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Diversity of local genetic resources of watermelon *Citrullus lanatus* (Thunb.) Matsum and Nakai, in Sudan

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Abstract

Morphological and molecular characterization were carried out in this study to estimate genetic diversity within the genus Citrullus collected from Sudan, with assistance of passport data to examine if the site of collection has any effect on the diversity of the species. Number of 30 accessions was chosen for this study. These accessions were collected from six different regions of the country representing North, West and Central Sudan. The experiment was carried out in the field of the Agricultural Research and Technology Corporation (ARTC) in Sudan. A descriptor list locally developed by the Plant Genetic Resources (PGR) unit of the (ARTC) was used for morphological characterization. Morphological data approved high variability for fruit and seed characters and referred to characters which may be considered valuable for plant breeders. The cluster obtained from morphological characterization separates the studied accession into four different morphotypes. Accessions from the western part of the country grouped together regardless of the specific site of collection inside the western region. SSR markers and RAPD markers were used for molecular characterization. The cluster obtained from molecular characterization separates the accessions into four groups with 71% similarity coefficient. Some of the accessions were appear to have high level of similarity which may facilitate findings of duplication. However the molecular groups did not coincide with the morphological groups and the site of collection has no effect on this grouping.

Key words: characterization, *Citrullus lanatus*, genetic diversity, Sudan

Contents

Introduction

The *Cucurbitaceae* is a large plant family found mainly in the warmer parts of all continents. It consists of 119 genera with altogether 825 species (Schipper 2002). Fruits of *Cucurbitaceae* have a considerable economic value. One of the main uses of the cucurbits apart from their fruits, leaves, flowers and occasionally their root is that of its seeds. The seed kernels of the *Cucurbitaceae* family found in markets through out West Africa are an important source of oil used for food. Those oil-rich seeds are found in a range of genera of which the most important are *Citrullus* (watermelon), *Cucurbita* (pumpkin), *Cucumeropsis* (egusi melon), *Lagenaria* (bottle gourd), *Cucumis* (melon), *Telfairia* (fluted pumpkin) and *Luffa* (sponge gourd) respectively (Schipper 2002).

Watermelon originated close to the Kalahari Desert where wild forms can still be found. It has been in cultivation for at least 4000 years in Egypt. It spread through the Sahara Desert region to the Middle East during antiquity. By the 10^{th} century it was introduced to China, which today is the world greatest producer and consumer of watermelon. Watermelon was grown in Europe by the 13^{th} century, and it was introduced into North America during the 17^{th} century (Jeffrey 1975; Whitaker and Davis 1962)

Watermelon accounts for 6.4% of the world area devoted to vegetable crops (FAOSTAT 2005). China is the largest producer of watermelon with 69.3 million tons of the total world production. Other major producing countries are Turkey, Iran, Brazil, the United States, Egypt, Russia and Mexico (FAOSTAT 2005).

Watermelon is grown throughout the world for human food; it is consumed as a dessert fruit, as a source of drinking water or for its edible seeds. It is also used as animal feed in some areas. Although watermelon is primarily eaten fresh, it is also eaten as cooked vegetable in Africa. In Russia, watermelon is eaten after being pickled or used for production of syrup by boiling the sugary flesh. In China, firm fleshed cultivars are cut into slices and dried for pickled or glass candy. In Sudan and Egypt the seeds are roasted, salted and eaten. Another use of watermelon is the use of the fruits as a source of drinking water during drought seasons, which is a well known use in parts of Sudan and Nigeria (van der Vossen 2004).

Sudan is a country of diversified ecological conditions including climate, vegetation and soil, which result in an enormous wealth of diversified indigenous genetic resources of crops of which watermelon is an example. The Western part of Sudan is an important region for the diversity of watermelon

where different cultivars and uses are known, especially in the Kordofan region.

Genetic resources use can be highly enhanced through characterization and evaluation. Characterization of genetic resources is regarded as an important activity for genebanks. It involves determining the expression of highly heritable characters, ranging from morphological features to seed proteins and possibly including molecular markers. Such characters enable easy and quick discrimination among phenotypes and allow simple grouping of the accessions (Engels and Visser 2003). In addition to molecular markers, scoring of such characters allows establishment of systematic relationship among accessions and even crops including their evolutionary relationships. This directly facilitates utilization of collections and allows detection of misidentification (Bretting and Widrlechner 1995). It also results in a better insight in the composition of a collection and the coverage of genetic diversity.

Characterization data should be linked to passport and evaluation data. Passport data comprise the information about the site and the environment from which an accession originated; accurate passport data including site description are useful as bases for correlating the origin with environmental parameter (Hawtin and Colin 1999).

To facilitate the standardization of information obtained during characterization and evaluation, the International Plant Genetic Resources Institute (IPGRI) has published a number of descriptor lists for different crop species in order to have a universally understood language for plant genetic resources (PGR) data (Engels and Visser 2003).

Research rationale

Despite the extent of its distribution and cultivation, and its importance, watermelon is a poorly described species. No standard descriptor list has been developed and published yet by the genetic resources community. Only 10 well defined morphological markers have been characterized and just few informative isozyme markers are available (Lee *et al.* 1996). Hence, available information on watermelon genetic diversity within the global germplasm holdings is very scarce.

The present study aims to contribute to the knowledge of variability within the genus *Citrullus* collected and conserved in the Plant Genetic Resources unit in Sudan through;

1. Morphological characterization: recording morphological characters that could be described easily, be of high heritability and show little genotype x environment interaction.
2. DNA characterization: DNA based technique is a complementary strategy to the morphological approach for assessment of genetic diversity, the major advantage being that it allows studying the variation at the DNA level, disregarding environmental influences.
3. GIS techniques: 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.

The results of this study are expected to improve documentation of *Cirullus* germplasm collected from Sudan, thereby enhancing utilization by different users and especially plant breeders.

General review

Introduction to the species

Taxonomy

According to the last taxonomic treatment of Jeffrey (1990), the family C*ucurbitaceae* consists of 118 genera and 825 species. The genus C*itrullus* is the most economically important crop in the family; it belongs to the subfamily *Cucurbitoideae,* tribe *Benincaseae* and sub tribe *Benincasinae.*

The genus *Citrullus* contains four diploid species, with basic chromosome number of (2n=22, n=11). (*Cirullus lanatus* Thumb.). Matsum and Nakai, which is divided into two botanical varieties, (C*itrullus lanatus* var. *lanatus*) that thrives in west Africa and is called egusi melon, and the preserving melon (*Citrullus lanatus* var. *citroides*) that is grown in Southern Africa (Whitaker and Bemis 1976) and is called tsamma melon.

Citrullus colocynthis (L.) Schrader, a perennial species, with globes fruits of 5-10 cm in diameter. The fruit of this species is bitter and even poisonous (Schipper, 2002), it grown in sandy areas through out Northern Africa, South West Asia and the Mediterranean (Jarret. *et al.* 1997). *Citrullus ecirrhosus* Cogniaux, a perennial species, grown in Southern Africa and West Namibia, it grows without tendrils and with woody deeply penetrating taproot.

The fourth species, *Citrullus rehmii* De Winter, an annual wild species, its distribution is confined to the western escarpment in Namibia (Schipper 2002).

It resembles *citrullus lanatus* but can be distinguished by its pink to orange mottled surface of the rind. All the species in the genus C*itrullus* are cross compatible to each other. *Citrullus lanatus* and C*itrullus ecirrhosus* appear to be more closely related to each other than either is to *C. colocynthis* (Navot and Zamir 1987).

There are two other closely related species: *Praecitrullus fistulosus* (Stocks) from India and Pakistan, the genus has a basic chromosome number of n=12 (Schipper 2002). Tinda varieties with their green-fleshed fruits that found in Kenya, Zimbabwe, and Ghana are belong to this species. The other species is *Acanthosicyos naudinianus* (Sond) a wild species native to southern Africa.

