Diversity of landraces and wild forms of watermelon (*Citrullus lanatus*) in southern Africa

A synopsis of the PhD Study

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Summary

The genus *Citrullus*, belongs to the Cucurbitaceae family. Among the four species in this genus, a single species *Citrullus lanatus* (Thunberg) Matsum. & Nakai, commonly known as watermelon, is grown throughout the drought-prone southern Africa as a staple food (edible seeds), a dessert food (edible flesh), and for animal feed. The fruit can be eaten fresh or cooked and the seeds can be roasted. Its uses are however, multifaceted and vary depending on the customs of the humans growing this crop. In addition, the fruit can serve as a source of water, especially in deserts or where drinking water is contaminated. Several morphotypes of watermelon are found in southern Africa. *Citrullus lanatus* exhibits expansive polymorphism in southern Africa and the species occurs in the following forms: wild populations distributed throughout the country in a wide range of habitat types; sweet watermelon, cooking melon and seed melon landraces of the traditional agrosystems; and possibly introgressed types which are regarded as agronomic weeds. Farmers' traditional onfarm practices contribute to the maintenance of watermelon landraces, and therefore play a role in nurturing local-level diversity. The wild watermelon is widely distributed in Africa and Asia, but originates from southern Africa occurring naturally in South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Zambia and Malawi. 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. This paper therefore is an attempt to summarise the work that has been done so far and the background literature, while providing the scope for the PhD study.

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1. General Introduction

The species *Citrullus lanatus* belongs to the Cucurbitaceae family, which consists of nearly 100 genera and over 750 species (Yamaguchi 1983). The genus *Citrullus* is among the major domesticated species (food plants) in Zimbabwe. Other domesticated species within the family Cucurbitaceae are *Cucurbita* (five species of squash & pumpkin), *Momordica charantia* (bitter melon), *Luffa* (two species of loofah) and *Lagenaria siceraria* (bottle gourd). Depending upon the species, virtually all parts of the plant can be used for food, including leaves, shoots, roots, flowers, seeds, and immature and mature fruits. Starch can be extracted from roots, and the seeds are a rich source of oils and proteins (Jacks et al. 1972).

The cucurbit family (also commonly referred to as the cucumber, gourd, melon, or pumpkin family) is a medium-sized plant family, primarily found in the warmer regions of the world. It is a major family for economically important species, particularly those with edible fruits. Some of these represent some of the earliest cultivated plants in both the Old and New Worlds. Some have medicinal and other uses. The family is distinct morphologically and biochemically from other families and is therefore considered monophyletic. General opinion is that it is closest allied phylogenetically with the Begoniaceae in the order Violales.

There is tremendous genetic diversity within the family, and the range of adaptation for cucurbit species includes tropical and subtropical regions, arid deserts, and temperate locations. A few species are adapted to production at elevations as high as 2000 m. The genetic diversity in cucurbits extends to both vegetative and reproductive characteristics. There is a considerable range in the monoploid (*x*) chromosome number (Jeffrey 1990), with 7 (*Cucumis sativus*), 11 (*Citrullus* spp., *Momordica* spp., *Lagenaria* spp., *Sechium* spp., and *Trichosanthes* spp.), 12 (*Benincasa hispida*, *Coccinia cordifolia*, *Cucumis* spp. other than *C. sativus*, and *Praecitrullus fistulosus*), 13 (*Luffa* spp.), and 20 (*Cucurbita* spp.).

The genus *Citrullus* has four species (*C. lanatus*, *C. ecirrhosus*, *C. colocynthis*, and *C. rehmii*), which occur mainly in southern Africa with the exception of *C. colocynthis*, which is distributed from northern Africa to southwest Asia. Cultivated watermelon (*C. lanatus*) is an annual, mostly monoecious diploid (2n=2x=22) (Shimotsuma 1963). The southern African region is the main center of diversity and the probable origin of most of the species

within *Citrullus*. The fruits and the leaves of the species within the genus are the main edible parts.

Within the genus *Citrullus*, a single species *Citrullus lanatus* (Thunberg) Matsum. & Nakai, commonly known as watermelon, is grown throughout the drought-prone southern Africa as a staple food (edible seeds), a dessert food (edible flesh), and for animal feed. The fruit can be eaten fresh or cooked. The rind can be pickled or candied. The juice from the fruit can be used fresh, made into a fermented drink, or boiled down into heavy syrup. Watermelon seeds are baked or roasted for consumption. The fleshy, juicy, sweet fruit provide a delicious refreshing dessert in hot weather. In addition, the fruit can serve as a source of water, especially in deserts or where drinking water is contaminated (Rubatzky 2001).

Genetic resources of indigenous plant species are important for crop and diet diversity. Variation of watermelons is predominant in farmers' fields and farmers have developed landraces for a variety of purposes: for dessert, oil, seed and porridge. The communities in southern Africa have not adopted modern varieties despite being exposed to these for decades. The status of traditional watermelon cultivation and onfarm conservation in this part of the world has been sparsely described. In traditional farming, watermelon is grown predominantly in low rainfall areas intercropping with cereals. There is a large potential in using the farmers' landraces of watermelon in traditional farming systems, as 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. Under the regional programme of Southern Africa Development Cooperation Genetic Resources Centre and National Plant Genetic Resources Centre Network, watermelon is considered as an underutilized crop, which implies that its full potential has not been explored. It is considered a mandate crop for conservation, and genetic studies are therefore critical for:

1. placement of germplasm into correct heterotropic 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.

The identification of germplasm is an important step for effective utilization of the available germplasm. It is in light of the above that a study will be carried out to determine diversity of the genus *Citrullus* in Zimbabwe and southern Africa and use DIVA GIS and FloraMap to map its distribution, and potential areas for further collections. 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 most of the species in the genus.

