Diversity of Landraces and Wild Forms of Watermelon (*Citrullus lanatus*)

Distribution and Implications for Conservation in Southern Africa, with emphasis on Zimbabwe

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Cover: Structure bar plot, leaves of sweet watermelon type (upper) and cow-melon type (below), watermelon distribution map of Zimbabwe, SSR electrogram, various fruits of watermelon, seeds of watermelon, RAPD banding patterns on agarose gel, 2-dimensional plot

(photos: C. Mujaju)

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Abstract

Watermelon (*Citrullus lanatus*) is commonly grown in traditional agrosystems throughout the drought-prone Southern Africa as a staple food (edible seeds), a dessert food (edible flesh), and for animal feed. Several morphotypes of watermelon are found in this area; sweet watermelon, cooking melon and seed melon landraces of the traditional agrosystems; and possibly introgressed types which are regarded as agronomic weeds. There has been little work on investigating the relationships between wild and cultivated forms, and to study amount and partitioning of genetic variation, to allow for better conservation strategies. Previous studies have reported relatively low levels of genetic diversity in cultivated watermelon but these have been based mainly on US plant introductions and modern watermelon cultivars linked to breeding programmes for disease resistance. By contrast, germplasm maintained in the putative centre of origin in southern Africa, can be expected to display considerably higher variability.

Three different sampling strategies were used to collect plant material of both wild and cultivated forms of cow-melons (*Citrullus lanatus* var. *citroides*) and of sweet watermelons (*C. lanatus* var. *lanatus*, only known from cultivation); (1) in-depth sampling in the fields of one village in Zimbabwe, (2) medium-scale sampling across the watermelon growing districts in Zimbabwe, and (3) broad-scale sampling across Southern Africa (Botswana, Namibia, South Africa, Zambia and Zimbabwe). Two molecular marker methods were used, random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) also known as microsatellite DNA. Similarity matrices obtained with RAPD and SSR, respectively, have been highly correlated, suggesting that for some applications, the less demanding RAPD can be a useful alternative, especially in developing countries. Considerable amounts of genetic diversity were found at all levels, including within-accessions (half-sib families). Sweet watermelon accessions appear to contain almost as much variability as cow-melon accessions. A genetic structure analysis divided the wild-weed-landrace complex collected in one village into three groups confirming the existence of three major forms with limited admixture. Defining the major forms into landraces and/or folk varieties was considered critical for identification of proper units for both on-farm and *ex-situ* conservation. Distribution of most watermelon accessions in Zimbabwe was associated with sandy loam and sand soils.

Keywords: *Citrullus lanatus*, genetic diversity, RAPD, SSR, folk variety, landrace.

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Dedication

To my wife and family,

Your endless endurance that has seen you through a harsh economic environment in Zimbabwe captivated with patience and long-suffering to see me through this four-year study period is unfathomable.

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Claid Mujaju

Contents

List of Publications

This thesis is based on the work contained in the following papers, referred to by Roman numerals in the text:

I Mujaju C., Sehic J., Werlemark G., Garkava-Gustavsson L., Fatih M. & Nybom H. 2010. Genetic diversity in watermelon (*Citrullus lanatus*) landraces from Zimbabwe revealed by RAPD and SSR markers. Hereditas 147, 142-153.

II Mujaju C., Zborowska A., Werlemark G., Garkava-Gustavsson L., Andersen S. B. & Nybom H. 2011. Genetic diversity among and within watermelon (*Citrullus lanatus*) landraces in Southern Africa. Journal of Horticultural Science & Biotechnology 86, 353-358.

III Mujaju C. 2011. Distribution patterns of watermelon forms in Zimbabwe using DIVA-GIS. International Journal for Biodiversity and Conservation 3, 474-481.

IV Mujaju C. & Nybom H. 2011. Local-level assessment of watermelon genetic diversity in a village in Masvingo Province, Zimbabwe: structure and dynamics of landraces onfarm. African Journal of Agricultural Research (Accepted).

V Mujaju C., Werlemark G., Garkava-Gustavsson L., Smulders M.J. & Nybom H. 2011. The dynamics of genetic diversity and farmers' use of a wild-weed-landrace complex of watermelon in Zimbabwe (Manuscript).

VI Mujaju C. & Sehic J. 2011. Assessment of EST-SSR markers for evaluating genetic diversity in watermelon accessions from Zimbabwe (Manuscript).

The contribution of Claid Mujaju to the papers included in this thesis was as follows:

I I planned the work and collected watermelon accessions from Zimbabwe and then planted them in the greenhouse at Balsgård. I extracted DNA, optimized the DNA analyses (RAPD and SSR), and performed a large part of the analyses including the band evaluations. I performed all the statistical analyses and produced the first draft of the paper, and wrote the final version together with the supervisors.

II I sampled accessions of watermelon from each of the selected SADC countries (Namibia, Botswana, South Africa, Zambia and Zimbabwe) and planted them at Balsgård followed by DNA extraction. I planned the laboratory work and evaluated SSR marker data. I did all the statistical analyses and wrote the first draft manuscript, which I further worked upon together with supervisors to produce the final manuscript.

III I collected passport data from all accessions housed in the National Genebank of Zimbabwe, made the analyses and wrote the first draft manuscript, which I further worked upon with advice from my supervisors.

IV I planned the work and collected watermelon accessions at farms within a single village and the associated information on indigenous knowledge systems. I grew watermelon accessions at Balsgård, followed by DNA isolation. I performed RAPD band evaluations and all the statistical analyses, and wrote the first draft manuscript which I then worked on together with supervisors to produce the final manuscript.

V I collected data on farmer-preferred traits, administered a survey questionnaire, planted watermelon samples at Balsgård and performed RAPD band evaluations. I did all the statistical analyses and then wrote the first draft manuscript which I further improved upon together with supervisors.

VI I was responsible for the planning of the work and collection of the seeds in Zimbabwe. I grew the watermelon seeds at Balsgård and isolated DNA. I performed all the statistical analyses and I wrote the manuscript together with my co-author.

1. Introduction

1.1 Taxonomy of the genus *Citrullus*

The genus *Citrullus* belongs to the family Cucurbitaceae, subfamily Cucurbitoideae, tribe Benincaseae and subtribe Benincasinae. It consists of four diploid species (2n=22), which are generally sprawling hairy vines with pinnately lobed leaves. The species *C. lanatus* (Thunberg) Matsumura & Nakai consists of two botanical varieties: *C. lanatus* var. *lanatus* the cultivated watermelon widely grown around the world and *C. lanatus* var. *citroides*, a wild form found in Southern Africa and also cultivated mainly for feeding animals. *Citrullus lanatus* is characterized by large green leaves with three to five deep lobes, or more rarely none, medium-sized monoecious flowers with short pedicels, medium to large fruit with smooth skin, and flesh with a high water content, and oval to oblong seeds of a white or brown colour. Previously, the cultivated species *C. lanatus* included three subspecies: (i) *lanatus*, (ii) *vulgaris* with two varieties, var. *vulgaris* and var. *cordophanus*, and (iii) *mucosospermus* (Fursa, 1981). Lately, two varieties have been recognized, i.e., var. *lanatus* for the sweet watermelons, and var. *citroides,* which consists of a wild type (var. caffer) commonly known as "Tsamma" and the cultivated citron type known as citron melon or cow-melon.

Citrullus colocynthis (L.) Schrader is a perennial species, with globular fruits of 5–10 cm in diameter. It grows predominantly in sandy soils throughout Northern Africa, Southern Asia and the Mediterranean region (Robinson & Decker-Walters, 1997) and bears fruits that are bitter and even poisonous. *Citrullus ecirrhosus* Cogniaux is a perennial species, growing in Southern Africa and West Namibia although previously thought to be endemic only to the coastal Namib Desert (Meeuse, 1962). The fourth species, *Citrullus rehmii* De Winter, is an annual wild species confined to the western escarpment of Namibia (De Winter, 1990).

Two other species appear to be closely related to the genus *Citrullus; Praecitrullus fistulosu*s (Stocks) Pangalo and *Acanthosicyos naudinianus* (Sond) C. Jeffrey. The former grows in India and Pakistan, is commonly known as "Tinda" and belongs to a genus with a basic chromosome number of n=12 (Schippers, 2002) while the latter is a wild species native to southern Africa.

1.2 Origin, distribution and domestication of *Citrullus*

Generally, watermelon is distributed in tropical and subtropical regions and is well-adapted to long, warm or hot summers. The domesticated watermelon, *C. lanatu*s var. *lanatus*, is grown in tropical and subtropical regions worldwide; the preserving melon, citron melon or cow-melon, *C.*

lanatus var. *citroides*, is grown in southern Africa; the perennial *C. colocynthis*, known as the bitter apple, is grown for medicinal purposes from Northern Africa to Southwest Asia; the perennial *C. ecirrhosus* and the annual *C. rehmii* are wild species endemic to desert regions of Namibia (Robinson & Decker-Walters, 1997). Among the wild watermelon species, *C. colocynthis* has the widest distribution; the plant grows in the Mediterranean basin, in North Africa and in Southwestern Asia in dry and sandy habitats. It is indigenous to coastal and desert regions of Israel although over the past decades it has become less abundant, especially in the northern coastal plains (Zohary, 1983).

The primary centre of origin for watermelon remains debatable owing to the fragmented knowledge regarding domestication routes. One proposition suggests that watermelon originates from southern Africa and occurs naturally in Botswana, Malawi, Mozambique, Namibia, South Africa, Zambia and Zimbabwe (FAO, 2009b). A number of distinct landraces, which are cultivated in the Kalahari region and its periphery, may represent early forms of domestication. In this regard, the distribution of the wild taxa in Southwest Africa point to Namibia or possibly Southern Africa as the centre of domestication for watermelon. Several studies (Whitaker & Davis, 1962; Esquinaz-Alcazar & Gulick, 1983; Mohr, 1986; Rubatzky, 2001; Dane & Lang, 2004) have supported southern Africa, in part (Kalahari Desert) or the entire region, to be the centre of origin. However, the presence of 5,000-year-old seeds of *C. lanatus* in Libya as archaeological evidence implies that domestication might instead have occurred in Northern Africa. The oldest published records of *Citrullus* remains come from Egypt, the tomb of Tutankhamum (ca. 1330 BC), while it was known in Sudan as early as 1500 BC (van Zeist, 1983). Records of cultivated watermelon are known around the Mediterranean from early 1st millennium BC and in Egypt, watermelon has been an important vegetable for at least 4000 years (Robinson & Decker-Walters, 1997). From Africa, watermelon spread to India and China, and further to Southeast Asia and Japan. It was introduced to the Americas in early post-Columbian times (van der Vossen *et al.*, 2004). The spread of watermelons worldwide has resulted in the formation of secondary centres of diversity. India became a strong secondary centre of watermelon diversity after the introduction in ancient times (Whitaker & Davis, 1962). Northeast Brazil has also been suggested as a secondary center of diversity for watermelon, after an introduction by African slaves around three centuries ago (Romão, 2000). Studying the geographical distribution of plant lineages can help to synthesize both history and current genetic exchanges and provide insights into the different factors that shape the genetic diversity of the species and crop origins (Avise, 2000; Gepts, 2003).