Morphology and Physiology

Watermelon is a warm season crop; it requires a long growing season. Flowering and fruit development are promoted by high light intensity and high temperature. Its growth habit is a trailing vine; the stems are thin, hairy, angular, grooved and have tendrils at each node (Robinson and Decker-Walter 1997). The stems are highly branched and up to 10m long, although there are dwarf types (dw-1 and dw-2 genes) with shorter, less branched stems, dwarfing is primarily related to shortened internodes (Mohr 1956). Roots are extensive but shallow, with taproot and many lateral roots growing within the top 2 feet from the soil (Robinson and Decker- Walters 1997).

Watermelon is the only economically important cucurbit with lobed leaves; all of the other species have a hole (none lobed) leaves. The leaves are pinnately divided into 3 or 4 pairs of lobes, except for an entire leaf (none- lobed) gene mutant controlled by nl (none-lobed) gene (Whenner 2005.)

Flowers are small and less showy than other cucurbits (Robinson and Decker-Walter 1997); flowering begins about 8 weeks after seeding. 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 (Whenner 2005). The first male flower formed on node 8-11 at 35-50 days after sowing. The first female flower formed at node 15- 25 at 45-60 days after sowing. The first female flowers often have poorly developed ovaries and fail to set fruits (van der Vossen *et al.* 2004). In many varieties the pistillate or perfect flower appears at every seventh node, with staminate flower at the intervening nodes. The flower

ratio of typical watermelon varieties is 7:1, staminate: pistillate but the ratio range from 4:1 to 15:1 (Whenner 2005). Flowers open shortly after sunrise and remain open only one day.

Fruit of wild plants range between 1.5- 20 cm in diameter, sub-globes, greenish, mottled with dark green; fruit of cultivated plants are up to 30x60 cm, sub-globes or ellipsoid, green or yellowish green, evenly colored or variously mottled or striped (Messiaen 1994) . Fruits vary considerably in morphology. Whereas the fruits of the wild forms are small and round, the cultivated forms are large oblong fruits. They vary from pale yellow or light green (wild forms) to dark green (cultivars), and with or without stripes. The pulp varies from yellow or green (wild forms) to dark red (cultivars).

Watermelon seeds are larger than those of melon and cucumber, with 7-20 seeds/g. They are very varied in color, from light to bright red to brown to black; seeds continue to mature as the fruit ripen and the rind lightens in color (Messiaen 1994). Seeds will be easier to extract from the fruit if the fruit is held in storage (in the shade or in the seed processing room) for a few days after removing them from the vine. If seeds are left so long in the fruit they will germinate *in situ*. There is no dormancy in watermelon seeds (van der Vossen. *et al.* 2004), but germination is 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 temperature of 17º C at night and 32º C at day time and also at constant temperature of 22º C. Seeds will not germinate at temperature below 15º C. light has an inhibitory effect (Whenner 2005).

Economic importance of the crop

Watermelon is a major cucurbit crop that account for 6.4%of the world area devoted to vegetable crops (FAOSTAT 2005), there are 3.4 million ha of watermelon grown in the world. China and the Middle Eastern countries are the major consumers of the crop. China is the largest watermelon producer with 69.3 million metric tons which accounts 71% of the total world production (FAOSTAT 2005). The other watermelon major producing countries are Turkey, Iran, Brazil, United state, Egypt, Russia and Mexico (FAOSTAT 2005).

In Africa, cultivars of which the edible portion is seeds are the mostly economically important, Western and Central Africa are the largest producer of egusi seeds, however statistics are scarce. FAO statistic includes melon seeds and several *Cucurbita* species, world production of melon seeds in 2002 was

567.000 t from an area of about 608.000 ha, from this total Nigeria contribute by 347.000 t from an area of 361.000 ha. Cameroon produce 57 000 t, Sudan produced 46 000 t; D.R C. produced 40 000 t; Central Africa Republic 23 000 t and Chad produced 20 000 t (van der Vossen. *et al.* 2004 from FAO 2002). Sudan exports about 27 000 mainly to Arab countries; however quantities fluctuate strongly from year to year. Unrecorded trade of bulbs and oil extracted from the seeds occur to African community in North America and Europe.

Uses

Citrullus lanatus comprises overlapping group of cultivars that yield seeds or edible fruits. Fruits of the wild or semi-wild plants are used in the Kalahari region as a source of drinking water, the same use is practiced in western part of Sudan during seasons of drought (van der Vossen. *et al.* 2004).

Most important in Africa are cultivars of which the only edible portion is seeds, the fruit pulp of these cultivars is too bitter for human consumption. In western Africa the seeds kernel are made into paste and added as thickener to soups (Schipper 2002), they are also fermented to produce sweetener, or they are roasted, pounded, wrapped in leaves and then boiled to produce another kind of sweetener. The pulp of roasted and salted seeds is eaten in Sudan and Egypt where it is called tesali. In the far northern part of Sudan seeds of some types are eaten whole including the seed coat (van der Vossen. *et al.* 2004). In Nigeria the seeds are boiled in salted water, or the roasted seed are ground and added to tsamma meal.

A highly prized vegetable oil is extracted from the seeds; this oil is used for cooking and cosmetics purposes and of interest to pharmaceutical industries. The residue from the oil extraction is made into balls that are fried to produce local snack in Nigeria, or is used as cattle feed. In Cameroon, dehulling (removing seed coats) is an important income generating activity, for those who cannot afford the product themselves (Schipper 2002).

Agronomy

Citrullus can grow in a wide variety of soil types but sandy, free draining soils are the preferred ones (Schipper 2002). In places where the water can not drain freely, diseases often become problematic. Before sowing, 200 kg 15-15-15, N-P-K compound fertilizer/ha should be applied, farmers usually sow 3-5

seeds/hole and retain the strongest plant. Spacing differs depending on variety with 75x100 cm for single plants, to 2x2m for one or two plants retained per station.

In Sudan watermelon grows in different types of soil, but commercial production is mainly concentrate in irrigated scheme and besides the rivers. In irrigated schemes soil should be raised in flats and the holes placed 20 cm from the ridge. Spacing is 50- 100 cm between holes. In flood lands it is not possible to raise flats, so seeds are placed in holes distance between plants is 200 cm and the seeds are in depth of 30 cm. Nitrogen fertilizer applied twice. First dose is after 30 days from sowing and the second dose after 30 days from the first one (Basheer 1995).

Seeds germinate after 4-7 days. The first flower can be expected after about 4 weeks and before the ground has been fully covered by the crop. Weeding is needed at an early stage until the crop covers the surface of the soil, when further weeding may damage the crop. Depending on temperature and the varieties, fruits become ripe 3-4 months after sowing. Ripe fruit can be recognized by their brown fruit stalks, changing of the outer skin color, the dull noise made when tapping the fruit by finger and dryness of the tendril opposite to fruit peduncle (Basheer 1995; Schipper 2002).

Excessive rainfall and high humidity give excessive vegetative growth and promote diseases infection, mainly leaf and fruit rot. 6-7 soil pH is adequate, at lower pH values soil born diseases *(Fusarium)* become a serious problem. A moderately rich soil is required for early and close vegetative cover, which is suitable to control weeds and erosion (Schipper 2002).

Genetic resources of the crop

Centers of origin and centers of diversity

Watermelon originated in southern Africa. It is growing wild throughout the area (Namibia, Botswana, Zimbabwe, Mozambique, Zambia and Malawi) and reaches its maximum diversity. The natives in Kalahari Desert region knew of sweet as well as bitter forms growing throughout the area, which is considered an evidence to prove that the species is indigenous to tropical Africa, more specifically the southern parts of Africa (Whenner 2005 c.f. De Candolle 1882). Southern Africa is a primary center of diversity, with wild relatives found in West Africa. China and India considered as secondary centers of diversity since diversity of related species occur in the area, in addition to areas of Middle East and Mediterranean (Bates and Robinson 1995).

Geographic distribution and domestication

Following first domestication of *Citrullus lanatus* in Southern Africa in prehistoric times, its cultivation became wide spread in the Mediterranean Africa. It was spread in the Middle East and West Asia for more than 3000 years ago. Introduction into India have occurred in ancient times and strong secondary center of diversity was developed there. *Citrullus lanatus* reaches China around the 10th century and was introduced into Europe during the 13th century, Japan in the 16th century; and it was introduced to America in the 17th century (Jeffrey 1975, Whitaker and Davis 1962).