2. Taxonomy of the genus *Citrullus*

The genus *Citrullus* belomgs 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 in other parts of the world mainly for feeding animals. It 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. According to Fursa (1981), the cultivated species *C. lanatus* includes three subspecies: (i) *lanatus*, (ii) *vulgaris* which has two varieties, var. *vulgaris* and var. *cordophanus*, and (iii) *mucosospermus*. Lately, a single variety, var. *lanatus*, has been recognized with the others treated as synonyms. *C. lanatus* var. *citroides* consists of a wild type (var. *caffer*) commonly known as 'Tsamma' and the *citroides* group, which are ancient cultigens derived from the tsamma melon. The *citroides* group consists of varieties commonly referred to as citron melons, which include var. *viridis* a 'giant' watermelon from Iraq and cultivar 'Black Tom Watson'; var. *albidus* in the *nigro-seminius* and *albidus* forms bred in the central areas of Iran; var. *variegatus*; var. *rotundus*; var. *pulcherrimus*; var. *shami*; var. *oblongus* with the common name 'Fairfax'; var. *virgatus*; var. *pumilus* which is called 'New Hampshire'; var. *caffe* a cultivated watermelon (Maheshwari 1978).

Citrullus colocynthis (L.) Schrader, a perennial species, with globular fruits of 5–10 cm in diameter. It grows predominantly on sandy soils in the north and southwest areas of Africa and Asia, and bear fruits which are bitter and even poisonous. It can be divided into two different races, one found on the Mediterranean coast and in Israel, the other found in the deserts of Negev and Sinai. *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). It is distinguished by the absence of tendrils during its

growth and a woody deeply penetrating taproot. The fourth species, *Citrullus rehmii* De Winter, is an annual wild species confined to the western escarpment in Namibia (Schippers 2002, De Winter 1990). It resembles *Citrullus lanatus* but can be distinguished by the pink to orange mottled rind surface.

All the species in the genus *Citrullus* are cross compatible with each other. *Citrullus lanatus* and *C. ecirrhosus* appear to be more closely related to each other than either is to *C. colocynthis* (Navot and Zamir 1987). Two other species appear to be closely related to the genus *Citrullus*. One is *Praecitrullus fistulosus* (Stocks) Pangalo from India and Pakistan commonly known as 'Tinda' and belonging to a genus with a basic chromosome number of n=12 (Schippers 2002). Tinda varieties of this species with their green-fleshed fruits are also found in Kenya, Zimbabwe, and Ghana. The other species is *Acanthosicyos naudinianus* (Sond) C. Jeffrey, a wild species native to southern Africa.

3. The Biology of Watermelon

3.1. Morphology, Anatomy and Physiology

Watermelon grows as a trailing vine; the stems are thin, hairy, angular, grooved and have tendrils at each node. The stems are highly branched and up to 10 m long. Although there are dwarf types with shorter, less branched stems, dwarfing is primarily related to shortened internodes (Mohr 1986). Roots are extensive but shallow, with one taproot and many lateral roots growing within the top 2 feet in the soil (Robinson and Decker-Walters 1997).

Watermelon is the only economically important cucurbit with lobed leaves; all of the other species have entire leaves. The leaves are pinnately divided into 3 or 4 pairs of lobes. Fruits vary considerably in morphology. Fruits of wild plants range between 1.5 and 20 cm in diameter, are sub-globose, pale yellow or greenish mottled with dark green; fruits of cultivated plants are sub-globose or ellipsoid, up to 30 x 60 cm, green or yellowish green, evenly colored or variously mottled or striped (Messiaen 1994). The pulp varies from yellow or green (wild forms) to dark red (cultivars).

The anatomy of watermelons has been studied mainly in *C. lanatus* var. *lanatus* (Whitaker and Davis 1962). Barber (1909) gave a generalized description of fruit histology of most Cucurbitaceae fruits, including cultivated watermelon *C. vulgaris,* which has been recently changed to *C. lanatus* var. *lanatus*. Anatomical features played a major role in determining transportation and storage properties of watermelon (Lal et al. 1977). Fruits characterized by thin outer mesocarp cell walls were prone to rupturing during storage and transportation. When investigating the mode of water conservation in the Tsamma melon, Botha (1982) reported that the pericarp was covered by stomata and that the stomata were plugged by some material after fruit abscission and attributed this to the apparent cessation of water loss in the fruit. The rind consists of four layers of tissue: epicarp, hypodemis, outer-mesocarp and middle-mesocarp. Studies of rind development have shown that differentiation is identical in the two varieties. The first layer to differentiate is the epicarp, followed by the hypodermis, outer-mesocarp and finally, the middle-mesocarp. The outer-mesocarp is the most distinct tissue in the rind, and is made up of tightly packed bands of brachysclereids. The band of sclereids is more extensive and broader in Tsamma melon than

in var *lanatus*. Rind anatomy, and particulariy the nature of the outer-mesocarp tissue have been associated with crack resistance. The outer-mesocarp tissue is likely to enhance keeping quality by minimizing cracking following mechanical shocks. Epicuticular waxes are likely to enhance keeping quality by minimizing chances of infection, injury caused by mechanical stress and chilling injury, and increasing the reflectance of heat radiation that may promote fruit decay. Sunken stomata enhance keeping quality of watermelon fruits by minimizing uncontrolled water loss through the cuticle, reducing chances of infection and injury, and facilitating stomatal plugs which completely seal off the fruit after abscission.