A recent study using chloroplast DNA to infer biogeographic and evolutionary relationships, origin and domestication history of watermelon, suggests that cultivated and wild watermelon have diverged independently from a common ancestor, possibly *C. ecirrhosus* from Namibia (Dane & Liu, 2007). Though not exhaustive, this is the most recent study that points to Southern Africa as a centre of origin with a subsequent spreading to the Mediterranean areas and in an easterly direction to India, and later also to other parts of Europe and the Americas.

1.3 Cultivation, breeding and production of watermelons

1.3.1 Cultivation and breeding

Watermelon has a long history of cultivation in Africa and the Middle East and has been planted in the Nile Valley since the second millennium BC (Zohary & Hopf, 1988). The time span for watermelon cultivation in Central Africa is over 5000 years, and in Egypt and the Middle East over 4000 years. By the 10th century it was introduced in China, which is today the world's greatest producer and consumer of watermelon. By the 13th century, watermelon was grown in Europe, and the crop was introduced into North America during the 17th century (Whitaker & Davis, 1962; Jeffrey, 1975). Several cultivated watermelon varieties have been recognized in different parts of the world, differing in size, shape and colour of fruit skin, colour of flesh (red, pink, white and yellow), and the colour and size of seeds (Maheshwari, 1978).

Cultivated watermelon types have traditionally been red-fleshed (only dessert type) and seeded. There is, however, genetic variation for flesh colour in the species, and colours can range from white or yellow to orange, depending upon the genetic constitution. Yellow-fleshed cultivars are now available, and there may be a market for white-fleshed cultivars if quality can be assured, since consumers tend to associate white flesh with immaturity. A relatively recent development in watermelon breeding has been the use of ploidy manipulations to produce seedless triploid genotypes (Kihara, 1951). A number of seedless cultivars have been developed, but they tend to be more susceptible to physiological problems such as poor seed germination and hollow heart. Seedless varieties are produced by crossing a tetraploid (2n=4x=44) inbred line as the female parent with a diploid (2n=2x=22) inbred line as the male parent of the hybrid. The reciprocal cross (diploid female parent) does not produce seeds. The resulting hybrid is a triploid (2n=3x=33). Triploid plants have three sets of chromosomes, and three sets cannot be divided evenly during meiosis (the cell division process that produces the gametes). This results in non-functional female and male gametes although the flowers appear normal. Since the triploid hybrid is female sterile, the fruit induced by pollination tend to be seedless.

Unfortunately, the triploid has no viable pollen, so it is necessary to plant a diploid variety in the production field to provide the pollen that stimulates fruit to form.

The latest development in watermelon breeding is the screening of watermelon accessions worldwide for disease-resistance genes. Watermelons have been extensively screened for resistance to papaya ringspot virus watermelon strain and zucchini yellow mosaic virus (Guner, 2004). Furthermore, a new source of resistance to gummy stem blight in watermelon has been evaluated (Gusmini *et al.*, 2005). Recently, watermelon germplasm has also been screened for resistance to powdery mildew, a disease caused by *Podosphaera xanthii* (*Sphaerotheca fuliginea*) (Davis *et al.*, 2007). The study evaluated among others, watermelon accessions from southern Africa. Analysis by geographical origin revealed that 36% and 15% of the 93 most resistant accessions were from Zimbabwe and Zambia, respectively. These accessions constituted only 9% and 4% of the U.S. *Citrullus* species PI collection. Further work on the same material is now focused on inheritance studies and identification of multiple resistance genes in order to pyramide resistance sources into a single cultivar to obtain greater resistance stability.

Molecular markers have been used in breeding programmes particularly to map traits of interest, which include agronomic traits as hardness of rind, Brix of flesh juice (measure of sugar content), flesh colour (red and yellow) and rind colour. The initial linkage maps for watermelon were linked to the identification of genes associated with fruit bitterness and fruit colour (Navot *et al.*, 1990), and genes associated with diseases such as fusarium wilt resistance (Levi *et al.*, 2001c). More recently, an extended linkage map for watermelon based on SRAP, AFLP, SSR, ISSR and RAPD markers using a test population [(Griffin 14II3 x NHM) x PI 386015] produced 12 large linkage groups (II9.6–373 cM), and 7 small to medium-sized linkage groups (47.9–91.4 cM) (Levi *et al.*, 2006). Molecular mapping is helpful to the breeder, since it contributes to the development of marker-assisted selection (Staub *et al.*, 1996; Perin *et al.*, 1998). Genetic maps are also useful in gene cloning and in analyzing complex traits (Lee, 1995). Many more markers are still required for construction of a saturated map that can be used effectively in watermelon breeding programmes, and for locating genes that control important traits like fruit quality and resistance to diseases and pests.

1.3.2 Watermelon production and Southern Africa

Globally, watermelon is a major cucurbit crop that accounts for 10.7% of the world area devoted to vegetable production in 2009; it is grown on over 3.8 million ha producing more than 100 million metric tons of fruit. Watermelon is produced in more than 55 countries worldwide, with China accounting for 67.7% of the total production. Other leading countries are Turkey (3.8%), Iran (3.1%), Egypt (1.5%) and Russia (1.4%) (FAO, 2009a).

The African countries produced a total of 4,662,393 tones of which only 1.6% was contributed from Southern African, all of it commercially produced in South Africa. Do these production figures reflect an absence of watermelon production in the other countries within the region? Indeed not. But watermelon in Southern Africa is mostly grown in traditional agrosystems for subsistence. In these environments, production statistics are not captured. While watermelon is mainly cultivated as a monocrop using improved varieties and agro-chemical inputs in a formal commercial production system, this crop is instead typically grown under rainfed conditions intercropped with cereals or root crops by small-scale farmers in many African countries (Matanyaire, 1998) with almost no fertilizer or chemical application. Watermelon is a day length neutral crop grown from lowlands up to 2000 m above sea level, and naturally occurs in drier environments. It prefers a mean annual rainfall from 400 to 1000 mm and performs better under savannah environments in well-drained sandy loam soil (van der Vossen *et al.*, 2004). With climate change expected to adversely affect agricultural production in Africa, large areas of the continent are projected to become drier than at present (ICSU, 2008).

Rainfall is one of the most important resources in Zimbabwe and parts of Southern Africa. It feeds the rivers and groundwater from which water is extracted for agriculture. Changes in the supply of rainfall in total volume or in frequency and reliability have enormous consequences for a wide range of agricultural activities and hence people's livelihoods. Because agricultural production remains the main source of income for most rural communities in the region, adaptation of the agricultural sector is imperative to protect the livelihoods of the poor and to ensure food security. A better understanding of farmers' perceptions together with a molecular evaluation of the existing indigenous watermelon genetic resources could be very helpful for developing conservation and adaptation measures in view of the impending climate change. This is envisaged to assist the decision-making process to inform policies aimed at promoting successful adaptation strategies for the agricultural sector.

1.4 Nutritional status and uses of watermelons

Watermelon is almost free of fat, sodium and cholesterol: the fruit flesh contains 93–95% water, 5% carbohydrate, 0.5–1% protein, and 0.2% fat (Rubatzky & Yamaguchi, 1997). Watermelon has a high lycopene content in the red-fleshed cultivars: 60% higher than in tomato. Lycopene in the human diet helps to prevent heart attacks and certain types of cancer. Recently, watermelon rind has been found to contain an important natural compound called citrulline, an amino acid that the human body makes from food. Citrulline, found in high concentration in the liver, promotes energy and assists with the immune system (Perkins-Veazie *et al.*, 2001). One of the

key roles of citrulline is to create another amino acid, argenine, which plays an important role in wound healing, detoxification reactions, immune functions, and promoting the secretion of several hormones including insulin and growth hormone (Flynn *et al.*, 2002). Watermelon is also an excellent source of beta-carotene and vitamin C, while the seeds are high in vitamin E and in the antioxidant minerals zinc and selenium.

Worldwide, watermelon is primarily grown for dessert purposes and it is being consumed in greater amount than any other cucurbit (Robinson & Decker-Walters, 1997). The fruit of sweet types of *C. lanatus* make a delicious and refreshing dessert and can also be transformed into juice. In countries where the seeds are consumed, the 'Egusi' types of watermelons are the most important for cultivation. 'Egusi' type watermelons are prevalent in West Africa, where they have been selected for their edible seeds, and are appreciated as an important source of vitamin E, edible oil, protein, fat and carbohydrates (Badifu, 1993). The seeds provide an essential food source to supplement nutrient deficient diets of subsistence farming (Maggs-Kölling & Christiansen, 2003). In addition, 'Egusi' crops are used to generate household income, household food and gifts to relatives (Achigan-Dako *et al.*, 2008).

In sub-Saharan Africa, cow-melon (*Citrullus lanatus* var. *citroides*) is cultivated for its dried seeds reported to be rich in nutrients ~ 60% lipids and ~30% lipids (Loukou *et al.*, 2007). Furthermore, the rind is used to make pickles or conserves, and the fruits are fed to livestock.

1.5 Genetic diversity and conservation of *C. lanatus* in Southern Africa

1.5.1 Phenotypic diversity

The study of morpho-agronomic variability remains important for assessing genetic diversity for plant breeders. In Southern Africa, a study on the diversity of landraces, agricultural practices and traditional uses of watermelon (*C. lanatus*) in Mozambique, revealed three main types: a dessert type with sweet, white to red, spongy flesh; a seed type with white either firm or spongy flesh, and a cooking type with yellow, firm flesh (Munisse *et al.*, 2011). A study to assess morphological diversity undertaken in Namibia (Maggs-Kölling *et al.*, 2000) exhibited various morphotypes based on gross morphology, ecology and usage, and supported the indigenous classification system with three distinct groups (seed, cooking and fresh-eating types). Commercial watermelon cultivars formed a distinct cluster. Wide variation was found within groups of the local types whereas the genetic basis of the commercial type appeared to be narrow. Introgressed types regarded as agronomic weeds were found growing

together with the cultivated landraces (Maggs-Kölling *et al.*, 2000).