Watermelon is thought to have been domesticated at least 4000 years ago, the plant was grown as a crop in the Nile Valley since the second millennium B.C (Bates and Robinson 1995). *Citrullus colosynthis* is considered to be the wild ancestor of watermelon.

While the distribution pattern of the species and its wild relatives proposed that it is domesticated in southern Africa, archeological information provides new probability (Bisognin 2002). The species was cultivated in the Nile Valley when farming was not practiced in South West Africa (Zohary and Hopf 2000). Seeds of C*itrullus colosynthis* appear in several Egyptian, Libyan and Near Eastern sites. Thus the species was probably used by human there prior to its domestication in Southern Africa.

The domestication selection in cucurbitaceae was for fruit shape, less bitter flesh, larger and fewer seeds and larger fruit sizes. This selection resulted in high genetic diversity within and among cultivated species. Breeding history indicates how artificial selection speed up changes in fruit characteristic to attend specific uses and increase adaptation to a variety of environmental conditions in which cucurbitaceae is growing world wide (Bisognin 2002).

Genetic erosion

The continuing adaptive changes and development in agriculture has always been associated with genetic erosion – the loss of formerly favored crop varieties or genes and alleles; although there are varied reasons for the loss of genetic diversity, there is widespread agreement that one major reason is replacement of local crop varieties by new cultivars. At present, few quantitative data exist to define the extent and rate of genetic erosion of crops and their wild relatives (Ceccarelli *et al.* 1992). Farmers in traditional system will routinely and intentionally discard component of local crop varieties as normal part of their management practices (Wood and Lenné 1997).

The report state of plant genetic resources (FAO 1996) also list number of other causes of such genetic erosion, including:

- Change in the agricultural system and the abandonment of traditional crops in favor of new ones;
- Overgrazing and excessive harvesting;
- Deforestation and clearance, which is cited as being the most frequent cause of genetic erosion in Africa;
- Adverse environmental condition such as drought and flooding;
- The introduction of new pest and diseases;
- Population pressure and urbanization;
- War and civil strife, and
- Policy legislation (for example until recently the cultivation of farm landraces was discouraged in Europe).

Western Sudan is considered rich in crop genetic resources, especially for watermelon germplasm. Unfortunately great loss is caused by desertification and the armed civil conflict especially in Darfur region. For a period of time there is no regular agricultural production, due to displacement of the local inhabitants and destabilizing their whole life patterns including crop cultivation and animal rearing activities. The same effect could be realized in the southern part of Sudan where the civil war continued for more than 20 years, although there is peace agreement now but still there are no accurate surveys to measure the loss or the presence of genetic diversity.

Migrations of inhabitants from rural areas to cities and big towns due to insecurity and / or economical factors and consequence abandoning of farming and shifting to other jobs, has been negatively affecting the original agrobiodiversity used to be utilized and conserved by the local people. In addition the new development and constructions in the Northern Sudan (dams) and in the Western Sudan (oil exploration fields), which disturb the life pattern of the local people and hence has negative impact on the agrobiodiversity.

In the Northern region of Sudan where the old horticultural practices are applied and the search by farmers for superior cultivars led to an early and continuous introduction of exotic materials, accordingly great loss has resulted in watermelon local types and other vegetables grown in the area. While in Kordofan region the case is different, the local types are gaining economic value in the markets for its seeds and edible fruits. More over the exotic cultivars in these regions are susceptible to fruit cracking, while the local types are crack – resistance (Hassan *et al*, 1984).

Characterization

Definition

Characterization can be defined by recording descriptors which are highly heritable, can easily seen by eyes and can expressed in all environments (Painting *et al.*1995). There are main four subcategories of characters: morphological; botanical; agronomical and chemical characters (Simpson and Withers 1986). These characters can be recorded on plants or their products (for example seeds) grown only in one environment. For this reason characterization may begin during exploration and collection and continue in the laboratory before or after multiplication (Perrino *et al.*1991).

Characterization is always linked with evaluation; however evaluation can be defined as recording characters which are susceptible to environmental differences (fruit yield, drought susceptibility) and usually controlled by one or more genes and estimated to be important for breeding programs or for direct use, but usually have strong genotypic- environmental interaction (Perrino *et al.*1991). In practice when an accession is being characterized, a limited number of evaluation characters are recorded at the same time (so called preliminary evaluation characters). These are generally straight forward to record and are of general interest to user of a particular crop.

Characterization can be classified as the third stage of genebank operations. The first stage concern with exploration and collection, the second stage bay more attention to improve multiplication technique and storage capacity. On the third stage emphasis placed on characterization, evaluation and documentation because distribution and utilization activities which constitute the fourth stage depend on a thorough knowledge of the value of the materials in the collection (Perrino *et al.* 1991).

A number of different procedures are used for characterization and preliminary evaluation depending on the species. The procedures vary according to operation and labor intensity. The characterization or preliminary evaluation can be performed at the same time with regeneration or multiplication, which is not the same case with evaluation because different environment are required to carry out evaluation process (Simpson and Withers 1986).

Constrains to characterization and evaluation

The International Board for Plant Genetic Resources (IPGRI) suggested three main categories of evaluation data: characterization of morphological and

agronomic descriptors of high hereditability; preliminary evaluation on special agronomic characters and full or secondary evaluation (several useful characters). From these three categories morphological descriptors are the most used ones by genebanks, however in the global scale, breeders and general user were not making sufficient use of the plant genetic resources maintained in genebanks. A survey showed that the reason why breeders were not making more use of the data and the materials from genebanks was that the important information required was not always available. According to Hawkes (1985) the information they sought was in order of priorities.

- Pest and disease resistance data,

- Stress tolerance and adaptation information,

- Information on maturity,

- Plant height measurements,

- Any rare or interesting data,

- Some morphological data,

Although crop breeding has different objective according to the species and to the knowledge of its genetic system (the higher the genetic knowledge, the more sophisticated the breeding approach). The priorities listed above indicate clearly the importance of information on biotic and a biotic stress resistance and on qualitative traits.

Purposes of characterization and evaluation

Characterization and evaluation work in genebanks have four main objectives:

- To study genetic variability of certain characters in relation to their geographical distribution in order to develop new and more adequate collecting strategies for further collection of useful germplasm in the same or similar areas.

- To study genetic variability present in the collections, especially within samples, and develop the most appropriate technique and strategies for maintaining the genetic integrity of such diversity; studies on genetic drift and the physiology of seed aging are the most appropriate.

- To widen genetic diversity of crop through intra-specific and inter-generic hybridization and mutation; other engineering technique maybe of interest.

- To screen the collections for traits which from time to time are considered important for breeding programs aiming to improve agriculture in a given country, regions or geographical area; this include studies on seed quality, resistant to diseases and best and adaptation to adverse soil conditions.

The role of passport data

Passport data are one of a large set of data sets and data sources used by genebanks, it comprises the information about the site and the environment from which an accession originated; accurate passport data including site description are useful as bases for correlating the origin with environmental parameter (Hawtin and Colin 1999).

Passport data provide collection curators and users with convenient ways to group collection materials into logical and more manageable units. In addition it provides linkage between genebanks and the external data sources. For example, taxonomic passport data can link genebanks data to a large variety of biological data sources. The combination of genebank data with external data sources adds considerable depth to biodiversity studies upon which genebanks and their user's community base strategy for conservation and use (Hazekamp 2002).

Passport data are used by genebanks for a variety of tasks mainly three tasks involve direct use of passport data

- Germplasm collection,

- Germplasm management,

- Germplasm use,

An ecogeographic survey is commonly used as the bases for a conservation strategy. Passport data of existing collections (both genebank and herbaria) are an important source of information since they help to establish the historical occurrence of the species in particular areas and the sampling it has been subject to in the past.

