAPD data and on the eight farmer-preferred morphological traits data, respectively, were highly correlated ( $r = 0.833$ ,  $P < 0.001$ ), suggesting that farmers' knowledge of local forms of watermelons, for the most part, was supported by genetic differentiation as measured by the RAPD analysis. A cluster analysis based on the farmer-preferred morphological traits retrieved three distinct clusters of sweet watermelons, cultivated cow-melons and wild weedy melons (Fig. 3). In contrast to the RAPD-based dendrogram, the main dichotomy now occurred between the cultivated watermelons (sweet watermelon and cow-melon) on the one hand and wild-weedy melons on the other hand. A major cluster of sweet watermelons and cultivated cow-melons was thus differentiated at 27% similarity from the wild-weedy melon cluster. The cultivated cow-melon subcluster branched off at the 46% similarity level. The putatively hybridogenous accession VAR23 clustered with the cow-melons but branched off at 57% similarity level. The cophenetic correlation between the similarity distance matrix and the dendrogram was 0.922, suggesting a very high goodness of fit. A Kruskal-Wallis test in which the three sub-clusters were treated as factors and morphological traits as response variables, showed that most of the traits, except for predominant seed colour (DSC) and secondary seed colour (SSC), were able to distinguish ( $P < 0.001$ ) the three sub-clusters.

## Discussion

### *RAPD markers, farmer-preferred traits and multivariate analysis*

Some scholars have raised concern about reproducibility, primer competition and the inability to distinguish heterozygotes from homozygotes in estimating genetic diversity with RAPD markers (Nybom, 2004; Weising et al., 2005). However, in our recent study in watermelons, RAPD markers proved reliable and informative for assessing genetic diversity, yielding results that were very similar to those obtained with microsatellite DNA analysis of the same set of samples (Mujaju et al., 2010). The degree of genetic differentiation of sweet watermelon group (*Citrullus lanatus* var. *lanatus*), cultivated cow-melon and wild-weedy melon groups (*C. lanatus* var. *citroides*) at molecular and farmer-preferred trait levels revealed different patterns. The RAPD-based dendrogram showed a major dichotomy between sweet watermelons on the one hand, and cultivated cow-melons and wild-weedy melons on the other hand. By contrast, the farmer traits differentiate strongly between cultivated (sweet watermelon and cow-melon) and wild-weedy forms, and this may or may not coincide with the delimitation of the two botanical varieties *lanatus* and *citroides*. This could be linked to conscious selection of planting materials (Mujaju and Nybom, 2011), which results in farmer traits contributing to the observed dichotomy between the cultivated forms (sweet watermelon cultivation and cow-melon groups) and the wild-weedy melon forms. Both the cultivated forms are selected for larger size fruits against the wild-weedy melon forms.