Schippers (2002) noted the existence of diverse wild forms of watermelons in the Kalahari Desert. Studies by the Botswana NPGRC (SPGRC, 2004) also highlighted the presence of various domesticated and wild watermelons. Domesticated watermelons in Botswana include landraces such as "Magapu", whose pulp is eaten fresh with colour varying from white to red, and "Marotse", whose pulp is cooked fresh or dried, and with seeds that are sometimes roasted and eaten as a snack. The Marotse type exists also in various forms of which some are known as "Sesowane" with seeds of high oil content and "Senowane". A hybridogenous type known as "Mekatse" was observed and suggested to be derived from a cross between two genetically diverse parents belonging to the "Magapu" and "Marotse" types (SPGRC, 2004).

The "Tsamma" melon is found in the wild and is regarded as an important source of water in the Kalahari Desert. In addition, its pulp is eaten after the flesh has been pounded, and seeds are consumed as roasted snacks or ground and prepared into a coarse meal. Ground seeds have also been used as a cosmetic when smeared over the body.

Preliminary investigations of the collections in Zimbabwe and the collection trips carried out around the country also bear witness to the existence of diverse forms of watermelons. The forms of *C. lanatus* in Zimbabwe can be broadly distinguished by their taste:

a. "Manwiwa", "Mavisi", "Mabvembe", "Makhabe" – these forms are referred to as sweet watermelons, and are consumed fresh.
b. "Mashamba", "Majodo" – these forms are referred to as cow-melons and are inspid in taste, only consumed after boiling and cooking to produce a meal called Nhopi in the Shona language or simply fed to animals.
c. "Kiriwani", "Ganganwiwa" – These forms probably arise from crosses between the sweet watermelons and cow-melons. The term "Kiriwani" is also used for wild-weedy melons which have comparatively smaller fruits. These forms, if kept by farmers, are mostly used as animal feed.

However, the relationship between farmer classification and different forms of watermelon requires further investigations. Wild watermelons from the desert have the ability to withstand severe drought conditions, and therefore are potential sources of genes for watermelon improvement programmes.

1.5.2 Molecular diversity

Molecular markers can be an effective means to determine genetic relatedness among cultivars and among selections used in watermelon breeding programmes. They are suitable in assessing how much allelic

diversity is present in a crop and have the potential for providing unique DNA fingerprints for each genetically distinct genotype, which is a useful means of identifying different forms of watermelon. Previous studies designed to examine genetic diversity and phylogenetic relationships among watermelon cultivars have used both isozymes and DNA-based methods (hybridization- and PCR-based). The thrust of most of the watermelon molecular studies have been on modern cultivated varieties from the developed world, mostly United States of America and Asia, mostly China and South Korea, and there has been limited studies associated with landraces in traditional agroecosystems. Most of these genetic diversity studies have only included southern African material encoded as US Plant Introductions, and as such because of adaptation to US environment, the material may not truly represent the existing diversity in southern Africa.

Isozyme polymorphism in *C. lanatus* and *C. colocynthis* exhibited little variation within ecotypes, and the commercially grown cultivars were monomorphic at all loci except for one *C. lanatus* accession which carried alleles of *C. colocynthis* (Zamir *et al.*, 1984). Another isozyme-based study on the genus clustered *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides* in the same group separating them from *C. colocynthis* (Navot & Zamir, 1987). The groupings observed were consistent with the variability in six seed-protein bands and with the crossability relations among the examined species. Based on isozyme data, a South African germplasm was considered to be the wild progenitor of cultivated watermelon (Navot & Zamir, 1986). However, the use of isozyme/protein based markers was short-lived and overtaken by DNA-based markers due to the limited number of polymorphic isozyme loci detected in watermelon (Biles *et al.*, 1989).

The much used random amplified polymorphic DNA markers (RAPD) have produced a limited number of polymorphisms in analysis of genetic diversity in watermelon (Lee *et al.*, 1996; Levi *et al.*, 2001a; Levi *et al.*, 2001b). Contrary to RAPD markers, the other dominantly inherited markers used, inter-simple sequence repeat (ISSR) and amplified fragment-length polymorphism (AFLP) markers, were highly effective in differentiating among watermelon cultivars or elite lines with limited genetic diversity (Levi *et al.*, 2004).

A widely preferred DNA-based marker type is the simple sequence repeat marker (SSR). SSR markers detect polymorphisms based on the repeat length of microsatellite sequences and are hypervariable, mutiallelic, co-dominant, and easily detectable by simple PCR procedures. Jarret *et al.* (1997) determined genetic variation among PI accessions of *C. lanatus* var. *lanatus*, *C. lanatus* var. *citroides* and *C. colocynthis* using SSR markers and delineated 4 groups: the largest group consisted of *C. lanatus* var. *lanatus*, the second of wild and cultivated *C. lanatus* var. *citroides*, the third of a hybrid

accession between *C. lanatus* var. *lanatus* and *C. lanatus* var. *citroides* and the fourth group of *C. colocynthis*. Low levels of genetic diversity in cultivated and elite watermelon varieties have been reported except when genetic variability and differentiation of watermelon accessions could be attributed to broad geographical distribution of material that may have been subjected to local adaptation and selection.

In Southern Africa, SSR markers have been evaluated on watermelon accessions from Mozambique; three groups with different genetic background were differentiated relative to agro-ecological conditions (Munisse, 2011). Of the three groups, the seed type watermelons (*Citrullus lanatus* var. *citroides*) and the dessert types (*Citrullus lanatus* var. *lanatus*) were clearly distinct, whereas the third group presumably contains products of admixture between the two main types. However, other forms of watermelon, particularly the wild-weedy melons, were not included in this study. In other Southern African countries like Zimbabwe where neither phenotypic nor molecular studies have been performed previously, there is a need to study genetic variation within the genus *Citrullus*. Through comparative analysis at the molecular level of wild and cultivated accessions of *C. lanatus*, relationships among these forms can be revealed, which may help to understand the domestication and agronomic development of the species.

1.5.3. Conservation of watermelon in southern Africa

With recognition of the current and future contribution of genetic resources of indigenous plant species to livelihoods of rural communities, Southern African Development Community (SADC) countries initiated the establishment of the Regional Programme on plant genetic resources conservation in 1989. The major thrust of the programme was the establishment of Southern Africa Development Cooperation Genetic Resources Centre (SPGRC) to hold the base collection and coordination of a network of National Plant Genetic Resources Centres (NPRCs) in all the SADC member countries. Each member country established its own national plant genetic resources centre as desired to meet local conditions. Bioversity International (then IBPGR) played a major role in supporting the process by providing technical and scientific advice at various levels while the Nordic Genebank served as the technical consultant to the project.

SPGRC was mandated to conserve and promote sustainable use of plant genetic resources with special focus on indigenous species and endangered species in the region. It's role included co-ordination of all activities related to plant genetic resources conservation and sustainable use in the region; maintaining the regional base collection on behalf of member states,

arranging for safe duplication of materials, and developing and maintaining the SADC database system for *ex-situ* as well as *in-situ* plant genetic resources. National plant genetic resources centres maintain an active collection of their local germplasm for use in the various national programmes.

1.5.4. Aims of this study

Watermelon has been considered as an underutilized crop within the regional conservation programme of SPGRC and NPGRCs. This implies that the full potential of this crop has not yet been explored. The status of traditional watermelon cultivation and on-farm conservation in the SADC region has been sparsely described. There is a large potential in using the farmers' landraces of watermelon in traditional farming systems, since most of the landraces are drought tolerant and widely adaptable. However, very limited information is available on the diversity of the genus *Citrullus* and the extent of its distribution in Southern Africa. It is considered a mandate crop for conservation, and genetic studies are therefore critical for:

1. placement of germplasm into correct heterotic groups through genetic diversity studies,
2. the management of genebank collections by refining the core subsets,
3. development of a regional database of watermelon characterization, and
4. the identification of gaps in collections and/or sources of potential novel forms of watermelon.

It is in the light of this regional initiative that a study to investigate amount and partitioning of genetic variability of watermelon in Zimbabwe and southern Africa, and identify potential areas for further collections, was considered critical. Information generated by the study will be used in developing *in-situ* and *ex-situ* conservation strategies. This information will also help in promoting better utilization of different forms of watermelon.

2 Objectives

The objective of this study was to assess the diversity of landraces and wild forms of watermelon and consider implications for conservation strategies in Southern Africa. In addition to the accessions from Zimbabwe, some watermelon germplasm from Namibia, Botswana, South Africa and Zambia was also studied. An attempt was also made to map and/or predict environment patterns in which the watermelon forms are distributed in Zimbabwe; and to determine the degree of genetic relationships and gene flow among the different forms of watermelon using molecular markers.

For the Southern Africa region and international community, this study will provide information to aid (i) breeders in the placement of watermelon germplasm into correct heterotic groups, (ii) germplasm curators (both at regional level and in each member country) in the management of genebank collections, (iii) scientists in understanding the gene flow among watermelon types in Southern Africa, (iv) the development of a regional database of watermelon characterization, and (v) the identification of gaps in collections and/or sources of potential novel forms of watermelon in Zimbabwe.

The specific objectives were:

1. To estimate the diversity of watermelon (*Citrullus lanatus*) accessions, using SSR and RAPD markers, in Zimbabwe across the agro-ecological regions
2. To assess the diversity of watermelon and the contributory environmental and socio-economical factors in a selected community in Zimbabwe
3. To evaluate diversity of watermelon in some countries in southern Africa: Zimbabwe, Zambia, Namibia, South Africa and Botswana.
4. To evaluate the dynamics of genetic diversity and structure of a wild-weed-landrace complex of watermelon in a selected community in Zimbabwe
5. To assess the distribution pattern of watermelon using DIVA-GIS, applying geo-reference data to correlate origin with environmental parameters, and study if the site has an effect on the distance or the similarity between accessions and its implications in conserving Zimbabwean watermelon accessions.

3 Materials and methods

3.1 Plant material and DNA extraction

Watermelon germplasm has been obtained mostly from Zimbabwe and selected Southern African countries, which include Botswana, Namibia, South Africa and Zambia.

In paper I, seeds from 10 watermelon accessions were obtained from the National Plant Genetic Resources Center of Zimbabwe (also known as National Genebank). The accessions were collected across the country in districts inhabited by two distinct groups of people, the Shona (Mashonaland and Masvingo provinces) and the Ndebele (Matabeleland and Midlands provinces). Unfortunately, two of the sweet watermelon accessions never produced any seedlings and a third accession only produced one seedling. A total of 81 plants were chosen for this study.

Seedlings from 25 watermelon accessions were used in paper II. These accessions represented the two major forms of watermelons (sweet watermelon and cow-melon) obtained independently from each of Namibia (8), Botswana (2), South Africa (4), Zambia (4), and Zimbabwe (4). In addition, three commercial varieties of sweet watermelon were obtained from the Harris Moran Seed Company (Davis, USA) for comparison. All the African accessions were obtained from local gene banks, except for Botswana, where the two accessions were obtained directly from farmers. A total of 243 plants (~10 plants per accession) were chosen for this study.