A detailed knowledge of the eco-geographical distribution of the target taxon helps to identify potential collecting site. With link to GIS and molecular marker technology a significant contribution can be made to development of more effective collecting procedure as part of the overall conservation strategy. In another type of studies, Green et al, (1999) showed that as a part of the evaluation of the newly collected germplasm, the passport data in combination with GIS were successfully used to help curator with post collection decision, particularly which of the newly acquired samples to include in the genebank collection.

Passport data with descriptors such as species name, geographical origin and ecological descriptors enables genebanks to make linkage to external data sources such as standard reference systems for scientific names or eco-geographical coordinates, which is originated from other biological or eco-geographical discipline.

World wide, 50% of the germplasm collections consist of duplicated accessions. Eliminating this type of duplication has often been suggested as a way to reduce the cost associated with the operation of genebanks. Methodologies based on passport data have been used to assist in the identification of replicated germplasm collections. Van Hintum and Knupffer (1995) concluded that passport data were useful in giving a first indication of probable duplicates. But in a very few cases would lead to direct and definitive identification of duplicates. In most cases definitive verification require additional data from field observations and genetic marker.

Due to extensive collections that have taken place during 1970-1980, large a mount of the materials in the collections were not introduces to characterization and evaluation process, simply because resources is not available. Hence users have to use more indirect criteria to select germplasm from the collection. The geographic origin of an accession is regarded as a very useful criterion. This is based on the understanding that plant populations are adapted to local conditions and that accessions from similar geographical origin probably share a larger part of their genetic makeup than more distant accessions. However some care should be taken in using eco-geographical factors as predictors of the pattern of diversity.

Another development that needs mentioning in relation to enhancing the use of germplasm collections is the development of core collection. Different methodologies exist to designate accessions to a core collection. In general a mixture of passport, characterization and evaluation data are used to determine whether an accession should be part of the core set. Never the less passport data and particularly the geographical origin, are usually one of the first criteria used to determine selections, however the methodologies to designate a core

collection are still relatively new and experience with the use of core collection is limited.

The role of molecular markers

The study of morphoagronomic 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. However with molecular markers techniques, powerful tools have been developed so that genetic resources can be accurately assessed and characterized.

Molecular markers are efficient because various and numerous points can be accessed along the genome and also because of their variable nature. It is therefore possible to study nuclear chloroplast or mitochondrial DNA and to conduct different types of evolutionary studies. Such multiple and complementary information will subsequently enhance the value of genetic resources.

One major application of molecular markers is for monitoring plant materials and assisting in management of the germplasm collections. One objective is to determine the breadth of the genetic base of germplasm collections used by breeders using molecular marker analysis. These markers are suitable for assessing how much allelic diversity is present in a crop and they have the potential for providing unique fingerprinting for each genetically distinct genotype, a useful means of identifying different cultivars.

An assessment of genetic diversity based only on morphoagronomic traits might be biased, because distinct morphotypes can results from few mutations and share a common genetic base, as has been demonstrated in coca "distinct morphotypes has similar genetic background"; the opposite also may be true these is demonstrated by sorghum "cultivars grouped together according to morphotypes, isozymes studies revealed genetic differentiation between them".

Core collection is strategy aims to organize germplasm collections so that potential of accessions are fully characterized and be useful for breeders. It is also an approach used by curators to organize the diversity and variability existing within these collections. Molecular markers are essential for exploiting whether existing genetic variability, which is assessed by measuring morphoagronomic traits, related to genetic diversity, which is assessed by measuring allelic frequencies using molecular marker. This information can be used to construct core collections.

Diversity studies provide useful information for breeders about genetic relationships and distances between individuals. Molecular markers can exploit the hidden diversity to obtain the benefit from the potential of each species. Therefore molecular markers provide classification of cultivars which allows better use of the genetic resources of the species.

Materials and Methods

Sources of materials

A number of Altogether 30 accessions were chosen from the stock of watermelon germplasm conserved in the Plant Genetic Resources unit for this study. These accessions were original materials collected from six different regions in Sudan representing the western, northern and central parts of the country (Table.1).

Table (1).Sources of watermelon germplasm

No	Acc Number	Collection Site	province
1	HSD 0196	Nyala	Darfur
2	HSD 0320	Nyala	Darfur
3	HSD 1535	Wadaah	Darfur
4	HSD 4014	Abukarainka	Darfur
5	HSD 4013	Aldaine	Darfur
6	HSD 0318	Zalingi	Darfur
7	HSD 3306	Sharafa	Gezira
8	HSD 0185	El Obeid	N. Kordofan
9	HSD 3016	Kabur	N. Kordofan
10	HSD 3021	Kabur	N. Kordofan
11	HSD 3655	Abu Heraz	N. Kordofan
12	HSD 5086	Merawi	Northern state
13	HSD 5097	Tengesi	Northern State
14	HSD 0164	Abu Gebaiha	S. Kordofan
15	HSD 0165	Abu kersheola	S. Kordofan
16	HSD 0172	Rashad	S. Kordofan
17	HSD 0182	Rashad	S. Kordofan
18	HSD 4454	Alsandara	S. Kordofan
19	HSD 4455	Um Fakrain	S. Kordofan
20	HSD 6088	Awlad Younis	S. Kordofan
21	HSD 6089	Kojoria	S. Kordofan
22	HSD 3037	Um kaib	W. Kordofan
23	HSD 3039	Um khairan	W. Kordofan
24	HSD 3047	Almigaisim	W. Kordofan
25	HSD 3053	Aial Bekheet	W. Kordofan
26	HSD 3065	Alnihood	W. Kordofan
27	HSD 3079	Gibaish	W. Kordofan
28	HSD 3354	Alnihood	W. Kordofan

Site and location of the experiment

The study was conducted in the Gezira research station farm of Agricultural Research Corporation-Wad Medani, Sudan. Wad Medani is located at latitude 14:24° N, longitude 33:29° E, and altitude 406.9 m (above see level). The climate of the area is hot, semi arid. The soil is vitriol-soil with clay content ranging between 40 to 65 %, pH value ranging between 8 and 9.6, with less than 1% organic carbon, 300 ppm total nitrogen and 406 to 700 ppm total phosphorus (Ishag and Said 1985).

Land preparation and cultivation practices

The land was prepared for sowing by deep ploughing using disk plough, harrowing using disc harrow and leveling. Flats were raised 7 meter in length and 3 meter in width. Materials were sown on 2nd August 2006, on one side of the flat, each accession represented by two flats, 10 plants per flat, with a spacing of 50 cm between plants. About three seeds were placed in each hole and they were thinned during the 4th week to one plant.

Weeding and raising of ridges were carried out regularly, these two processes started one week after germination and continued prior to irrigations. Intervals between irrigations were ranged between 7-10 days and slightly more in case of rainfall. Nitrogen fertilizer was applied twice in form of urea 46% at the rate of 1 N (50 kg /Feddan)[1], first dose was applied 30 days after sowing date and the second dose was 30 days later.

The plants were highly infested by *Aphis gossypii* during the whole season. There was also an infestation by cucurbit red bug, which caused damage of the shoot tips of the plants. Treatments included Folimat 50 or Mossiplan at severe infestations, and were made by the Plant Protection Unit. Fungicide protective spray was conducted for powdery mildew and other expected fungal infestations.

Harvesting of fruits and extraction of seeds

Indicators for fruits ripening were described by:

- Dryness of the tendril opposite to peduncle,

- Change of the color of the outer skin of the fruit, the part contacting the soil from white to yellow.

- The hollow sound made by tapping the fruit with finger.

The first harvest took place 3 months after planting, and the harvested fruits were labeled by the entry number, plant number, fruit number and harvesting date.

[1] Agricultural land measuring unit in Sudan it is equal to 4200 m²

Seeds were extracted manually from the fruit flesh. They were left in a pot to the next day in order to ferment, after which they were cleaned, washed and spread in a wire- netted sieves and left to dry in the room temperature. After drying, which took 1-3 days; they were packed in paper envelopes and left at room temperature for further drying.