Most plants inhabiting dry environments circumvent severe water loss by utilizing the Crassulacean acid metabolism (CAM) photosynthetic pathway (Levitt 1980, Moore et al. 1995). CAM plants have a photosynthetic pathway that is temporally separated and thus, open their stomata during the night and close them during the daytime. This mechanism ensures that the stomata only open at night when the temperature is lower and humidity much higher than during the day, thus preventing water loss. Given the environmental conditions under which Tsamma melon occurs and the hydration that it exhibits, one would expect it to utilize the CAM pathway. However, this is not the case. Botha (1982) found that Tsamma melon does not utilize the CAM pathway, but is a typical C_3 plant with stomatal opening during the day and closed at night, a mechanism that is less effective in minimizing water loss by transpiration.

The development of flowers and fruits are promoted by high light intensity and high temperature. Flowers of watermelon are staminate (male), perfect (hermaphroditic), or pistillate (female), usually borne in that order on the plant as it grows (Messiaen 1994). Monoecious types are common, but there are andromonoecious (staminate and perfect) types mainly in the older varieties or the accessions collected from the wild. The pistillate flowers have an interior ovary and the size and the shape of the ovary is correlated with final fruit size and shape. The developmental sequence of floral parts in the staminate and pistillate flower is similar in both var. *lanatus* and *citroides.* In the pistillate flower, the sepals are the first organs to form in a unidirectional phyllotactic pattern, followed by the petals and stamens. The anthers fail to develop to maturity, but remained as staminodes. The carpe1 primordia is initiated next, alternate and equidistant to the three staminodes. The nectary ring forms last at the base of the style. The identical development of the pistillate flower in the

two varieties suggest that differences observed in keeping quality of mature fruits likely occur after, and not before pollination. In the staminate flower, like in the pistillate flower, the sepals are the first organs to form, followed by the petals and then the stamens. The anthers develop elaborate connective tissues, filaments and thecae. The nectary arises last at a very late stage of floral development. There are no rudimentary carpels in the staminate flower. The order and initiation of organs in the staminate flower suggests that the male flower in *C. lanatus* is not bisexual during ontogeny.

The first male flower forms at nodes 8–11 at 35–50 days after germination. The first female flower forms at node 15–25 at 45–60 days after germination, and they often have poorly developed ovaries and fail to set fruits (van der Vossen et al. 2004). In many varieties, pistillate or perfect flowers appear at every 7th node, with staminate flowers at intervening nodes. Flower ratio of typical watermelon varieties is 7:1, staminate:pistillate, but ranges from 4:1 to 15:1 (http://cuke.hort.ncsu.edu/cucurbit/wmelon/wmhndbk/wmtaxonomy.html). Flowers open shortly after sunrise and remain open only one day.

Fruit surface morphology and anatomy in var. *lanatus* and var. *citroides* (Tsamma melon) share a number of general features, but differ in stomatal morphology and anatomy, and in the extent of the outer-mesocarp tissue. These differences are responsible for the enhanced keeping quality of the Tsamma melon compared to cultivated watermelon. In var. *lanatus* the stomatal complex has one pair of subsidiary cells, and is situated at the surface of the epicarp during anthesis to fruit maturity. In Tsamma melon, the stomatal complex, which consists of three pairs of subsidiary cells is raised above the epicarp during anthesis, and become sunken with fruit growth. At maturity, a layer of amorphous wax covers the fruits of both varieties. Wax deposition increases after abscission in both varieties. Because of the sunken nature of the stomata in Tsamma melon, a waxy bloom covers the entire stomatal complex and blocks the stomatal pores. A previous study by Botha (1982) reported stomatal plugging in Tsamma fruit, but did not determine the nature of the material clogging the stomata. Rind anatomy of the two varieties is remarkably similar.

Watermelon seeds are relatively large, with 7–20 seeds/g, varying in color i.e., white, green, yellow, grey, tan, brown, red, black as well as mixed colours. Seeds continue to mature as the fruit ripens and the rind lightens in color (Messiaen 1994). Seeds are easier to extract from the fruit if the fruit is held in storage (in the shade or in a seed processing room)

for a few days after removing them from the vine. If seeds are left too long in the fruit they will germinate *in situ*. According to van der Vossen et al. (2004), there is no dormancy in watermelon seeds, germination can only be retarded under high temperature regimes. Germination can be accelerated by pre-soaking in water for 24 hours after scarifying the seed at one end, especially for cultivars which have a hard seed coat. Seeds germinate best at temperatures of 17 ºC at night and 32 ºC at day time, and also at a constant temperature of 22 ºC. Seeds do not germinate at temperatures below 15 ºC.

3.2. Ecology

Watermelon is a warm season crop; it requires a long growing season and does best on a rich sandy loam, although it will grow in most soil types provided that the soil is well drained. The plants are drought resistant and prefer full sun, and hot, dry air. Humid, moist climates put the plants at greater risk for disease, and for that reason, long periods of crop rotation in moist climates is key for maintaining healthy crops. Watermelon grows best at a soil pH of 5.6 to 7.0, but will tolerate a pH as low as 5.0. In low pH soils, manganese toxicity can be an issue. The optimum air temperature range for growth is between 21 and 30 ºC.