Passport data for all the 89 watermelon accessions housed in the National Plant Genetic Resources Center of Zimbabwe representing the two major forms of watermelons (45 sweet watermelons and 44 cow-melons) were used in paper III.

In paper IV, 29 watermelon accessions were collected from farms in Chitanga village representing a single Zimbabwean community. Ten plants per accession amounting to a total of 290 plants were chosen for this study.

In paper V, 43 watermelon accessions collected from the Chitanga village in Zimbabwe, of which 29 were landraces (described in more detail in paper IV), one putative hybrid between sweet watermelon and cultivated cow-melon, and 13 wild-weedy melons, were used. Ten plants per accession resulting in a total of 430 plants were chosen for this study.

For paper VI, seeds from thirty-four watermelon accessions representing the two major forms of watermelons (sweet watermelon and cow-melon) from different regions of Zimbabwe were obtained from the National Genebank. In addition, three commercial varieties of sweet watermelon were obtained from the Harris Morgan Seed Company (Twin Falls, ID, USA) for comparison. A total of 154 plants with an average of 4 plants per accession (the range was 1-5 plants per accession), were chosen for this study.

In all papers above excluding paper III, seeds from the collected accessions were germinated at Balsgård in Sweden. DNA extraction was done from young leaf tissue (10 μg) using the E.Z.N.A.™ SP Plant DNA Mini Kit (Omega Bio-Tek, Norcross, GA, USA) (paper I) or using the Qiagen Dneasy™ Plant Mini Kit (QIAGEN AB, Sollentuna, Sweden) following the manufacturer's protocol (papers II, IV, V & VI). DNA concentrations and sizes were estimated visually using the DNA low mass ladder™ (Invitrogen, Life Technologies Carlsbad, CA, USA) by electrophoresis in 2% (w/v) agarose gels stained in 3μl of ethidium bromide (EtBr).

3.2 Methods and data scoring

3.2.1 RAPD Analysis

Based on polymorphism, reproducibility and clarity of the obtained banding patterns, oligonucleotide primers from Operon Technologies were selected for RAPD analysis in papers I, IV and V. The PCR protocol followed in all papers is described in detail in paper I. Binary data matrices were produced, based on manual scoring with presence "1" or absence "0" of a band.

3.2.2 SSR and EST-SSR Analyses

Ten SSR primer pairs (MCP1-03, MCP1-07, MCP1-12, MCP1-13, MCP1-14, MCP1-21, MCP1-28, MCP1-32, MCP1-37 and MCP1-33) previously designed for watermelon (Joobeur *et al.*, 2006) were used in papers I and II. The choice was based on previous screening of African watermelon DNA at the Department of Agricultural Sciences, University of Copenhagen, Denmark. To separate DNA fragments and determine sizes, all primer-pairs were fluorescently labeled at the 5´-end with either FAM (primers MCP1-07, MCP1-13, MCP1-21, MCP1-32, and MCP1-37) or HEX (primers MCP1-03, MCP1-12, MCP1-14, MCP1-28, and MCP1-33). For paper VI, ten EST-SSR primers developed for watermelon (Levi *et al.*, 2009) were chosen. The selection was based on high polymorphism when evaluated among 25 American watermelon heirloom cultivars and 13 US Plant Introductions (PIs) of *Citrullus* sp. by Levi *et al.* (2009).

The PCR protocol for SSR and EST-SSR primers used a total volume of 10 μl, with details for the reaction mixture and PCR cycling profile given in each paper. Successful amplification was checked on 2% agarose gels with subsequent visualization of fragments using UV illumination.

PCR products were separated and analysed using capillary gel electrophoresis. Fragments were sized relative to the internal ROX size standard (500ROXTM Size Standard, Applied Biosystems) and scored with

manual bin setting using the software program GeneMapper® Software v 3.0 (Applied Biosystems, Carlsbad, CA, USA) (paper I) or GeneMarker® Software version 1.85 (SoftGenetics, State College, PA, USA) (paper II & VI). The amplified SSR fragments were either letter coded (paper I) or scored in terms of allele sizes (paper II) for evaluating single-locus profiles. In addition, the fragments were scored phenotypically, with "1" or "0" for alternate homozygotes, and "0.5" for heterozygotes (Staub *et al.*, 2000). Similarly, the amplified EST-SSR fragments were scored in terms of allele sizes (paper VI) for evaluating single-locus profiles.

3.2.3 Statistical analysis

3.2.3.1. Genetic diversity estimators

Genetic diversity within accessions or groups of accessions was evaluated using different parameters for RAPD, genomic SSR and EST-SSR data. Four gene diversity estimators of within-accession variation were used with RAPD data: mean percentage polymorphic bands, mean Jaccard similarity, the expected heterozygosity which is equivalent to Nei's unbiased gene diversity H_S (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). 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. For SSR and EST-SSR data, gene diversity estimators included percentage polymorphic alleles within accessions, expected heterozygosity H_E, observed heterozygosity H_O, and the Shannon index.

Variation among accessions was calculated as the coefficient of genetic differentiation G_{ST} (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). 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, and between and within accessions for either RAPD or SSR or EST-SSR data sets. To investigate the informativeness of primers used, a polymorphic information content (PIC) value for each locus (SSR and EST-SSR data) or band frequency (RAPD data) was calculated according to the formula: $PIC = 1 - \sum P_i^2$, where P_i is the frequency of the i-th allele (Smith *et al.*, 1997). A marker index for each of the RAPD primers was obtained by multiplying the PIC-value by number of polymorphic loci.

3.2.3.2. Genetic similarity and relatedness

Levels of similarity among and within accessions were also investigated using multivariate methods, employing cluster analysis (papers I, II, IV, V and VI) and multidimensional scaling (papers I, IV and V) or principal coordinate analysis (paper II, VI). As specified in each paper (I, II, IV, V and VI), a genetic similarity matrix was used to construct a UPGMA cluster analysis with NTSYS-pc version 1.80 (Rohlf, 1993). The distortion effect was estimated using a cophenetic correlation analysis.

As a means of verifying groups derived from the cluster analysis, an ordination analysis (multidimensional scaling or principal coordinate analysis (PCO)) was computed in GenAlEx 6 or NTSYS-pc. While clustering methods show a hierachical, categorical structure which is inherently incapable of describing gradients or multiple patterns in data (Crisp & Weston, 1993), ordinations are designed to reveal multiple, continuous, and overlapping patterns of variation (Sneath & Sokal, 1973) and are most appropriate under a nonhierarchical model of infraspecific variation (Swofford & Berlocher, 1987). Where two different markers were used (paper I: RAPD and SSR, paper V: RAPD and farmer-based traits), correlation between the two similarity matrices was investigated with a Mantel test (MXCOMP in NTSYS-pc, using 9999 permutations to compute the significance of a given correlation).

A model-based structure analysis for clarifying genotypic ambiguity and identify cases of gene flow (Falush *et al.*, 2007) was performed with the computer program STRUCTURE version 2.3.3 (Pritchard *et al.*, 2000). Details of the computations are explained in paper V.

3.2.4 Farmer-preferred traits and socio-economic survey

Farmer-preferred morphological characterization was recorded for 43 watermelon accessions (29 landraces, a single putative hybridogenous accession and 13 wild-weedy forms) collected from a single village during the harvesting period of April–May 2009. With the help of a field extension officer, a group of 17 respondents was formed comprising thirteen females above 40 years and four males above 59 years. Paper V explains the details but, to summarize, farmers selected eight qualitative characters as being important for distinguishing forms of the wild-weed-landrace complex of watermelon in the field.

A single 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. In order to guard against male dominance in focused group interviews, a government female extension worker moderated the discussions. Questions were asked as open-ended to allow farmers to discuss widely on their landrace perceptions. The survey findings were generally descriptive, and where statistics were involved,

respondents would provide estimates in terms of percentages (%) or numbers. The documentation included socio-demographic information about respondents, seed source and cropping systems, and watermelon uses.

3.2.5 DIVA-GIS analysis

Analysis of the spatial distribution of 89 watermelon accessions collected throughout Zimbabwe was conducted based on geo-referenced data using DIVA-GIS version 5.2 (Hijmans *et al.*, 2002). The analysis involved generating maps of the distribution pattern of watermelons in relation to altitude and soil type, with the help of a point-to-grid option applying simple method.

4 Summary of results and discussion

4.1 Genetic diversity in watermelon

4.1.1 Genetic diversity across Zimbabwe (papers I & VI)

For paper I, main objectives were to assess levels of intra- and inter-accession diversity in some *C. lanatus* samples collected in various districts across Zimbabwe and to estimate relatedness among these accessions, and to investigate the level of congruence between RAPD- and SSR-based findings.

Results obtained from estimating DNA marker-based diversity among 81 seedlings from 8 accessions of watermelon (5 accessions of cow-melons (*Citrullus lanatus* var. *citroides*) and 3 of sweet watermelons (*C. lanatus* var. *lanatus*)) using two molecular marker methods (RAPD and SSR) were highly correlated. However, only RAPD was able to provide each sample with an individual-specific DNA profile (Fig. 1). Ten RAPD primers produced 138 markers of which 122 were polymorphic whereas nine SSR primer pairs detected a total of 43 alleles with an average of 4.8 alleles per locus. The polymorphic information content (PIC) ranged from 0.47 to 0.77 for the RAPD primers and from 0.39 to 0.97 for the SSR loci. Dendrograms and multidimensional scaling (Fig. 2) produced two major clusters; one with the five cow-melon accessions and the other with the three sweet watermelon accessions. One of the most variable cow-melon accessions took an intermediate position in the MDS analysis, indicating the occurrence of gene flow between the two subspecies. Analysis of molecular variation (AMOVA) attributed most of the variability to within-accessions.

Contrary to previous reports where higher levels of genetic diversity have been reported within *C. lanatus* var. *citroides* compared to *C. lanatus* var. *lanatus* (Jarret *et al.*, 1997; Navot & Zamir, 1987), genetic diversity estimators revealed that sweet watermelon accessions apparently contain diversity of the same magnitude as the cow-melons.