Genotype characterization

Development of a descriptor list

Characterization data were collected using a descriptor list locally developed for watermelon. This descriptor list was developed by the Plant Genetic Resources unit-Sudan, by consultation with other scientists/specialists and by searching in literature. It was continuously improved during the course of many studies and specially when practicing characterization in the field and in the laboratory. This descriptor list constituted a number of 35 discrete and continuous characters, involving different vegetative, inflorescence, fruit and seed characters.

Component of the descriptor list

The descriptor list used for data collection constitutes of 27 qualitative characters and 8 quantitative characters these characters were measured as in table (2).

Data collection

Characterization data was collected at two different growth stages:

- Vegetative and inflorescence characters were recorded in the field immediately when flowering and during plant development until fruit ripening. For leaf characters middle leaves in the plant were examined.

- Fruit and seed characters were recorded after fruit harvesting and seed extraction in the laboratory.

Data on qualitative descriptors were recorded after consulting a panel of two or three persons, while quantitative data were recorded using the measuring devices as required.

Table (2) Measuring methods for qualitative and quantitative characters

Qualitative characters	
Descriptor state	**Measuring method**
Leaf shape	Three figures were used to record this descriptor
Leaf size	Three categories were used (small≤7cm, medium≥7-10cm, large>10cm)
Leaf color	Five colors were determined to specify leaf color
Leaf pubescence density	Determined using figures
Leaf pubescence texture	Determined using finger tips
Internode length	Three categories were used for the length between the third and the fourth node(short ≤5 cm, intermediate ≥5-8 cm, long >8 cm)
Stem pubescence density	Determined using figures
Stem pubescence texture	Determined using finger tips
Plant canopy coverage	Described by fruit exposure or coverage within plant canopy.
Sex type	Determined on male and female flowers
Flower color and flower color intensity	Using horticultural color chart (Wilson, 1938)
Flower size	Three categories were used for corolla diameter (small ≤ 2 cm, medium≥ 2-3 cm, large>3 cm) for male and female flower
Ovary length	Categorized as (short ≤ 1 cm, medium≥1-2cm, long >2cm)
Ovary pubescence	Using finger tips
Fruit shape	Three shapes were determined to specify fruit shape
Primary skin color	Three color were used to determined primary skin color
Secondary skin color	Two colors were used to determine secondary skin color
Design produced by secondary skin color	Determined using figures
Flesh color	Horticultural color chart
Flesh texture	Pressing and touching the flesh by fingers
Seed size	Categorized as (small ≤0.7 cm, medium ≥0.7-1 cm, large >1 cm)
Dominant seed color	Determined using range of colors
Secondary seed color	Determined using range of color
Quantitative characters	
Fruit diameter, fruit length and rind thickness	Measured in cm
Total soluble solid (TSS)	Determined using hand held refractometer
Fruit weight	Determined in kilograms by using balance
Days to 50%flowering	Days from sowing till 50% of plants in the entry showed at least one open male flower
Seeds number /kg fruit	Seed number per fruit divided by fruit weight
Hundred seed weight	Measured using sensitive balance

Data analysis

Quantitative and qualitative variables were scored on an individual plant bases. For quantitative characters mean value was calculated for each descriptor state in an accession. For qualitative characters presence of a descriptor state in an accession considered as homogeneous and presence of two or more descriptor state in an accession considered as heterogeneous.

Frequencies of occurrence were calculated for each descriptor state for the qualitative characters. The occurrence level of each descriptor was categorized as very rare, rare, medium, common and abundant.

Descriptive statistics (mean value, minimum and maximum, standard deviation and coefficient of variation) were calculated for the quantitative characters in order to compare the level of phenotypic variation.

Qualitative data was in nominal scale and numerically coded (painting *et al* 1995). The scale is usually ranged from (1-9), a series of pre-defined descriptor state. for example for leaf color descriptor state, number 3 used to refer to the green color, number 5 used to refer to the dark green color,… etc. the data matrix of numerical codes for qualitative character and the quantitative characters is used for principle component and cluster analysis. Principle component analysis (PCA) was used to identify characteristic that is contributed significantly to the variability among accessions on the bases of the weight or the loading assigned to the original variables.

Cluster analysis, a mathematical method for displaying the similarity or differences between pairs of subjects in a set, was conducted for grouping genotypes that showed similarity in several traits or responding pattern. The standardized data matrix of qualitative and quantitative characters was used to generate similarity indices based on Euclidian distances. Clustering was carried out using hierarchal analysis from GENSTAT.

DNA characterization

DNA extraction procedure

A procedure developed by Gusmini and Whenner (2005), for isolation of genomic DNA from watermelon leaves was followed. Young leaves were collected from the field. The leaves were washed and dried at room temperature; dried leaves were ground using pistil and mortar to a fine powdered tissue. Small quantities of these products were transferred to 1.5

ml eppendorf tube. 700µm of hot CTAB extraction buffer was added to the powdered tissue and vortexed thoroughly. The samples were incubated at 65° C for 15 minutes and shaken 3-5 times. The homogenate was extracted with an equal volume of chlorophorm: Isoamyle (24:1), and 3µm RNaseA (10mg/ml) was added. The mixture was shaken for 5 minutes and centrifuged at 13.000 rpm for 10 minutes to separate phases. The upper supernatant layer containing DNA was transferred to a fresh 1.5 ml tube. DNA was precipitated with cold 400 ml isopropanol for 5 minute and centrifuged for 10 minute at 13.000 rpm. The supernatant was poured and the pellet was suspended in 500µm of 70% ethanol. The tubes were centrifuged for 10 minutes at 13.000 rpm. The supernatant was poured and the pellet was dried in at the room temperature, and it was suspended in 100µm TE. The extracted DNA was stored at 4º C, its concentration and purity measured using spectrophotometer.

Primers selection

Nineteen Single Sequence Repeat (SSR) primers were evaluated by Guerra, (2002), for their ability to detect polymorphisms within the genus *Citrullus*. For the purpose of this study 9 of these 19 primers were selected to study the diversity within the genus *Citrullus*, in addition to 11 RAPD primers which have been used in similar studies. Table (3) showed the primers used and their products.

PCR amplification

PCR reactions of 20 µm contained 2 µm of reaction buffer, 0.4 µm Taq polymerase enzyme, 0.5 µm dNTPs, 0.5 µm primer, 1 µm DNA template and 15.1 of double distilled water. Amplification was carried out using a touch down program, with a profile as follow:

1. Initial denaturation at 94 ºC for 5 minutes,
2. Denaturation at 94 ºC for 30 seconds,
3. Annealing at Tm of the specific primer for 30 seconds
4. Extension at 72 ºC for 1 minute**,**
5. Step 2 and 3 repeated for14 cycle,
6. Extension at 72 ºC for 1 minute,
7. Step 2 repeated for 29 cycles,
8. Extension at 72 ºC for 5 minute.

This program was applied for both SSR and RAPD analysis with changes in annealing temperature according to each primers needs.

PCR Products were separated by gel electrophoresis on 2% agaros gel in TAE buffer for the RAPD products, while SSR products were separated in 2% metaphor agaros gel in TBE buffer and visualized after staining in ethidium bromide (0.1 μg/ml), using a gel documentation system.

Data analysis

The amplification products were scored as 0 or 1 for absence or presence of bands, based on this scores a data matrix was constructed and analyzed using the unweighted pair-group method with arithmetic average (UPGMA) clustering method from the NTSYSpc software, a dendrogram was constructed to analyze the degree of similarity of the studied accessions.