4. Origin and Geographical Distribution

Generally, watermelon is distributed in tropical and subtropical climates worldwide and is cultivated and adapted to warmer parts of the world. Modern-day cultivated varieties are commonly grown in any climate with long, warm or hot summers (www.fao.org/ag/AGP/AGPC/doc/GBASE/Safricadata/citlan.htm). The domesticated watermelon, *C. lanatus* var. *lanatus*, is grown in tropical and subtropical regions worldwide; the preserving melon or citron, *C. lanatus* var. *citroides*, is grown in southern Africa; perennial *C. colocynthis*, known as the bitter apple, is grown for medicinal purposes from northern Africa to southwest Asia; perennial *C. ecirrhosus* and annual *C. rehmii* are wild species endemic to desert regions of Namibia (Robinson and Decker-Walters 1997). Among the wild watermelon species, *C. colocynthis* has the widest world distribution; the plant grows in the Mediterranean basin, in North Africa and in Southwestern Asia in dry and sandy habitats. It is a perennial species indigenous to coastal and desert regions of Israel although over the past decades it became less abundant, especially in the northern coastal plains (Zohary 1983).

The primary centre of origin for watermelon is not known. 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). Despite the economic importance of watermelon, domestication events and phylogeographic relationships have only recently attracted scientific attention. Knowledge regarding the path of domestication, however, is fragmentary and various scenarios have been postulated for the origin of the domesticated watermelon from its progenitor, wild *C. lanatus.*

The wild watermelon is widely distributed in Africa and Asia, but originates from southern Africa occurring naturally in South Africa, Namibia, Botswana, Zimbabwe, Mozambique, Zambia and Malawi (www.fao.org/ag/AGP/AGPC/doc/GBASE/Safricadata/citlan.htm). A number of distinct landraces, which are cultivated in the Kalahari region and its periphery, may present early forms of domestication. Thus, the distribution of the wild taxa in southwest Africa point to Namibia or possibly southern Africa as the centre of domestication

for watermelon. Several authors (Rubatzky 2001, Mohr 1986, Whitaker and Davis 1962, Esquinaz-Alcazar and Gulick 1983, Dane and Lang 2004) have supported the notion that southern Africa in part (Kalahari Desert) or as a whole region is the centre of origin. However, the available archaeological information does not support this as only a few records of watermelon are known from southern Africa and all belong to the younger periods (8th–13th century AD). The presence of 5,000-year-old seeds of *C. lanatus* in Libya 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; Hepper 1990), 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. Consequently, the domestication process may also have occurred in the northern distribution range of the species, where a long history of agronomic activity has been recorded. Thus Mallick and Masui (1986), and Guner and Wehner (2004a) suggests that watermelon is native to central Africa where it was domesticated as a source of water, a staple food crop, and an animal feed. Zeven and de Wet (1982) suggested that the centre for domestication of the watermelon was in Hindustani, an area encompassing India, Nepal, Burma, Thailand and Pakistan. *Citrullus* cultivation may also have evolved independently in different regions and in different forms with whatever suitable plants were at hand. Whitaker and Davis (1962), furthermore, also describe the existence of a secondary diversification centre for the species of *Citrullus* in India. Romão (2000) supports Africa as the origin of watermelon, and furthermore Northeast Brazil as a secondary center of diversity for watermelon, after an introduction by African slaves around three centuries ago.

A recent study by Dane and Liu (2007), 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. Though not exhaustive, this is the most recent study that point 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.

4.1. Domestication traits

The fruit of the wild species are characterized by white flesh and an extremely bitter flavour. This bitter taste is caused by a high concentration of a substance called Cucurbitacine E. glycoside (Herrington et al. 1986) or colocynthine (Mohr 1988) and is controlled by a (Bi) gene which is dominant over the non-bitter character (Robinson et al. 1976, Navot et al. 1990). The bitter taste is also present in wild species of other Cucurbitaceae (Joubert 1980).

The red colour of the flesh is influenced by a recessive (red) gene, but according to Navot et al. (1990), the colour inheritance of the flesh is more complex and involves an epistatic effect. These characters have been the two most important ones in the domestication process as there is strong pressure for red flesh and a non-bitter taste.

5. Cultivation of *Citrullus lanatus*

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 and 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 (Jeffrey 1975, Whitaker and Davis 1962). Maheshwari (1978) recognizes 13 watermelon varieties cultivated in different parts of the world, e.g. India, Pakistan, Malaysia, Polynesia, Japan, China, Iraq, Europe, Africa, and South and Central America. Among other characters, such varieties differ in size, shape and colour of fruit skin, colour of flesh (red, pink, white and yellow), and the colour and size of seeds.

Cultivated watermelon types have traditionally been red-fleshed and seeded. There is genetic variation for flesh color in the species, however, 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 could 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.

In northwest China, edible seed watermelons are an important crop (Zhang and Jiang 1990); these melons are small in size (2.5 to 3.5 kg) with low soluble solids content, but have a high ratio of seed to flesh in the fruits. In Zimbabwe, the most preferred watermelons are larger sized ranging between 2.5 to 10 kg.

Globally, watermelon is a major cucurbit crop that accounts for 7.5% of the world area devoted to vegetable production in 2003; it is grown on over 3.7 million ha producing more than 83 million metric tons of fruit, with China and the Middle Eastern countries being the major producers and consumers (FAO 2003). Watermelon is grown in more than 96 countries worldwide, with China accounting for 70.3% of the total production. Other leading countries are Turkey (4.7%), Iran (2.3%), the United States (2.2%), and Egypt (1.7%) (FAO, 2003).

5.1. Nutritional Status

Watermelon has become an important part of the healthy diet since nutritionally it is almost free of fat, sodium and cholesterol. The fruit contains 93–95% water, 5% carbohydrate, 0.5–1% protein, and 0.2% fat (Rubatzky and Yamaguchi 1997). Watermelon has a high lycopene content in the red-fleshed cultivars: 60% more than tomato. Lycopene has been classified as useful in the human diet for prevention of heart attacks and certain types of cancer. Recently, it has been found that watermelon rind contains 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.