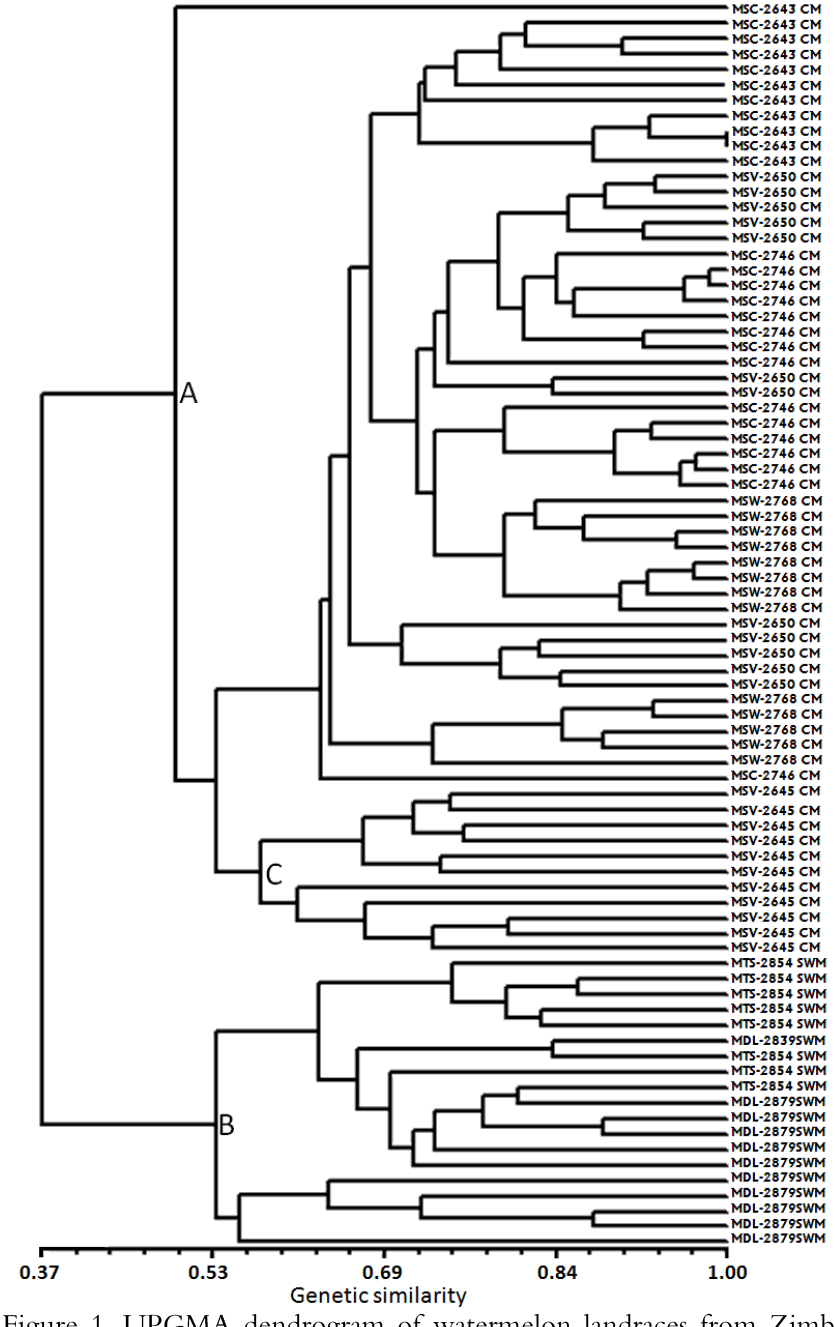

Figure 1. UPGMA dendrogram of watermelon landraces from Zimbabwe using RAPD data, showing two major clusters, **A** cow-melons (CM) and **B** Sweet watermelon (SWM). **C** is a sub-cluster of plants from accession 2645 (Figure 2, paper I).

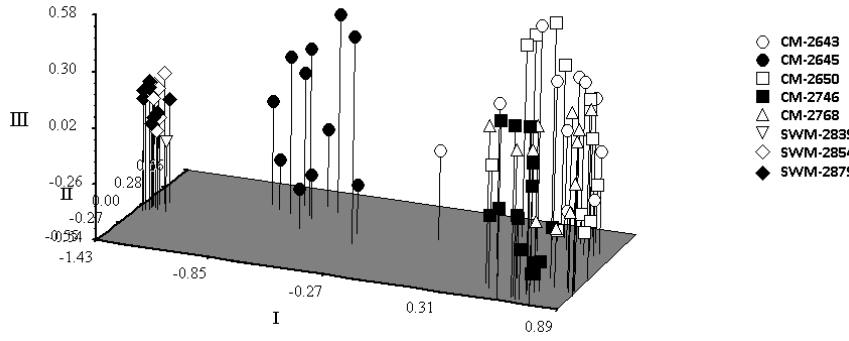

Figure 2. Three-dimensional plot of watermelon accessions using multi-dimensional scaling on combined RAPD and SSR data. CM refers to cow-melon and SWM to sweet watermelon (Figure 4, paper I).

For paper VI, the main objective was to assess the genetic diversity of a larger set of watermelon accessions. Genetic diversity was investigated in 37 watermelon accessions, 34 of which were collected from all the geographical provinces of Zimbabwe and 3 US commercial varieties, using 10 expressed sequence tag (EST)-derived simple sequence repeats (EST-SSRs). A total of 36 alleles were detected among all the watermelon accessions. For the 9 polymorphic EST-SSRs, the number of alleles per locus varied from 2 to 6, with a mean average of 4 alleles per locus. The values for the polymorphic information content increased as the number of alleles increased, and varied from 0.750 to 0.972 with an average of 0.884 suggesting sufficient discriminatory power. The genetic relationships among watermelon accessions based on the fruit EST-SSR markers (Fig. 3) were consistent with our previous studies based on RAPD and SSR markers (Mujaju *et al.*, 2010; Mujaju *et al.*, 2011). The two multivariate analyses strongly supported differentiation between the sweet watermelons and cow-melons, with significant variation (63%, $P < 0.01$) between these two forms attributed by an AMOVA partitioning of variation. Within the sweet watermelon group, two distinct sub-clusters formed, one of which consisted two of the three commercial varieties from the United States (US).

The EST-SSR markers revealed a somewhat higher diversity of sweet watermelon accessions in traditional agrosystems compared to that of cow-melons, the finding of which is contrary to previous reports which focused mostly on commercially cultivated sweet watermelon lines or varieties.

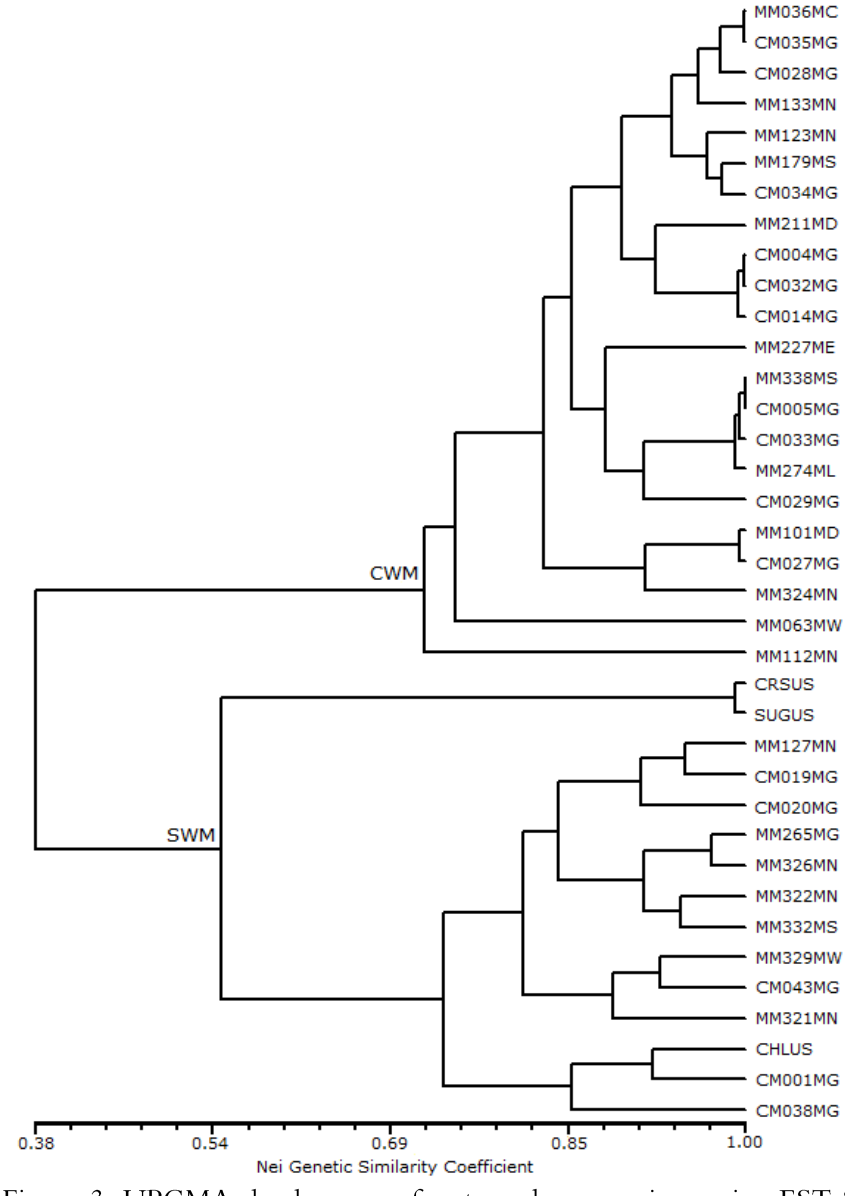

Figure 3. UPGMA dendrogram of watermelon accessions using EST-SSR data, showing two major clusters, **CWM** cow-melon group (cultivated & wild) and **SWM** sweet watermelon group. Accession code: initial two letters and three numbers correspond to the accession number, the last two letters denote province in Zimbabwe (MC Mashonaland Central; MW Mashonaland West; ME Mashonaland East; MD Midlands; MN Matabeleland North; MS Matabeleland South; MN Manicaland and MG Masvingo) or country (US United States of America) (Figure 1, paper VI).

4.1.2 Genetic diversity at village level (paper IV)

This study was stimulated by the confirmed significant differentiation between the two subspecies of *C. lanatus*, and the revealed considerable variation among and within watermelon accessions, for both cow-melon and sweet watermelon types (Mujaju *et al.*, 2010). 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. The need to further explore the organization of landrace diversity and the forces that shape and maintain within- and among-landrace diversity was considered critical. Using RAPD, this study investigated intra- and inter-landrace genetic variation in 29 landraces (20 landraces of sweet watermelon and 9 landraces of cow-melon), collected at four recently established farms in a single village.

AMOVA (Table 1) and ordination (Figure 4) 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 information about farmers' use of own seed or seed acquired from close family members, traditional myths and different cultivation practices, were concordant with the results from the RAPD analysis. Accordingly, it has been noted that different farmer management strategies as well as the seed source and soil conditions contribute to the differentiation of plant populations within a village (Brocke *et al.*, 2003). 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).