Table (3) primers used and their products

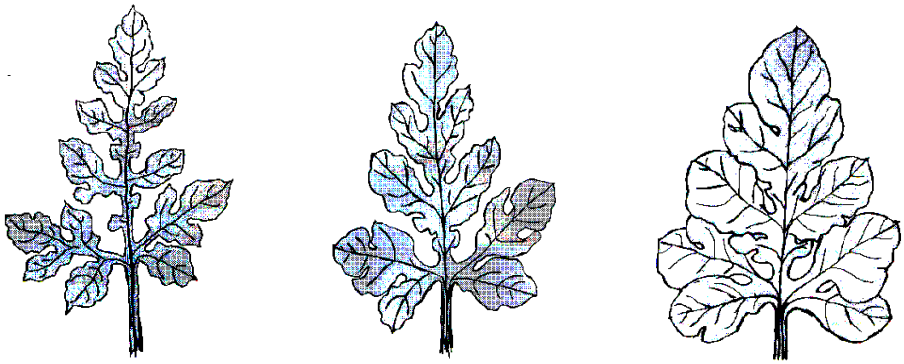
	SSR primers	Number of products	RAPD primers	Number of products
1	AF 2880421.1	2	UBC155	6
2	AB018561	2	A04	7
3	EST00691	3	A02	9
4	EST00507	2	A03	7
5	X04130.1	3	A012	3
6	AF008925.1	3	A01	4
7	D28777.1	3	A017	9
8	EST 00612	3	A05	8
9	EST00674	2	UBC222	7
10			UBC157	4
11			A06	5

Results

Qualitative characters

Distribution of frequencies (table 6- Appendix 1) was based on frequencies of occurrence (table 5- Apendix1). Clear variation was observed in the level of occurrence of the different descriptor state between the characterized entries.

Majority of the accessions (67%) had a medium lobed lamina area which is referred to as leaf shape (2), while (7%) of the accessions had either deeply lobed lamina area (leaf shape (1)) or slightly lobed lamina area (leaf shape (3)) (figure 1). The remaining percentage of the accessions (17%) expressed mixture of leaf shapes and was considered as heterogeneous accessions. Leaf size is commonly large (90%) or very rarely medium (3%). There was no small leaf size recorded.



Leaf shape (1) Leaf shape (2) Leaf shape (3)

Figure (1) different leaf shape of watermelon

Female flowers were commonly large or medium sized (47% and 27% respectively), or rarely small sized (10%). Male flowers were commonly medium sized (60%) or rarely small (10%) or large sized (13%). Sex type varied among accessions between medium for monoecious types (13%) and common for andromonoecious types (83%). There was no variation within or between accessions with respect to the number of female flowers per axle (i.e. 1) or flower color (i.e. sulphary yellow).

The high percentage of heterogeneity was calculated for fruit and seed characters and consequently some descriptor state were disappeared in the homogeneity's level even though they were recorded when characterization was based on individual plants. Figure(2) illustrates the difference between homogenous and heterogeneous traits.

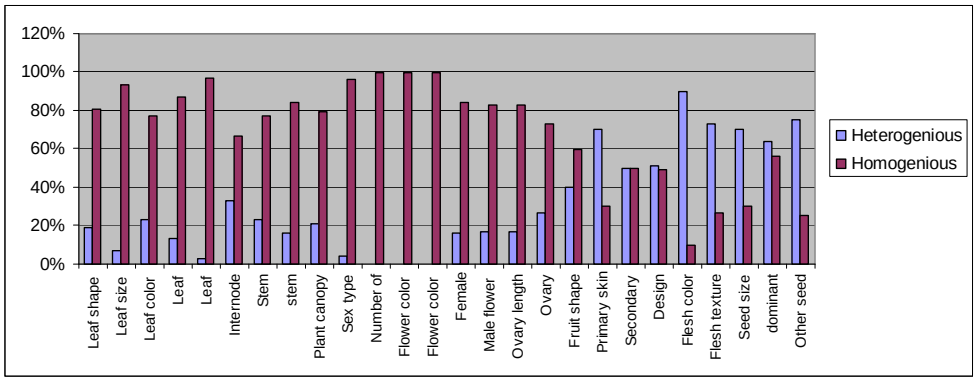


Figure (2) Comparison between homogeneity and heterogeneity within traits

Quantitative characters

Statistical summary of quantitative characterization data are presented in table (7). It was observed that the highest coefficient of variation was recorded for fruit weight (48.17%), followed by seed number /kg fruit (47.07%). Rind thickness (38.75%) and TSS (37.57%) were relatively high. Medium coefficient of variation was recorded for fruit length (20.16%), 100 seed weight (21.90%) and fruit diameter (17.74%), the lowest coefficient of variation was recorded for days to harvest (5.03%) and days to 50% flowering (5.1%).

Table (7) standard deviation and coefficient of variation for quantitative characters

Descriptor	Min	Max	Mean	Standard deviation	Coefficient of variation
Days to 50% flowering	62	73	66.37	3.43	5.71
Days to harvest	131.58	133.12	132.20	6.66	5.03
Fruit diameter(cm)	12.14	12.59	12.45	2.21	17.74
Fruit length (cm)	12.66	13.22	12.94	3.38	26.16
Rind thickness(cm)	0.76	0.83	0.79	0.31	38.75
Total soluble solid (TSS)	3.86	4.14	4.00	1.50	37.57
Fruit weight(kg)	1.16	1.28	1.21	0.58	48.17
Number of eeds/kg	375.34	440.83	395.81	186.29	47.07
100 seed weight	6.90	7.25	7.10	1.55	21.90

The first principle component (PC1) explained 65.50% of the total variation and was primarily accounted for by fruit flesh color. The second principle component (PC2) explained another 34.39% of the variation between accessions and was accounted for by the number of seeds/kg. Traits which

are responsible of most of the variation in the first and the second principle component are presented in the table (8)

Table (8) Character with high loading value in the first and the second PC

Percentage variation	PC1	PC2
	65.50	34.39
Loading value		
Days to 50% flowering	0.0025	-0.00239
Flesh color	0.00269	0.00238
Fruit length	-0.00057	-0.01279
Fruit diameter	0.00235	-0.01279
Seed No/kg	-0.68696	0.72615
100 seed weight	0.00258	-0.00239

A dendrogram was constructed from the similarity matrix of numerical and quantitative data (figure 3). The similarity coefficient for the 28 accessions was 60%. The dendrogram distinguished between 4 main distinct groups. The first group was characterized by 88% similarity within the group members and consist of cultivated accessions collected mainly from Western part of Sudan represent by Kordofan region (North, West, South) and Darfur region (North, West, South). This group could be further divided into 3 subgroups (Ia- Ib- Ic). The second group (II) was account for 95% similarity between the group members. It consists of two accessions their status classified as wild and they were collected from the Northern region. Group (III) represented by one accession from Darfur region group (IV) represented by one accession from the central region, its status was classified as wild.

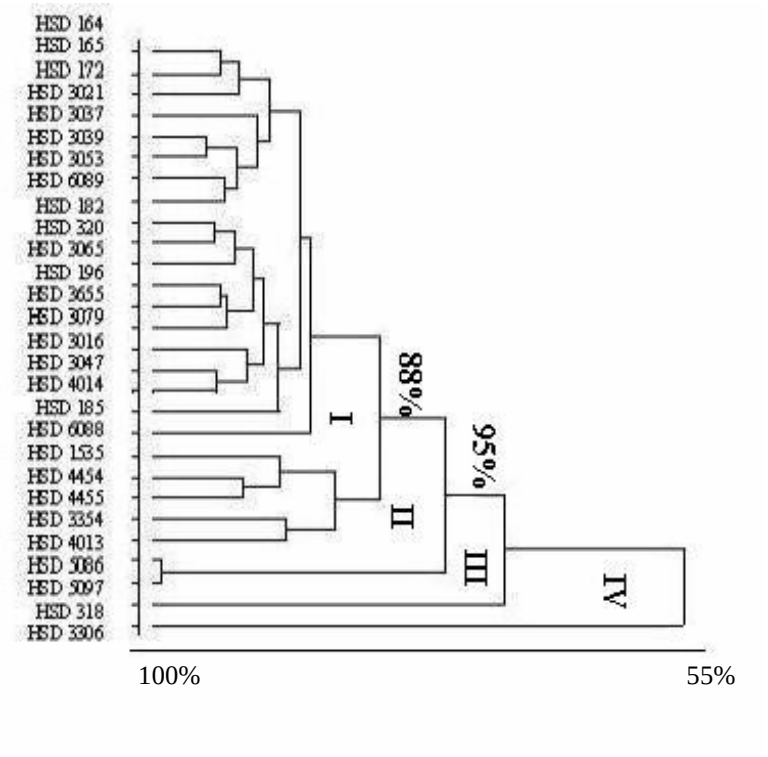


Figure (3) cluster analysis for morphological characters

Molecular characterization

Molecular characterization data was plotted in a dendrogram figure (6). The products of the SSR markers and RAPD markers (figure 4, 5) were plotted in on matrix. Primers used and numbers of product for each primer were shown in table (4). The similarity coefficient for the studied accession was (71%). Two distinct groups were observed. One of them can be further divided into 3 subgroups. The grouping seems not to be based on the origin of collection. However paired of accessions from the same origin appear to be closely related to each other. It could be realized that there is no similarity between the molecular and morphological grouping.