6. The Breeding of Watermelon

There are limited breeding efforts in southern Africa, particularly with *Citrullus* species; however, numerous local varieties have been developed by local farmers through a systematic gradual selection in the fields over time and space. Some of the resultant varieties are quite different from the traditional forms of watermelon.

Breeding of watermelon is largely conducted outside of Africa, in particular in the United States. Important objectives for watermelon breeding include desirable fruit shape, early maturation, high fruit yield, high sugar content, tough flexible rind, and desirable seed type. Watermelon breeding has affected some traits like shape of vines that match with size of fruit (i.e. short or medium-length vines well suited to varieties with small or medium-sized fruit); sex expression with monoecious varieties generally being preferred; yield as growers want high weight per acre of marketable size fruit, with a low percentage of culls (unwanted fruits). Early maturity is desirable because prices for watermelon are usually highest at the beginning of the local season. Other traits like fruit size, shape and rind pattern, external and internal fruit quality, seeds and seedlessness are also considered to meet market requirements. (http://cuke.hort.ncsu.edu/cucurbit/wmelon/wmhndbk/wmtraits.html).

Disease resistance is an important objective of most breeding programs. The major diseases affecting watermelons include *Fusarium* wilt**,** anthracnose**,** gummy stem blight, powdery mildew, yellow vine, bacterial fruit blotch**,** root-knot nematodes and virus diseases, and are a major limiting factor in commercial watermelon production worldwide. Virus diseases are destructive to the watermelon crop, and are difficult to control (Sherf and Macnab 1986). Fields may be infected with individual viruses, or with multiple viruses in combination (Davis and Mizuki 1987). The major viruses affecting watermelon are papaya ringspot virus watermelon strain (PRSV-W, formerly watermelon mosaic virus-1), watermelon mosaic virus (WMV, formerly watermelon mosaic virus-2), and zucchini yellow mosaic (ZYMV) (Adlerz and Crall 1967, Mohr 1986, Provvidenti 1991, Wehner et al. 2001). Plants infected with PRSV-W lose their photosynthetic capacity and subsequently display stunted growth, deformed fruit, and early mortality. Although resistance is generally pathogen-specific (Grumet 1989), the most economical method is to improve genetic

resistance. The latest development in watermelon breeding is the screening of watermelon accessions worldwide for disease-resistance genes. Munger et al. (1984) tested seven watermelon Plant Introduction (PI) accessions for resistance to PRSV-W using an unidentified isolate of PRSV-W. Hojo et al. (1991a) used an aggressive isolate, Ab-081, to screen some Brazilian watermelon accessions for virus resistance. Only one Brazilian resistant accession, BT-8501, a wild, bitter-fruited watermelon from Africa, was identified (Hojo et al. 1991b). More recently, watermelon was extensively screened for resistance to papaya ringspot virus watermelon strain and zucchini yellow mosaic virus (Guner and Wehner 2004b, Guner et al. 2004). Gusmini et al. (2005) have evaluated new sources of resistance to gummy stem blight in watermelon.

Recently, Davis et al. (2007) screened watermelon germplasm for resistance to powdery mildew, a disease caused by *Podosphaera xanthii (Sphaerotheca fuliginea).* 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. These accessions constituted only 9% and 4% of the U.S. *Citrullus* species PI collection respectively. Further work on the same material is now focused on inheritance studies and identification of multiple resistance genes in order to pyrmaide resistance sources into a single cultivar to obtain greater resistance stability.

7. Genetic diversity in the genus *Citrullus*

7.1. Phenotypic characterization and diversity

The study of morpho-agronomic variability is the classical way of assessing genetic diversity for plant breeders. For many species, especially minor crops; it is still the only approach used by breeders. *Citrullus lanatus* species exhibit expansive polymorphism in southern Africa and the species occurs in the following forms: wild populations distributed throughout the country in a wide range of habitat types; sweet watermelon, cooking melon and seed melon landraces of the traditional agrosystems; and possibly introgressed types which are regarded as agronomic weeds but are often found at vast distances from human habitation (Maggs-Kolling et al. 2000).

Shippers (2002) noted the existence of diverse wild forms of watermelons in the Kalahari Desert. Studies by the Botswana NPGRC (SPGRC Network News 2004) highlighted the presence of various domesticated and wild watermelons. Domesticated watermelons in Botswana include landraces such as 'Magapu', where the pulp is eaten fresh and colour varies from white to red, and 'Marotse', where the pulp is cooked fresh or dried, and with seeds that are sometimes roasted and eaten as a snack. The Marotse type exists also in diverse forms of which some are known as 'Sesowane' with seeds of high oil content and 'Senowane'. 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 watermelons. They are sweet and are consumed fresh.
b. 'Mashamba', 'Majodo' – these forms are referred to as cow-melons and are consumed after boiling and cooking to produce a meal called Nhopi in 'Shona' or in some areas fed to animals.

In the Kalahari region, wild watermelons are known by such names as 'Tsamma', 'Kgwengwe' or 'Mokate', and these names may denote different forms of wild watermelons. However, the relationship between vernacular naming and different forms of watermelon

remains a subject for speculation which requires further investigations. The 'Tsamma' melon is regarded as an important source of water in the Kalahari Desert and has other diverse uses. 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. Wild watermelons from the desert have the ability to withstand severe drought conditions, and therefore are potential sources of genes for watermelon improvement programmes.