This study demonstrates the usefulness of combining molecular studies with participatory socio-economic data in order to elucidate the observed genetic diversity patterns at local level. This approach is envisaged to provide holistic and additional social issues for investigating diversity of watermelons in marginal environments and is relevant for the development of *in-situ* management strategies for conservation of watermelon landraces at the village level.

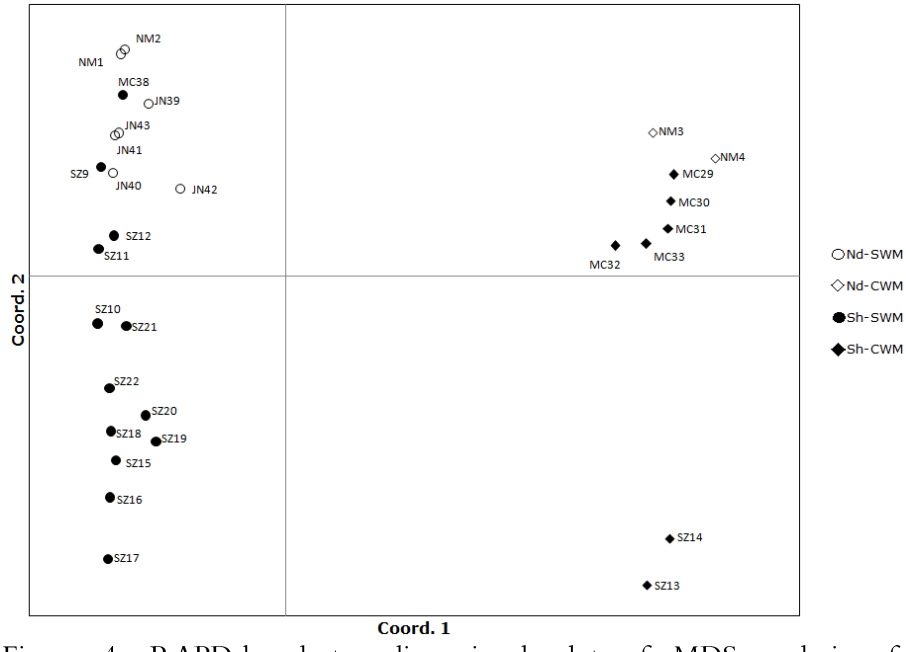

Figure 4. RAPD-based two-dimensional plot of MDS analysis of watermelon landraces from Chitanga village, Zimbabwe, collected from four farmers, belonging to two cultural groups, **Nd** Ndebele and **Sh** Shona, growing SWM (sweet watermelon) and CWM (cow-melon). The farmer's names are represented by the initials (NM, MC, JN and SZ), followed by accession number (Figure 3, paper II).

Table 1. 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 (Table 4, paper II).

Source of variation	
(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%, $P < 0.001$

4.1.3 Genetic diversity in selected southern African countries (paper II)

Low polymorphism in cultivated watermelon has been reported in previous studies, based mainly on US Plant Introductions and watermelon cultivars, most of which were linked to breeding programmes associated with disease resistance. Since germplasm sampled in a putative centre of origin (Southern Africa) may harbour considerably higher variability, genetic diversity in watermelon (*C. lanatus*) was estimated among 213 seedlings from 22 accessions collected in Botswana, Namibia, South Africa, Zambia, and Zimbabwe (Fig. 5). The accessions consisted of two types of watermelon landraces: sweet watermelon (*C. lanatus* var. *lanatus*) and cow-melon (*C. lanatus* var. *citroides*), also known as citron melon. In addition, three commercial varieties of *C. lanatus* var. *lanatus* from the USA were included for comparison.

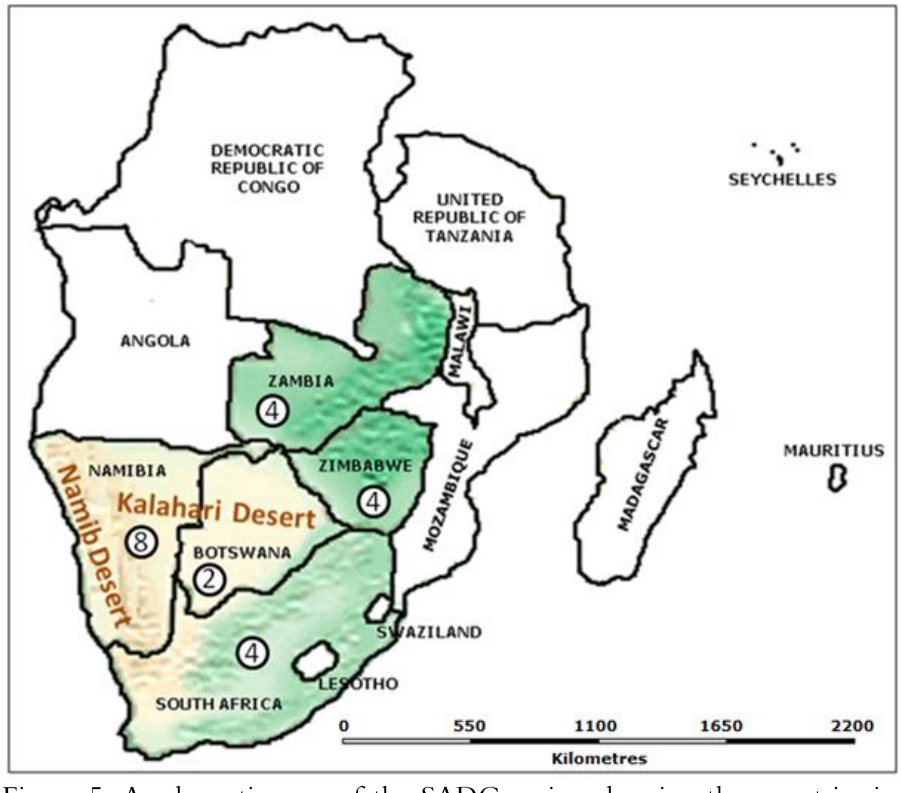

Figure 5. A schematic map of the SADC region showing the countries in which 22 accessions (circled) were collected, and indicating their close proximity to the Kalahari and Namib deserts regarded as the centre-of-origin for watermelon. In addition, three commercial varieties (Sugarbaby, Crimson Sweet, and Charleston) were obtained from USA (Figure 1, paper III).

Ten simple sequence repeat (SSR; microsatellite) loci detected a total of 153 alleles. The polymorphic information content (PIC) ranged from 0.833 to 0.963, suggesting sufficient discriminatory power. AMOVA demonstrated a significant differentiation between countries (15%; $P < 0.001$) but this was probably to a large extent caused by the uneven distribution of the two major groups of watermelon accessions between countries. Principal co-ordinate analysis (Fig. 6) as well as UPGMA clustering instead showed considerable overlap among accessions from different countries. Interestingly, the two sweet watermelon accessions from Botswana grouped adjacent to the sweet watermelon cultivars from the USA, suggesting that the latter may originate from that area. Some of the other accessions also grouped according to their country of origin, but others did not.

There was significant differentiation between accessions both when calculated across all accessions and within each of the two main forms, cow-melons and sweet watermelons respectively. Across all watermelon accessions, the estimates of among-accession differentiation ($\Phi_{ST} = 0.48$; $G_{ST} = 0.44$) were higher than values obtained for wild populations of annual ($G_{ST} = 0.40$) or short-lived perennial species ($G_{ST} = 0.31$), or for mixed breeding ($G_{ST} = 0.26$) or outcrossing species ($G_{ST} = 0.22$) (Nybom, 2004). The existence of the two strongly differentiated forms within our material possibly accounted for this observed discrepancy.

Within-accession diversity parameters showed that the sweet watermelon accessions found in traditional agrosystems were almost as genetically variable as the cow-melon accessions even when sampling was conducted at a regional level. H_O-values were lower than H_E-values in the majority of our watermelon accessions, with positive values for F_{IS}, indicating a lack of heterozygosity, assumed to be a result of the breeding system. In addition to outcrossing, watermelons can also self-pollinate, and sometimes accessions may therefore have been derived from pollination among related or less diverse plants (the Wahlund effect).

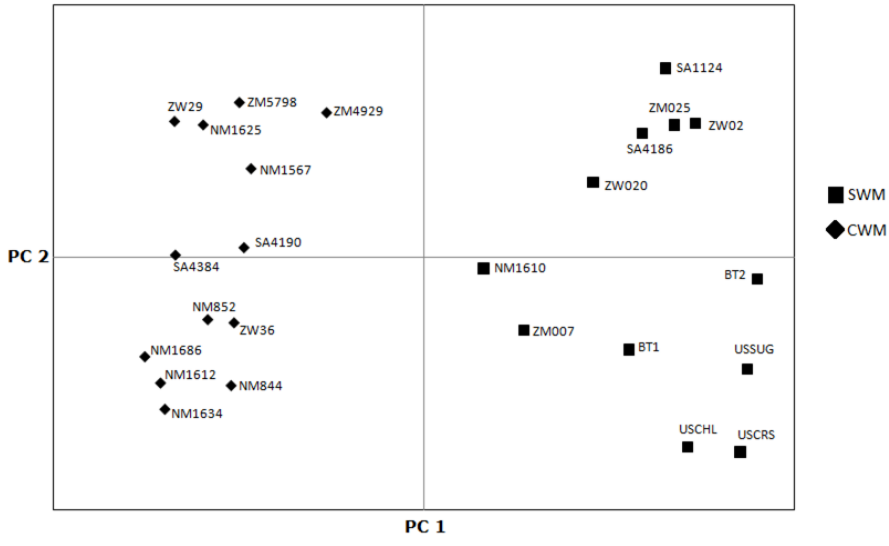

Figure 6. Two-dimensional plot of 25 watermelon accessions using principal coordinate analysis of the SSR data. CWM refers to cow-melons and SWM to sweet watermelons. The first two letters of the accession code denote the country of origin (US United States; BT Botswana; NM Namibia; SA South Africa; ZM Zambia; and ZW Zimbabwe) and the numbers are the accession number or name. The axes are principle component 1 (PC 1) and principle component 2 (PC 2) (Figure 2, paper III).