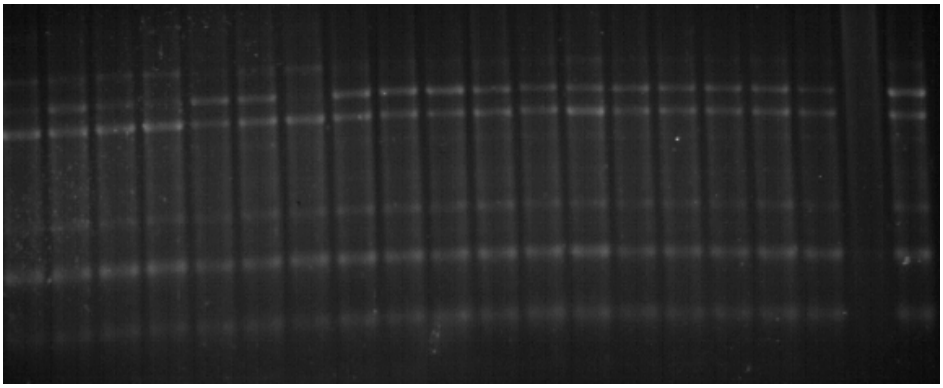


Figure (4) RAPD products

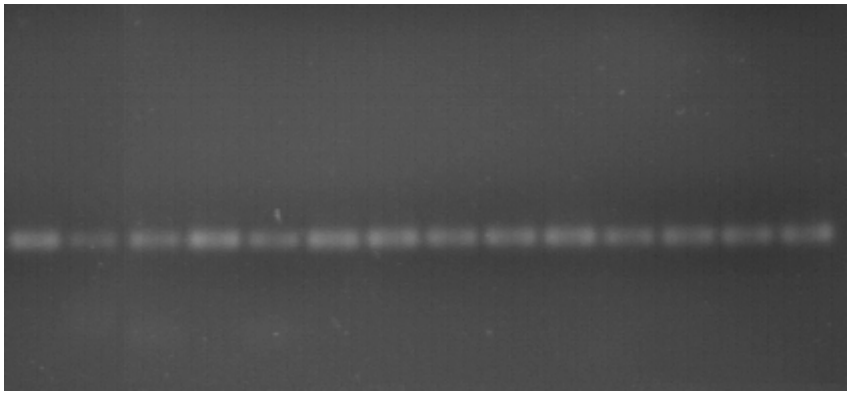


Figure (5) SSR product

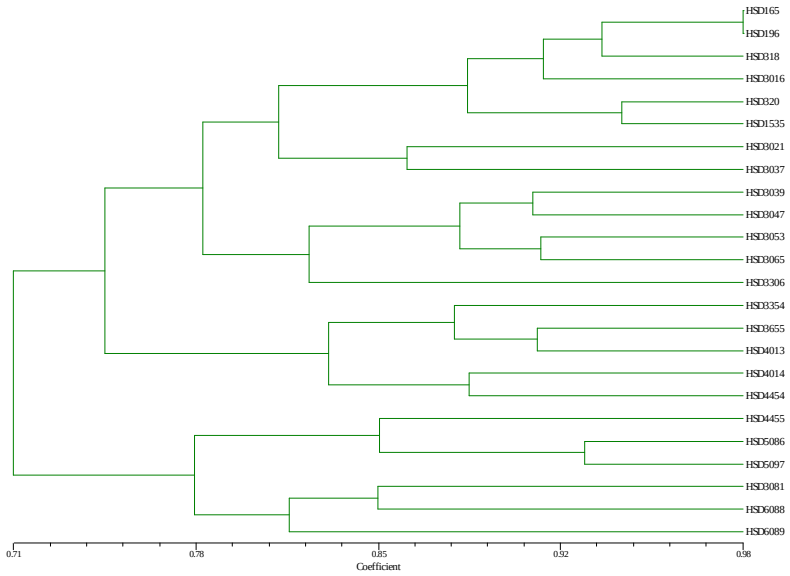


Figure (6) Dendrogram based on SSR and RAPD products

Discussion

The results obtained from this study showed that there is phenotypic variation in the local watermelon germplasm collected and conserved in Sudan (Appendix 2). Such phenotypic variation was evident for both qualitative and quantitative characters.

Three leaf shapes were recognized in the studied materials. Leaf shape (1) is deeply lobed and divided into small segments resulting in small lamina area, the dominant leaf shape (2), is medium lobed and has medium lamina area and leaf shape (3) which is slightly lobed with wide lamina area. Leaf shape (1) is expected to have more tolerance to drought than leaf shape (2) and (3), probably leaf shape has an effect on microenvironment and drought tolerance, since transpiration is expected to be less in leaves with small lamina area. Maxwell and Jennings (1980) mentioned that color and shape of leaf plants may remotely affect host selection behavior of insect pests and have also been associated with resistance to some diseases.

Khalifa and Gameel (1982) in an effort to control white fly (*Bemisia tapasia*) in cotton plants, introduced a deeply lobed okra leaf shape. This resulted in open

canopy coverage to the medium stable cotton variety grown in Sudan. It could therefore, be worthwhile to explore if leaf shape (1) could be used for controlling Aphids and whitefly in Sudanese watermelon germplasm.

The leaf size character is highly associated with plant canopy coverage, so the larger the leaf size the better the canopy coverage. Good canopy coverage has an advantage in covering fruits from exposure to sun burn which may affect the flesh quality in terms of nutrient value (vitamins and minerals).

In the germplasm studied a considerable percentage was observed to have short internodes. Four decades ago, Mohr (1965) discovered a mutant with short internodes resulting in dwarf plants. Dwarf plants in watermelon provide good possibility for developing cultivars for garden culture. In addition to that the occurrence of short internodes gives potential for adaptation to mechanical harvesting (Robison and Decker- Walter, 1997).

In the present study, majority of the accessions were observed to be andromonoecious, indicating that all the materials studied were landraces or less developed cultivars. Mohr (1986) and Messiaen (1994) indicated that most of the recent/advanced cultivars are monoecious types while the older varieties were generally andromonoecious. From the under study accessions 30% were observed to be monoecious which is possibly due to out cross pollinating characteristic, thus it may be explained by out crossing with monoecious cultivars.

There was a high correlation between ovary length and fruit shape. Long ovaries mostly resulted in elongate fruits while short ovaries resulted in globular fruits. Majority of the accession studied were globular cultivars (57%). Round fruited cultivars tend to be (quite) susceptible to hollow heart. This phenomenon was not observed in the Sudanese germplasm. The globular watermelon varieties in Sudan were observed to have lower percentage of cracking and blossom end rot than the elongate ones (El Mekki, 1991).

A range of phenotypic variability in flesh color was also observed (90% heterogeneous). This may be explained by the selection criteria, which are related to the user's behavior; either as a source of water or for seeds extraction (van der Vossen, (2004) without much concern with flesh quality (color and TSS content). The same could be applied for watery and spongy flesh texture.

Range of seed sizes and colors were observed in the studied germplasm, Mohr (1986) reported that seeds of watermelon ranged in size from very small seed size (size of tomato seeds) to large seed sizes (size of pumpkin seeds). Production of white colored seeds as well as large and medium sized seeds

attract farmers recently specially in the western part of Sudan, since interest is directed to such types for commercial purposes (FAO, 2002).