**Table 1.** Farmers' field characterization of watermelons in Chitanga village, Zimbabwe.

Acc. Code	Type Code	Farmer preferred traits for distinguishing watermelons							
		SS	DSC	SSC	TAF	SMF	FCP	SML	TFS*
CM1	SWM1	1	5	0	0	1	0	0	0
CM2	SWM2	1	9	0	0	1	0	1	0
CM3	CWM3	1	2	0	1	1	0	0	1
CM4	CWM4	1	4	0	1	1	0	0	1
CM5	WWM5	0	9	0	1	0	0	0	2
CM6	WWM6	0	4	0	1	0	0	0	2
CM7	WWM7	0	9	0	1	0	0	0	2
CM8	WWM8	0	8	0	1	0	0	0	2
CM9	SWM9	1	9	1	0	1	0	1	0
CM10	SWM10	1	5	0	0	1	0	1	0
CM11	SWM11	1	7	0	0	1	0	1	0
CM12	SWM12	1	5	0	0	1	0	1	0
CM13	CWM13	1	7	0	1	1	0	0	1
CM14	CWM14	1	7	0	1	1	0	0	1
CM15	SWM15	1	0	0	0	1	0	1	0
CM16	SWM16	1	0	3	0	1	0	1	0
CM17	SWM17	1	5	0	0	1	0	1	0
CM18	SWM18	1	9	0	0	1	0	1	0
CM19	SWM19	1	6	0	0	1	0	1	0
CM20	SWM20	1	9	1	0	1	0	1	0
CM21	SWM21	1	8	0	0	1	0	1	0
CM22	SWM22	1	5	0	0	1	0	1	0
CM23	Var23	1	9	0	1	1	1	1	1
CM24	WWM24	0	1	0	1	0	0	0	2
CM25	WWM25	0	1	2	1	0	0	0	2
CM26	WWM26	0	1	2	1	0	0	0	2
CM27	WWM27	0	1	0	1	0	0	0	2
CM28	WWM28	0	4	3	1	0	0	0	2
CM29	CWM29	1	4	2	1	1	0	0	1
CM30	CWM30	1	1	2	1	1	0	0	1
CM31	CWM31	1	7	0	1	1	0	0	1
CM32	CWM32	1	1	2	1	1	0	0	1
CM33	CWM33	1	1	2	1	1	0	0	1
CM34	WWM34	0	3	0	1	0	0	0	2
CM35	WWM35	0	4	2	1	0	0	0	2
CM36	WWM36	0	6	0	1	0	0	0	2
CM37	WWM37	0	4	2	1	0	0	0	2
CM38	SWM38	1	9	1	0	1	0	1	0
CM39	SWM39	1	9	0	0	1	0	1	0
CM40	SWM40	1	0	3	0	1	0	1	0
CM41	SWM41	1	6	0	0	1	0	1	0
CM42	SWM42	1	0	0	0	1	0	1	0
CM43	SWM43	1	0	3	0	1	0	1	0

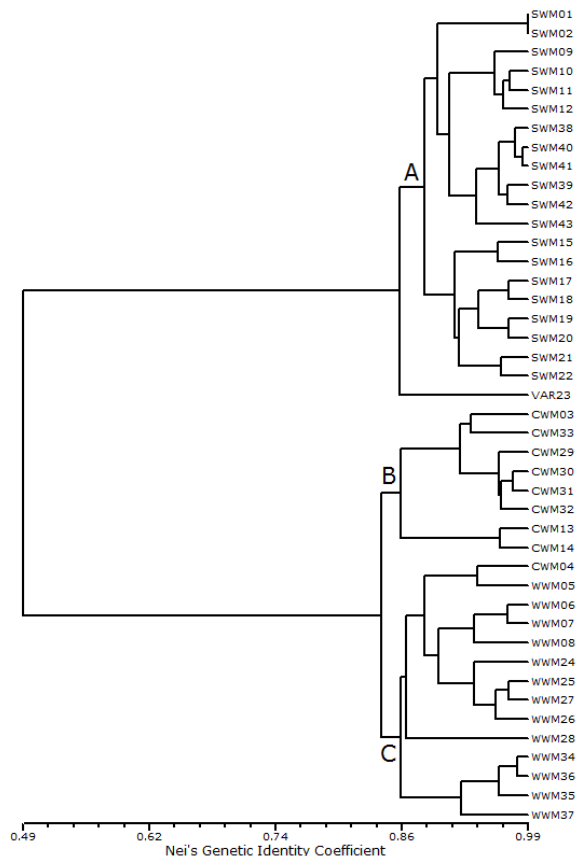
\* Acc. Code is accession code deposited at the Zimbabwe National Plant Genetic Resources Centre; Type Code indicates the same accessions with different watermelon forms (SWM - sweet watermelon; CWM - cow-melon; WWM - wild-weedy melon). Farmer-preferred traits: SS - seed size; DSC - dominant seed colour; SSC - secondary seed colour; TAF - taste when eaten fresh; SMF - size of mature fruit; FCP - fruit colour pattern; SML - shape of mature leaves; TFS\* - toughness of fruit skin.

Farmer-based classification of watermelons were able to distinguish ten types of landraces (seven seed types and three dessert types) in Mali (Nantoumé, 2011) and three main types (dessert, seed and cooking types) in Mozambique (Munisse et al., 2011). However, DNA studies using SSR markers indicated that all different types found in Mali belong to *C. lanatus* var. *lanatus* whereas those in Mozambique separated into the two botanical varieties *lanatus* (the dessert types) and *citroides* (the seed and cooking types).