4.2 Genetic structure and geneflow (paper V)

Farmers' fields have been associated with the occurrence of wild-weed-crop complexes, which might be indicative of a place of ongoing domestication (Beebe *et al.*, 1997). In addition, the existence of wild-weedy melons in farmers' fields among the diverse pool of landraces also provides a niche for development of hybridogenous variants. In this study, a combination of farmer-preferred morphological traits and RAPD markers, employing multivariate techniques and a model-based structural analysis were used to evaluate the dynamics of genetic diversity in 43 watermelon accessions at the level of a single village with a traditional farming system. The accessions constituted 20 sweet watermelons, 9 cultivated cow-melons, 13 wild-weedy melons and one putative hybrid between sweet watermelon and cultivated cow-melon. Multidimensional scaling and cluster analysis (Fig. 7) of RAPD data, revealed high differentiation among accessions and among watermelon forms. Population structure analysis also demonstrated the existence of the three major forms of watermelon, identified by a set of alleles predominant among each form (Fig. 8). However, two of the cultivated cow-melon accessions deviated strongly from the remainder while one accession among

the wild-weedy melons showed introgression from an unknown source. Only three watermelon accessions showed considerable genetic admixture between the main forms; the wild-weedy accessions WWM05 and WWM28 showed some influence of cultivated cow-melon and possibly of sweet watermelon, respectively, whereas the putatively hybridogenous accession VAR23 shared most of its alleles with the sweet watermelon group but also exhibited some influence from wild-weedy melon.

Microsatellite DNA-based STRUCTURE analysis of watermelon accessions from Mozambique also demonstrated the existence of admixtures between dessert types (equivalent of sweet watermelons) and seed types (equivalent of cow-melons) in the northern province of Cabo Delgado where two out of five accessions, morphologically classified as seed type, were placed between the two major types (Munisse, 2011). A study conducted in Mali also indicated the presence of admixture, as 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, personal communication).

Similarity matrices based on RAPD and farmer-preferred traits data were positively correlated according to a Mantel test. The RAPD-based dendrogram, however, showed a major dichotomy between sweet watermelon on the one hand, and cultivated and wild-weedy cow-melons on the other hand. By contrast, the farmer traits-based dendrogram (Fig. 9) showed the main dichotomy to occur between sweet watermelon and cultivated cow-melon on the one hand, and wild-weedy watermelon on the other hand. Although genetically most similar to wild-weedy plants, cultivated cow-melons obviously carry traits that make them more similar to sweet watermelon in the eyes of the farmers.

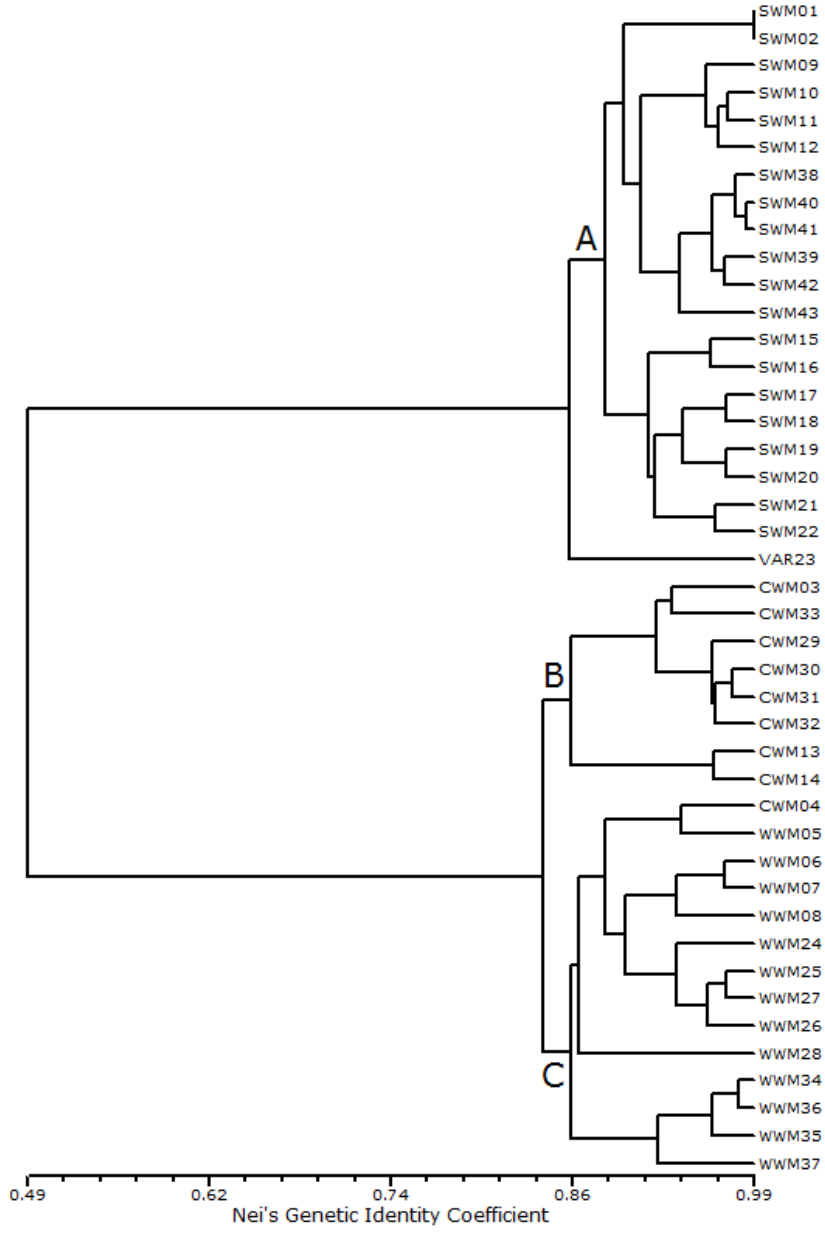

Figure 7. RAPD-based UPGMA dendrogram of watermelon accessions showing two major clusters: A sweet watermelons (SWM) and the putatively hybridogenous VAR23, and B cow-melons subdivided into cultivated cow-melons (CWM) and wild-weedy melons (WWM) (Figure 1, paper IV).

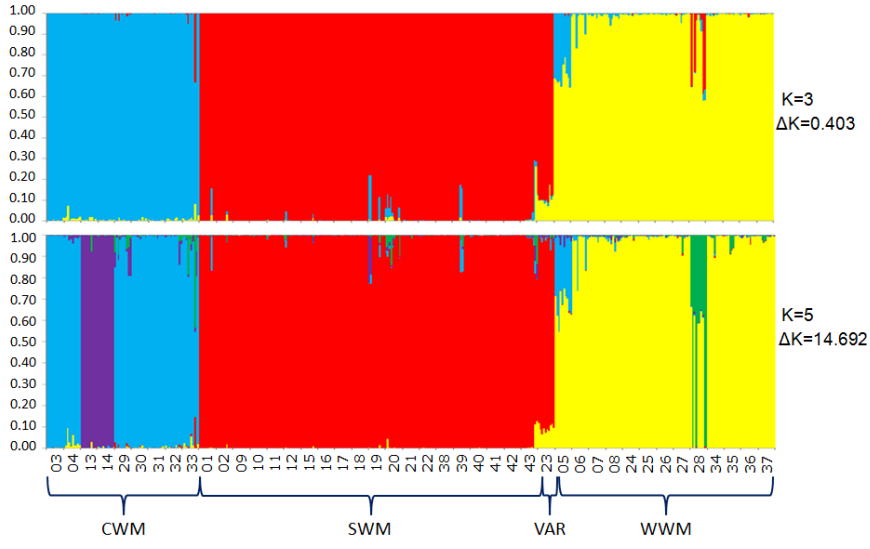

Figure 8. Inferred genetic structure of a wild-weed-landrace complex of watermelon in Chitanga village, Zimbabwe. Each plant is represented by a single vertical bar, which is partitioned into K (3 and 5) coloured segments. Each colour represents one cluster, and the length of the coloured segment shows 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 (Figure 2, paper IV).

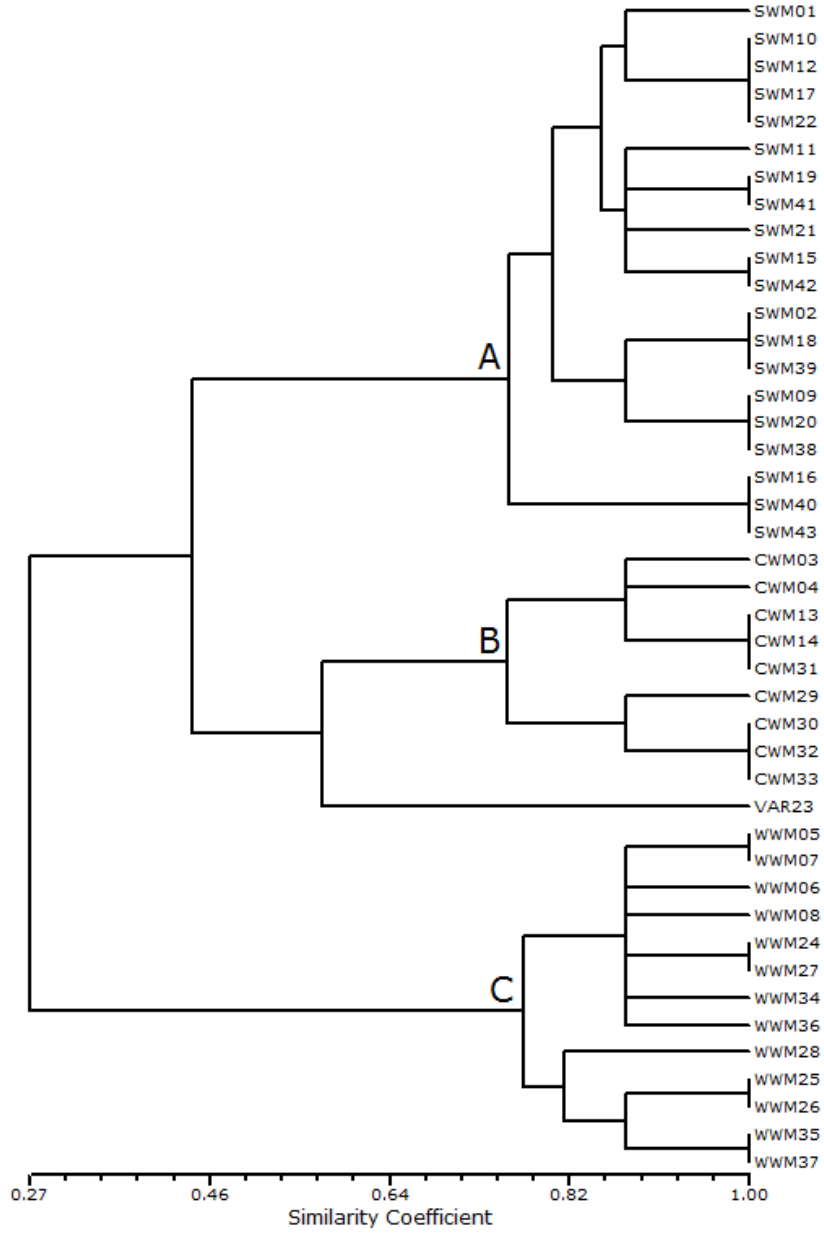

Figure 9. UPGMA dendrogram of watermelon accessions using farmer-preferred traits, showing three major clusters representing **A** sweet watermelons (SWM), **B** cultivated cow-melons (CWM) and the putatively hybridogenous VAR23, and **C** wild-weedy melons (WWM) (Figure 2, paper IV).