Natural hybrids between *C. lanatus* and *C. colocynthis* have already been found to exist (Singh 1978, Maheshwari 1978, Fulks et al. 1979, Herrington et al. 1986). Morphological and cytological observations suggest a close relationship between these two species. Singh (1978) found one of these hybrids in India, where both species are well represented in the semi-arid regions of Rajasthan. *Citrullus lanatus* locally knows an 'Matiro', *C. colocynthis* known as 'Tumba' and intermediate forms known as 'Khar' or 'Tatumba' have been found growing close to each other. Maheshwari (1978) also described a variety called 'Neri' as an almost intermediate form between *C. colocynthis* and *C. lanatus.* In Arizona, Fulks et al. (1979) found natural hybrids between *C. colocynthis* and *C. lanatus* 'cv. citron', which had white flesh, bigger fruit and no characteristic bitter taste. Furthermore, Shimotsuma (1963) obtained artificial hybrids. Natural hybrids have also been reported in Australia (Herrington et al. 1986) and in Texas, USA (Smith and Cooley 1973). Interspecific hybridization has produced a specific classification for plants with intermediary characters, using the nomenclature *C. lanatus* var. *citroides*. In southern Africa, the watermelon known as 'Mekatse' in Botswana is most likely derived from a cross between two genetically diverse parents (SPGRC Network News 2004), possibly 'Magapu' (paternal) and 'Marotse' (maternal) since a hybrid melon rind normally resembles mainly that of the maternal parent.

A phenetic analysis of morphological variation in *Citrullus lanatus* (Maggs-Kölling et al. 2000) for the various morphotypes supported the indigenous classification system used, with distinct groups (seed, cooking and fresh-eating types) based on gross morphology, ecology and usage. Commercial watermelon cultivars formed a distinct cluster. Wide variation was found within the local types whereas the genetic basis of the commercial type appeared to be narrow.

7.2 Molecular characterization

An assessment of genetic diversity based only on morpho-agronomic traits might be biased, because distinct morphotypes can results from only a few mutations while they share a common genetic base. Therefore, molecular markers have the potential to complement already existing estimations of diversity, and to be used, e.g., to construct core collections for effective genebank management.

Molecular markers can be an effective means to determine genetic relatedness among cultivars and among selections used in watermelon breeding programs. Molecular markers 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, a useful means of identifying different cultivars. Diversity studies provide useful information for breeders about genetic relationships and distances between individuals. For 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 Korea, and there have been virtually no studies associated with a study of landraces in traditional agroecosystems.

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* and was suggested to be a representative of a locally cultivated and highly polymorphic race grown for animal feed (Zamir et al. 1984). An isozyme-based phenetic study of the genus *Citrullus* was characterized by two main clusters: one with *C. colocynthis* and a second with *C. lanatus* and *C. lanatus* var. *citroides* (Navot and 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, Navot and Zamir (1986) considered South African germplasm to be the wild progenitor of cultivated watermelon. Based on immunochemical analyses, Fursa and Gavrilyuk (1990) argued that *C. lanatus* originated from var. *cordophanus*, a semi-cultivated variety found in Sudan.

A major problem with using isozyme/protein markers is that only a limited number of polymorphic loci are detected in watermelon, especially in the species *C. lanatus* (Biles et al. 1989, Navot and Zamir 1987, Zamir et al. 1984). The role of isozymes for discriminatorial purposes has now been taken over by DNA markers since the latter are able to provide much higher levels of polymorphism.

DNA marker-derived data can potentially be useful in determining whether closely related watermelon accessions have resistance to the same diseases and for establishing core germplasm collections with disease resistances that can be useful in watermelon breeding programs. 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, b). This method has been used mostly for estimating genetic relatedness among U.S. PIs (Levi et al. 2000, 2001a), but the data has also suggested that diversity is higher in the wild species *C. colocynthis* and in the wild subspecies *C. lanatus* var. *citroides*. Contrary to RAPD markers, other dominant 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).

SSR markers detect polymorphisms based on the repeat length of microsatellite sequences (Wang et al. 1994) and are often preferred in plant breeding as they 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 of *C. lanatus* var *lanatus*, second of wild and cultivated *C. lanatus* var *citroides*, third group 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 accounted for except where genetic variability and differentiation of watermelon accessions were attributed to the broad geographical distribution of the materials where it has been subjected to local adaptation and selection.

The limited number of informative and reliable DNA markers negatively affects progress in molecular genetic research in watermelon. Lee et al. (2007) developed sequence

characterized amplified region (SCAR) and cleaved amplified polymorphic sequence (CAPS) markers from amplified fragment length polymorphism (ALFP) markers, which together with SSR markers, were used to estimate genetic diversity of commercial varieties of watermelon. This study differentiated Korean watermelons into two major groups which were not significantly correlated with their morphological and physiological characteristics.

Restriction fragment length polymorphism (RFLP) was used to infer biogeographic and evolutionary relationships, and to study origin and domestication history of watermelon. Initially, a PCR-RFLP analysis was conducted in *Citrullus* species using many different chloroplast regions (Dane 2002). This study identified variability within *C. lanatus* at regions with high A+T contents, associated with indels and transversions as well as distinct chlorotype lineages separating the cultivated and egusi-type watermelon from var. *citroides* accessions, thus suggesting an ancient split from a common ancestor and haplotype fixation. This study also revealed that chloroplast divergence in watermelon is not associated with morphological divergence. However, Dane and Lang (2004) suspect that the present geographic distribution pattern might reflect patterns of survival more directly than patterns of origin. The limitation in many plant phylogenetic studies relying on sequences of cpDNA, to elucidate domestication routes, long-distance gene flow patterns and progenitor-derived relationships is the slow rate of sequence divergence. Potentially, single-copy nuclear gene sequences can be expected to cast more light on the radiation of *Citrullus* from its arid environments (Dane and Liu 2007).