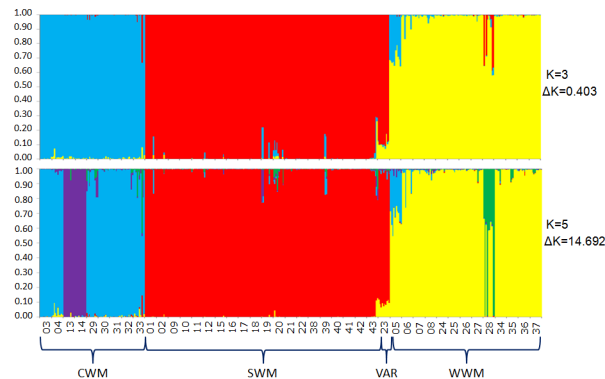
#### Genetic structure and gene flow

Cluster analysis and multidimensional scaling of molecular data differentiated the 43 watermelon accessions into two well-defined groups, the sweet watermelon group and the cow-melon group. A similarly strong differentiation between

these two forms has been noted also in other DNA marker-based studies of watermelon landraces in Africa (Mujaju et al., 2010; Mujaju et al., 2011; Mujaju and Nybom, 2011; Munisse et al., 2011). In our study, surprisingly few accessions showed evidence of genetic admixture (and then usually only at a low level), considering that this species is cross-pollinating with monoecious flowers (although within-plant pollination may be common), and that all accessions were obtained from the same village. Gene flow may be effectively limited due to large spacing distances among the different watermelon forms and to the close-knit rows of intervening intercrop plants of pearl millet and sorghum on the watermelon farms in Chitanga (Mujaju and Nybom, 2011). The presence of admixture between sweet watermelon and cow-melon was indicated also in one out of the eight analysed watermelon accessions collected from other areas in Zimbabwe (Mujaju et al., 2010). Hybrids between sweet



**Fig 1.** RAPD-based UPGMA dendrogram of watermelon accessions showing two major clusters: **A** sweet watermelons (SWM) and the putatively hybridogenous VAR23, and a second cluster with two sub-clusters: **B** cow-melons (CWM), and **C** wild-weedy melons (WWM).

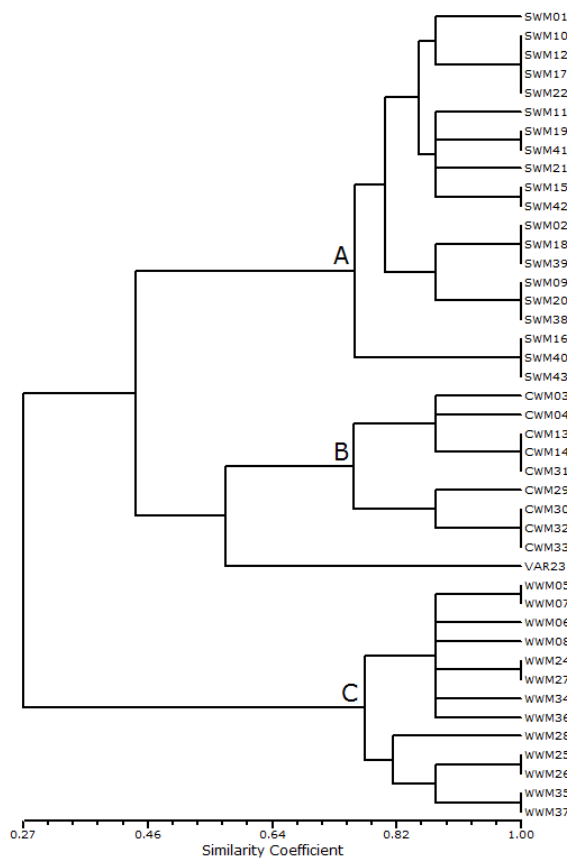


**Fig 2.** Inferred genetic structure of a wild-weed and landrace complex of watermelon in Chitanga village, Zimbabwe. Each plant is represented by a single vertical bar, which is partitioned into  $K$  (3 and 5) coloured segments. Each colour represents one cluster, and the length of the coloured segment shows the plant's estimated proportion of membership in that cluster as calculated by STRUCTURE in a typical run at that value of  $K$ . Ten plants per accession (accession numbers given below the bars) were analyzed. Scale of Y axis represents probability of log likelihood.