4.3 Distribution pattern of watermelon (paper III)

Identification of potential areas and regions for collection of watermelon germplasm is pivotal for better utilization of the available diversity. In this study, the DIVA-GIS tool was used on all watermelon collections in the National Genebank of Zimbabwe to explore the pattern of distribution of two forms of watermelons; sweet watermelon (*C. lanatus* var. *lanatus*) and cow-melon (*C. lanatus* var. *citroides*).

DIVA-GIS analysis revealed that most watermelon accessions have been collected at altitudes ranging from 160 to 1550 m above sea level, and that sandy loam and sand soils apparently are preferred (Fig. 10). Previously, GIS has been successfully used in identifying areas of high diversity i.e., for phaseolus bean (Jones *et al.*, 1997), wild potatoes (Hijmans *et al.*, 2000) and black pepper (Parthasarathy *et al.*, 2006). Masvingo province had the highest number of collections, mainly a result of a targeted collection mission in Chitanga village (Mujaju and Nybom, in press). Excluding watermelon collections from Chitanga village to provide a uniform sampling across the country, the watermelon distribution pattern becomes more or less uniform among the drier provinces, which include the two Matabeleland provinces, Midlands, Mashonaland West and Masvingo. The altitudinal range of 1081–1543 m above sea level constitutes the majority of sandy loam habitats from which the two forms of watermelons had been collected (Table 2): 20 sweet watermelons and 19 cultivated cow-melons. Watermelon has been reported to perform especially well under savannah environments in well-drained sandy loam soil (van der Vossen *et al.*, 2004). Cultivation of cow-melons and sweet watermelons is apparently important also for farmers in the altitudinal range 620–1081 m where 3 sweet watermelons and 13 cultivated cow-melons were collected.

In order to maintain watermelon diversity, promotion of *in-situ* or on-farm conservation of watermelon genetic resources using best practices in traditional farming system is recommended. Furthermore, this should be complemented by *ex-situ* conservation through gap collection missions in order to identify and document additional diversity hotspots.

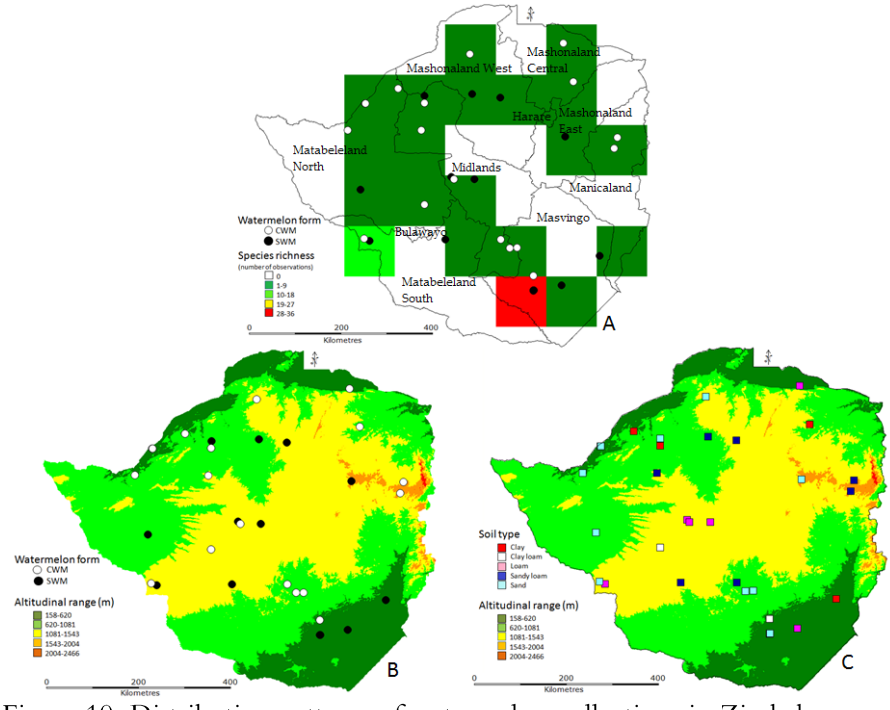

Figure 10. Distribution patterns of watermelon collections in Zimbabwe according to province (A), altitude (B) and soil type relative to altitudinal range (C) using DIVA-GIS. CWM cow-melon, SWM sweet watermelon (Fig 1, Fig 2 & Fig 3 in Paper V represented by the numbering A, B & C).

Table 2. Analysis of distribution patterns of two forms of watermelon with respect to soil type across altitudinal range and province.

Parameter	Sweet watermelon					Cow-melon				
	Clay	Clay loam	Loam	Sandy loam	Sand	Clay	Clay loam	Loam	Sandy loam	Sand
Altitude range (m)										
158-620	1		1	20 (20)					9 (9)	
620-1081		1		2		1	2	2	7	1
1081-1543		1	3	10	6	1	3	2	8	5
1543-2004				1		1			1	
2004-2466										
Sub-total	1	2	4	33 (20)	6	3	5	4	25 (9)	6
Province										
Mashonaland Central						1		2		
Mashonaland West					5				4	
Mashonaland East				1					1	1
Masvingo	1	1	1	20 (20)			2		11 (9)	
Manicaland										1
Midlands			2	2	1	1		1	1	4
Matabeleland North				6		1			4	
Matabeleland South		1	1	4			3	1	4	
Harare										
Bulawayo										
Sub-total	1	2	4	33 (20)	6	3	5	4	25 (9)	6

Numbers in parenthesis represent collections from Chitanga.

4.4 Implications for genetic conservation

Conservation of watermelon in the Southern Africa region is important since this is an underutilized crop species for which the potential has not yet been fully explored. Two strategies for conservation, particularly *ex-situ* conservation and on-farm conservation are considered most appropriate for conservation of watermelons. While *ex-situ* conservation is static, it can preserve germplasm for decades independent of the status of cultivation and utilization in the field, provided that viability of the propagation material can be maintained. On-farm conservation is mostly linked to the maintenance of landraces through farmers' cultivation practices, and it is dynamic since landraces continue to evolve. In Southern Africa, on-farm conservation strategies are strongly affected by the traditional agricultural

systems which are based on a high agro-biodiversity (Maxted *et al.*, 2002). Farmers' management of landraces on-farm thus 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).

Within the watermelons, variety types or forms reflect different modes of seed management and different levels of farmer involvement. As confirmed by our studies, watermelons can be genetically and morphologically differentiated into three groups (sweet watermelon, cultivated cow-melon and wild-weedy melon) and these can also be treated as three taxonomic units for conservation. 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. Such selection poses a potential threat since varieties with undesirable traits can be lost due to the increasing desire to satisfy the needs of buyers when income generation within farmer household becomes more important. 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.

Understanding whether the existing forms of watermelons reflect true landraces or folk varieties is a key step towards successful implementation of programmes for conservation and sustainable utilization. Lack of a clear-cut definition of the difference between landraces and folk varieties can obscure debates with regards to genetic erosion, on-farm conservation and seed-related policy issues (Berg, 2009). Farmers' seeds are most often lumped together and treated as 'landraces', even though the variety types or forms reflect different levels of farmer involvement. When a distinction is made, landraces refer to varieties adapted to environmental conditions of a particular locality where they have been grown for a long time with no intentional selection, as opposed to folk varieties, which are varieties consciously selected and maintained for one or more distinctive properties (Berg, 2009).

While the evolvement of different watermelon forms may originate from natural selection, intentional selection on-farm have certainly maintained and increased the distinctive properties of each form, and assisted in expanding the breadth of useful diversity within especially the sweet watermelon and the cultivated cow-melon. Particularly, the sweet watermelons should therefore 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 concurred 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.

The distribution pattern of watermelon forms demonstrates areas where very little explorations have been undertaken, suggesting gaps in collections. This has an implication on *ex-situ* conservation which should also focus on the need for comprehensive collection of watermelon germplasm in order to identify and document additional diversity hotspots.

5 Conclusion

Based on genetic diversity estimated by molecular markers and farmer-preferred characterization, cultural values and distribution patterns of watermelon, the following conclusions can be drawn:

I Considerable amounts of genetic diversity was found at all levels, including within-accessions (half-sib families), and sweet watermelon accessions appear to contain almost as much variability as cow-melon accessions.

II RAPD marker data compared with SSR marker data produced highly correlated similarity matrices, suggesting that for some applications, the less demanding RAPD can be a useful alternative, especially in developing countries.

III At village level, AMOVA and ordination revealed much variation across the landraces, and strong differentiation between the two main forms of sweet watermelons and cow-melons; with landraces from the same farm forming well-separated sub-clusters.

IV The farmers' perceptions with regards to farmers' use of own seed or seed acquired from close family members, traditional myths and different cultivation practices, were concordant with the results from the RAPD analysis at village level.

V Population structure analysis demonstrated the existence of the three major forms of watermelon in traditional farming system, identified by a set of alleles predominant among each form (sweet watermelon, cultivated cow-melon and wild-weedy type), with only three out of 43 watermelon accession showing considerable genetic admixture.

VI DIVA-GIS analysis revealed that most watermelon accessions have been collected at altitudes ranging from 160 to 1550 m above sea level, and that sandy loam and sand soils apparently are preferred.

6 Recommendations and future prospects

I To improve on a country-wide diversity assessment, it is prudent to further explore the organization of landrace diversity and the forces that shape and maintain within- and among-landrace diversity. Thus, more research should be undertaken to further assess variability within each of the subspecies using more accessions, and investigate possible associations with utility values, geographical origin, and/or socio-economic patterns. As gene-specific markers become available, these should be used to gain further knowledge about the traits of different landraces.

II Further studies to identify the main forces that determine genetic diversity at local village level. 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.

III Seed germination was very low or non-existent in a large number of the seed samples obtained for our study. Insufficient seed viability may have biological reasons but can also result from technical problems during seed storage, like frequent electricity outages. Conservationists or genebank managers need to consider establishing regeneration programmes for watermelon accessions with low viability to avert loss of diversity, and consequently their long-term adaptive potential which is useful for plant breeding.

IV A detailed GIS study based on more accessions and including also climatic data is warranted to predict the climatic and ecogeographic conditions required for watermelon and also to forecast the distribution of this crop in other parts of Zimbabwe.

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