7.3. Chromosomal Mapping

Molecular markers have been instrumental in the construction of linkage maps. Application of a molecular marker in this regard, makes it possible to locate and manipulate individual genetic factors associated with complex traits. Defining the genetic control of these traits will assist breeders and subsequent molecular mapping will contribute to the development of marker-assisted selection (Perin et al. 1998, Staub et al. 1996). High density genetic linkage maps are useful for positioning and tagging genes of interest to facilitate marker-assisted breeding in an increasing number of crop plants. Genetic maps are also useful in gene cloning and in analyzing complex traits (Lee 1995). It is further envisaged that many markers are still required for construction of a saturated map that can be used effectively in

watermelon breeding programs, and for locating genes that control important traits like fruit quality and resistance to diseases and pests.

The first linkage map for watermelon (Navot and Zamir 1986) was based on a cross between an accession of the wild species *C. colocynthis* and the watermelon cultivar Mallali, and described linkage relationships among 19 protein coding genes. The map was extended to 24 loci (including 22 loci, the locus for fruit bitterness, and the locus for flesh colour) that segregated in linkage groups, covering 354 cM (Navot et al. 1990). Hashizume et al (1996) using a BC1 population derived from a cross between an inbred line (H-7; *C. lanatus*) and a wild accession (SA-1; *C. lanatus*) subsequently constructed a genetic map containing 58 RAPD markers, one enzyme, one restriction fragment length polymorphism (RFLP) and two morphological markers segregating in 11 linkage groups, covering 524 cM. Hawkins et al. (2001) constructed two linkage maps containing 26 and 13 RAPD markers segregating in two and five linkage groups, covering 112.9 and 139 cM, respectively. Levi et al. (2001) constructed a genetic linkage map using a BC1 population [PI296341-fusarium wilt-resistant x New Hampshire Midget (fusarium wilt-susceptible)] x 'New Hampshire Midget' containing 155 RAPD markers, and a 700-base pair SCAR marker. The SCAR marker corresponds to a fragment produced by the RAPD primer GTAGCACTCC reported to be linked (1.6 cM) to race 1 fusarium wilt resistance in watermelon. The markers segregated into 17 groups covering 1295 cM. However, most RAPD markers could not be mapped with confidence, since a significant proportion of the markers in the F2 and F3 populations did not segregate in the expected 3:1 and 5:3 ratios, respectively. BC1 population also showed segregation patterns skewed away from the expected 1:1 ratio (at $P = 0.05$) (Levi et al. 2001c). In addition, a large number of markers in that map were closely linked (0–2.7 cM), indicating the presence of chromosomal regions with low recombination events.

In an effort to avoid skewed segregation and to examine low recombination events, Levi et al. (2002), constructed a genetic linkage map for watermelon derived from a testcross population. The linkage map consisted of RAPD and ISSR markers present in the wild accession (Griffin 14113) or in the cultivated watermelon (NHM), which in total revealed 25 linkage groups. Even then, skewed segregation in the testcross population was observed although not as high (18%) as in previous maps constructed for watermelon using an F2 and an F3 population (47.5% and 48% skewed markers, respectively; Hawkins et al. 2001), or in

the map constructed for watermelon using a BC1 population (25.7% skewed markers; Levi et al. 2001c).

Very few QTL loci have been added to the watermelon linkage maps. Hashizume et al. (2003) attempted QTL mapping using a linkage map constructed with RAPD, RFLP, inter-simple sequence repeats (ISSRs) and isozymes in an F2 population derived from a crossing between a cultivated inbred line (H-7; *C. lanatus*) and an African wild form (SA-1; *C. lanatus*). Linkage analysis revealed that 554 loci could be mapped into 11 linkage groups that covered 2,384 centimorgans (cM). A QTL analysis was applied by means of interval mapping for locating such agronomic traits as hardness of rind, Brix of flesh juice (measure of sugar content), flesh color (red and yellow) and rind colour with the relative order of markers essentially the same as that on the linkage map in the F2. Levi et al. 2006 constructed an extended linkage map for watermelon based on SRAP, AFLP, SSR, ISSR and RAPD markers using the JoinMap 3.0 mapping program and produced linkage groups with marker order consistent with those reported in previous mapping studies.

8. Greenhouse Cultivation and Postharvest

8.1. Cultural practice

In general, very little documentation is provided for studies involving greenhouse production of watermelon. Greenhouse temperatures ranged from 23 to 43 ^{o}C (day) and from 12 to 24 ^{o}C (night) (Guner 2004) and a seed germination mix of peat, vermiculite, and perlite was used (Gusmini 2005). However, through experience, temperature can be controlled and maintained by adjusting the lights. Seeding of watermelons was done directly in plastic pots (100×100 mm size, 600 mL volume) filled with a soilless mix of peat moss, perlite, vermiculite, processed pine bark (Gusmini 2005). In addition, it can also be done on ground beds, plastic bags or pots containing the growth medium, or in various liquid media such as ebb and flow benches or nutrient film technique (Wehner 2008). If pots or bags are used, different container sizes should be evaluated to ensure a proper plant size. Plants grown in large containers like 10(~25 cm) or 12-inch (~30 cm) diameter pots produce longer vines that are more difficult to train and prune, larger fruit, and more seeds per pollination (http://cuke.hort.ncsu.edu/cucurbit/wmelon/wmhndbk/wmflowering.html).

Greenhouses have been instrumental in facilitating controlled experiments in watermelon. Controlled pollinations can be made easily in an insect-proof greenhouse since there is no need to cover individual flowers to protect them from pollinating insects such as bees. These pollinations are usually done by hand, preferably in the morning since the highest success rate occurs when pollinations are made between 9:00 a.m. and 10:00 a.m. (Jeffrey 2005). Adequate pollination of a single female flower is achieved when pollen from at least three male flowers is used and through hand pollination success is achieved in at least 50% of the time under favorable conditions (Jeffrey 2005).