watermelon and any of the other two forms are often insipid in taste, as demonstrated by their local name 'Ganganwiwa' which when translated simply means insipid taste. Generally, farmers often remove wild-weedy melons when close to sweet watermelons and more rarely when growing in proximity to cultivated cow-melons. Gene flow is thus expected to predominantly occur between the wild-weedy melons and cultivated cow-melons. Microsatellite DNA-based STRUCTURE analysis of watermelon accessions from Mozambique also demonstrated the existence of admixtures between dessert types (sweet watermelons) and seed types (cow-melons) in the northern province of Cabo Delgado where the two out of five seed type accessions were positioned between the two major types (Munisse, 2011). A study from Mali alluded to the presence of admixtures, since a large proportion of the watermelon landraces could be grouped into dessert and seed types (non-sweet types used for seed extraction and cooking) but with no clear separation between them (Nantoumé, 2011). The seed types in Mali, however, have been suggested to be separate and distinct from the seed types found in southern Africa (Andersen, pers. com.), which would be consistent with the finding based on SSRs that they are genetically all var. *lanatus*, not *citroides*.

#### Units of conservation and their implications

Plant varieties maintained as farmers' seeds are often treated under the term 'landraces', although they may have resulted from variable levels of conscious farmer action (Berg, 2009). Within the watermelons, variety types or forms reflect different modes of seed management and different levels of farmer involvement. The sweet watermelon form is generally treated as a valuable asset for household use and income generation, whereas the treatment of cultivated cow-melons in traditional farming systems varies across cultural groups. Sweet watermelons are actively selected for better traits to meet the market demand (Mujaju and Nybom, 2011). Cow-melons, when cultivated are mainly used for on-farm consumption and animal feed, are subjected to less selection pressure. Our recent study noted that in Zimbabwe, Shona farmers value cow-melons higher compared to the Ndebele farmers, and consciously select seeds from large-sized fruits for sowing (Mujaju and Nybom, 2011). Ndebele farmers exercise seed selection only on sweet watermelons. Across all cultural groups, local farming practices often seek to weed off the wild-weedy melons and/or restrict them to the distal parts of the fields. While the evolution of different watermelon forms may be a result of natural selection, intentional selection on-farm have certainly maintained and increased the distinctive properties of each form, and contributed to the breadth of useful diversity within especially the sweet watermelon and the cultivated cow-melon. The sweet watermelons should certainly be classified as folk varieties, whereas cultivated cow-melons can be considered either as landraces or folk varieties depending on the extent of farmer involvement. Finally, the wild-weedy melons can be regarded as very primitive landraces, allowed to exist in the outskirts of the cultivated fields, and being used only in times of acute need. The existence of both landraces and folk varieties in the same community, depending on crop type and applicable technology, has been described in the literature (Berg, 2009). Conservation programmes, whether on-farm or *ex-situ*, should therefore recognize the correct identity of watermelon forms, the varying levels of stable and recognizable units within these forms, as well as the possibility of genetic admixture within and between the major forms.



**Fig 3.** UPGMA dendrogram of watermelon accessions using farmer-preferred traits, showing three major clusters representing **A** sweet watermelons (SWM), **B** cow-melons (CWM) and the putatively hybridogenous VAR23, and **C** wild-weedy melons (WWM). Sweet watermelons belong to *C. lanatus* var. *lanatus*, cow-melons and wild-weedy melons belong to *C. lanatus* var. *citroides*.