In the greenhouse, watermelon plants are usually trained vertically onto supports such as strings held by overhead wires to save floor space and make better use of available light. Stem length of most watermelons usually requires that plants be trained up the string to the trellis wire, and back down again. The overhead wire should be aligned is such a way to permit workers to freely perform their operations. Plants should be given sufficient floor space in the greenhouse to grow and flower. Wehner (2008) preferred at least 2 square feet (0.185 m^2) per plant for elite varieties and breeding lines and at least 4 square feet (0.37 m^2)

per plant or more for wild accessions. Plants should be pruned to one main stem, usually with no branches. Because of their weight, fruit must be supported either on a surface or in a sling.

8.2. Fruit harvest

It takes some practice to be able to judge when a watermelon is mature enough to harvest at the edible stage. The edible maturity stage is judged by several factors by farmers in the field, such as: (1) the spot in contact with the soil turns a yellowish colour, (2) the tendril on the vine nearest the point of fruit attachment is dried up, (3) the rind has a dull or rough appearance compared to smooth, glossier, immature fruit, and (4) a hollow sound is made when tapping the fruit with a finger. The tapping or thumping method needs experience. Though seeds are ready to harvest at the edible maturity stage, seeds continue to mature in the fruit for several weeks, a process called "after-ripening." Allowing the seed to after-ripen gives a higher quality seed. For this reason, seed extraction is best delayed for several weeks. For the best quality seed, the best time to extract the seed is between 10 and 30 days past eating maturity.

8.3. Seed extraction

Watermelon seed can be extracted by cutting the melon in pieces and scooping out the seed and spreading them on a sheet of paper for drying. Farmers in the field extract the seeds by eating the watermelons (as a single or community effort) and spitting out the seed into bowls. This method is practical in traditional agriculture for seed saved for home use. For commercial purposes, the seeds are extracted and put in suitable containers used for fermentation and then either macerated by hand or by use of a hand mortar mixer. Another method is to slice the fruit into wedges and rub the wedges over a ½-inch hardware cloth stretched on a wooden frame that is placed on top of a wheelbarrow used to catch the pulp and seed. A third method is to run slices of the wedges through an apple grinder. Most farmers prefer seeds that have fermented first. Fermentation is a process which kills viruses and separates the good seed from the bad seed and fruit pulp. After two to four days, the good viable seeds will sink to the bottom of the container while the pulp and bad seed float. Farmers then pour off the pulp, water, bad seed and mold. The good seed is then cleaned,

washed and spread on a screen or paper towel to dry at room temperature. After drying, which takes 1–3 days; the seeds are packed in paper envelopes, plastic containers and/or bottles for safekeeping.

9. The Current Study on Watermelon Diversity

My Ph D project is an attempt to contribute to the knowledge about genetic variation within the genus *Citrullus* in Zimbabwe and southern Africa. Through comparative analysis at the molecular level of wild and cultivated accessions of *C. lanatus*, studies have been initiated to reveal relationships among wild and domesticated forms, which may help to understand the domestication and agronomic development of the species.

9.1. Overall Objective

The objective of this project is to assess the diversity of landraces and wild forms of watermelon in their growing environments and the implications for the various conservation strategies in southern Africa. This entails the determination of genetic variation in watermelon germplasm material from southern Africa, e.g, Namibia, Botswana, South Africa and Zimbabwe, and map and/or predict environments in which the landraces and wild forms are adapted; to determine the degree of genetic relationships and gene flow among the different forms of watermelon; to characterize the watermelon germplasm of the region, and to determine their relationships using molecular markers.

For the SADC region and international community, this project will provide information to aid (i) watermelon breeders in the placement of germplasm into correct heterotic groups, (ii) germplasm curators (both at regional level and in each member countries) in the management of genebank collections by refining the core subsets, (iii) watermelon scientists in understanding the gene flow of watermelon in southern Africa, (iv) 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.

The specific objectives are:

- To assess the diversity of watermelon (*Citrullus lanatus*) accessions using SSR markers and RAPD markers in Zimbabwe across the agro-ecological regions
- To assess the diversity of watermelon and the contributory environmental and socio-economical factors in a selected community in Zimbabwe

- To assess diversity of watermelon in selected regional countries which include Zimbabwe, Zambia, Namibia, South Africa and Botswana.
- Application of GIS techniques, particularly the use of 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.

9.2. The First Study

The first study has now been finished, and a manuscript has been submitted (November 2009), entitled 'Genetic diversity in watermelon (*Citrullus lanatus*) landraces from Zimbabwe revealed by RAPD and SSR markers'.

The abstract runs as follows:

'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 in southern Africa may harbour considerably higher variability, DNA marker-based diversity was estimated among 81 seedlings from 8 accessions of watermelon collected in Zimbabwe; 5 accessions of cow-melons (*Citrullus lanatus* var. *citroides)* and 3 of sweet watermelons *(C. lanatus* var. *lanatus).* Two molecular marker methods were used, random amplified polymorphic DNA (RAPD) and simple sequence repeats (SSR) also known as microsatellite DNA. Ten RAPD primers produced 138 markers of which 122 were polymorphic. 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. Similarity matrices obtained with SSR and RAPD, respectively, were highly correlated but only RAPD was able to provide each sample with an individual-specific DNA profile. Dendrograms and multidimensional scaling (MDS) 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, and contrary to previous reports, sweet watermelon accessions apparently contain diversity of the same magnitude as the cow-melons.'

10. References

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