## Materials and methods

### Study site, plant materials and farmer-preferred traits

Forty-three watermelon accessions were collected from the Chitanga village in Zimbabwe (21°17'S, 30°45'E), of which 29 were landraces, one putative hybrid between sweet watermelon and cultivated cow-melon, and 13 wild-weedy melons. Each accession consisted of a batch of seed from a single plant grown on-farm and obtained from a local farmer during the harvesting period of April–May 2009. Prior to collecting any samples, 43 accessions of watermelon were identified on the basis of morphological characters used by farmers to classify the material in the field (Table 1). To avoid male-dominance, the identification of characters was initially done separately for females and males. The final characters were then chosen by the whole group together, after demonstrating the applicability of each one of them in the field. In total, farmers selected eight qualitative characters as being important for distinguishing forms of the wild-weed and landrace complex of watermelon in the field: (1) seed size (SS) based on length: 0 small (< 1cm), 1 large (> 1cm) (1; (2) dominant seed colour (DSC) occupying at least 70%

of seed area: 0 white, 1 green, 2 light green, 3 grey, 4 yellow, 5 light brown, 6 brown, 7 red, 8 dark red, 9 black; (3) secondary seed colour (SSC) occupying less than 30% seed area: 0 none, 1 white, 2 brown, 3 black; (4) taste when eaten fresh (TAF): 0 sweet, 1 not sweet; (5) size of mature fruit (SMF) after weighing: 0 small (< 5kg), 1 large (> 5kg); (6) fruit colour pattern (FCP): 0 defined or solid, 1 mixed; (7) shape of mature leaves (SML): 0 semi-divided, 1 deeply divided; (8) toughness of fruit skin (TFS) when tapped with a hand: 0 soft, 1 medium, 2 tough.

### DNA extraction and RAPD analysis

Seeds from the 43 accessions were germinated at 25 °C in a greenhouse at Balsgård in Sweden, and a total of 430 plants (10 per accession) were chosen for this study. DNA was extracted from five-to seven-day young leaf tissue using the Qiagen Dneasy™ Plant Mini Kit (Applied Biosystems) following the manufacturer's protocol. Choice of RAPD primers, PCR protocol, and subsequent DNA fragment evaluations followed Mujaju et al. (2010).

### Data scoring and analysis

#### RAPD data

Each RAPD band was considered as an independent locus, and polymorphic bands were scored as absent (0) or present (1) for all the 430 plants. Levels of similarity (relatedness) among and within accessions were quantified with cluster analysis and ordination using NTSYS-pc, version 1.80 (Rohlf, 1993). For cluster analysis, Nei's pairwise genetic identity matrix generated from GenAEx 6 was used to construct a dendrogram using the unweighted pair-group method with arithmetic averages (UPGMA) algorithm. The distortion was tested using a co-phenetic variation analysis. A multidimensional scaling (MDS) was performed on the same dataset for comparison since this analysis is not hierarchical in nature like a cluster analysis. A model-based structure analysis for clarifying genotypic ambiguity (Falush et al., 2007) was performed with the computer program STRUCTURE version 2.3.3 (Pritchard et al., 2000) as a means to explore, verify and identify clusters existing in the wild-weed and landrace complex of watermelon. STRUCTURE was run with number of clusters or groups (K) from 2 to 10. Each K was run 10 times with a 'burn-in period' of 10,000 rounds, assuming an admixture model. The most likely number of genetic clusters was estimated by the statistical ad hoc method using  $\Delta K$  (Evanno et al., 2005). An individual accession was attributed to a given cluster when the proportion of its genome in the cluster (qK) was higher than an arbitrary commonly used cutoff value of 80% (Vigouroux et al., 2008).

#### Farmer-characterized data

Kruskal-Wallis non-parametric analysis of variance was performed using Minitab 16 to test the significance of each trait among the forms of watermelon. All variables were combined to describe the structure of morphological diversity applying cluster analysis using NTSYS-pc. An average similarity matrix was computed and UPGMA was used to construct a dendrogram. Correlation between the matrices based on RAPD data and morphological traits data, respectively, was investigated with a Mantel test (MXCOMP in NTSYS-pc), which applies 9999 permutations to the significance of a given correlation.

## Conclusion

This study revealed the existence of three major groups; sweet watermelon, cultivated cow-melon and wild-weedy forms in a traditional farming system in Zimbabwe. Since only a few accessions showed admixture, a limited gene flow among watermelon groups is suggested. Additional studies with more watermelons from more villages are needed to explore the relationships among the different forms, and should be useful in exploring and maintaining germplasm for future plant improvement endeavours.

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