The selection during domestication in watermelon was for the shape, larger or fewer seeds and large fruit size resulting in high diversity within and among the species. This variation was associated with a wide range of uses that require different shapes and sizes. In western part of Sudan, the main uses recorded were as a source of water and for seed production. Hence high variation was recorded for fruit weight and seed number/kg. Fruit weight is highly correlated with fruit diameter and fruit length, characters which were responsible for most of the variation in the first and in the second principle component

Site of collection had no great effect on the groups clustered in the dendrogram. However the first group (I) which constitutes majority of the accessions was mainly collected from the western region (Kordofan- Darfur). Accessions from this group gathered together regardless the collection site; and this may be due to the fact that the western parts of the country to some extent share the same climatic zones and most likely the same growing conditions. Farmer to farmer seed exchange may also assist in the distribution of the seeds from area to area, in addition to seed dispersal by movement of animal and humans while pasturing which is considered one of the major activities for the inhabitants. All the accessions in this group were cultivated materials. Three sub-groups could be recognized in further separation. The first and the second sub-groups (1a-1b) appear to be closely to each other, while the third subgroup (1c) is some how not closely related to them.

The second group (II) represented by two accessions was collected from the northern region. The morphological feature differentiating them is the orange mottled surface which according to Shipper (2002) resembles *Citrullus rehmii* De Winter. The third group (III) was represented by single accession collected from the Darfur region. This accession was different from the others by its monoecious flowers, its leaf shape, size and elongated fruit shape. The fourth group (IV) was also represented by an accession from the central region of the country; it is characterized by medium leaf size with globes small striped fruit with bitter taste, hard flesh and small black seeds. These characters possibly resemble either *Citrullus lanatus var. citroides* (Baily) Mansf. or *Citrullus colocynthis* (L) Schrad. Navot and Zamir (1987) suggest that *Citrullus lanatus var. citroide* is likely to be the wild ancestors of *Citrullus lanatus var. lanatus*. This maybe due to the fact that most of the alleles detected in the var. *lanatus* are also common in var. *citroide*. F. Dane and J. Liu (2006) suggested that cultivated watermelon *C. lanatus var. lanatus* and *C. lanatus var. citroide* probably have diverged into separate lineages and appear to have evolved independently from common ancestors.

The clusters obtained from the molecular characterization data showed a minimum similarity coefficient of 71% between the studied accessions. Two major groups diverged from the trunk of the tree and one of the two groups could be further divided into three subgroups. Grouping was not related to the origin of the accessions, however some branches did group closely and they were in general from the same origin. For example both HSD 5086 and HSD 5098 were from Northern part. HSD 320 and HSD 1535 were from Darfur region and HSD 3039 and 3047 were from western Kordofan and HSD 3053 and HSD 3065 were also from western Kordofan. Van Hintum and Knupffer 1995 concluded that passport data can be useful in giving indication of probable duplicates. Duplication encountered tended to be more within site of collection. Ofori et al. (2006) suggested that repeated collection within the same area without proper documentation could account for such a situation.

The reduction of diversity during crop evolution must have happened as early as with initial cultivation of plants. This is similarly maybe the case in watermelon evolution. Very little diversity was detected among cultivated watermelon accession. . Navot and Zamir (1987) relate the low level of isozyme polymorphism detected in *Citrullus lanatus var.lanatus* to the domestication process away from its centers of origin.

As found in this study molecular diversity need not to be directly associated with morphological diversity. This was similarly observed with other species (Petit, et al. 2002). Levi et al (2001), while analyzing polymorphism in five accessions of *Citrullus lanatus* var. *lanatus*, recognized a low level of polymorphism even though the cultivar studied represented a wide range of horticultural traits.

Conclusion

In literature, characters like leaf shape, leaf size and plant canopy coverage might be important with respect to insect resistance. In watermelon, however these characters need to be studied further study to test their validity. The morphological characterization of the studied germplasm provided knowledge about traits that might be valuable for plant breeders.

Traits occurring at rare and very rare frequency deserve attention during regeneration and multiplication, as they may easily be lost during such operations. Splitting the germplasm into homogenous lines could be a good strategy for saving valuable materials from complete loss and extinction.

An obvious effect of selection is the enlargement of the desired product, in this case the fruit. The size has been magnified considerably when compared to the small sub-globose fruits of the wild population. Seeds of the cultivated forms are substantially larger with range of varied colors.

The cluster obtained from morphological characterization gave an opportunity of separating the accessions into different morphotypes. Such separation will support genebank curator with information with regard to the appropriate site for collection and the proper methods for management Also the cluster obtained gives a sense of the relationships among accessions and can help the selection of parents needed to maintain adequate diversity in the breeding program. It can also help determine which varieties to evaluate in which localities, as information is available on morphotypes and their performance with regards to their origin.

Plant populations are adapted to local condition and those accessions from similar geographic origin probably share larger part of their genetic makeup than more distant accessions.

Grouping constructed from molecular marker data did not coincide with the grouping constructed from morphological data. However molecular markers may facilitate the finding of duplications. The low similarity coefficient calculated for the morphological and molecular cluster indicate the narrow genetic base for the genus *Citrullus*, which was evident in number of studies.

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Appendix 1. Frequency of occurrence

Table (4) frequency of occurrence (N: 28 accessions)

Descriptor	Descriptor state	Frequency %	Heterogeneity %
1-Leaf shape	Shape 1	7	19%
	Shape 2	67	
	Shape 3	7	
2- Leaf size	5- Medium	90	7%
	7- Large	3.3	
3-Leaf color	3- Light green	10	23%
	5- Green	60	
	7- Dark green	7	
4- Leaf pubescence density	3- Slight	70	13%
	5- Medium	10	
	7- Dense	7	
5- Leaf pubescence texture	3- Soft	3	3%
	5- Intermediate	87	
	7- Coarse	7	
6- Internode length	3- Short (=<5cm)	27	33%
	5- Intermediate (5-8cm)	40	
7- Stem pubescence density	3- Slight	47	23%
	5- Medium	23	
	7- Dense	7	
8- Stem pubescence texture	3- Soft	50	16%
	5- Medium	27	
	7- Coarse	7	
9- Plant canopy coverage	3- poor	13	21%
	5- Fair	23	
	7- Good (Vigorous)	43	
10- Sex type	3- Monoecious	13	4%
	5- Andromonoecious	83	
11- Number of female/ perfect flower per axil	1	100	0%
12- Flower color	Sulphery yellow	100	0%
13- Flower color intensity	1/2	86%	0%
	1/3	7%	
	1	7%	
14- Female flower size	3- Small (=>2cm)	10	16%
	5- Intermediate (2-3cm)	47	
	7- Large(<3cm)	2	
15- Male flower size	3- Small (=>2cm)	10	17%
	5- Intermediate (2-3cm)	60	
	7- Large(<3cm)	13	
16- Ovary length	3- Short (=<1cm)	70	17%
	5- Intermediate (1-2cm)	10	
	7- Long (>2cm)	3	
17- Ovary Pubescence	3- Slight	10	27%
	5- Medium	30	
	7- Dense	33	

Descriptor	Descriptor state	Frequency %	Heterogeneity %
18- Fruit shape	1- Globular	57	40%
	5- Elongate	3	
19- Primary skin color	3- Light green	20	70%
	5- Green	10	
	7- Dark green	3	
	9- Orange	7	
20- Secondary skin color	3- No	3	50%
	5- Green	7	
	7- Dark green	40	
21- Design produced by secondary color	1- No	3	51%
	3- Stripes	43	
	5- streaks	3	
22- Flesh color	White	26.4	90%
	Granium lake 20/3	3.3	
23- Flesh Texture	1- Watery	7	73%
	5- Spongy	13	
	7- Hard	7	
24- Seed size	3- Small(=>7mm)	10	70%
	5- Medium(7-10mm)	7	
	7- Large(<10mm)	13	
25- Dominant seed color	Black	10	64%
	Brown	3	
	Red	3	
	Tan	17	
26- Other seed color	Tan	3	75%
	Yellow	3	
	Brown	3	
	Light brown	3	
	No	13	

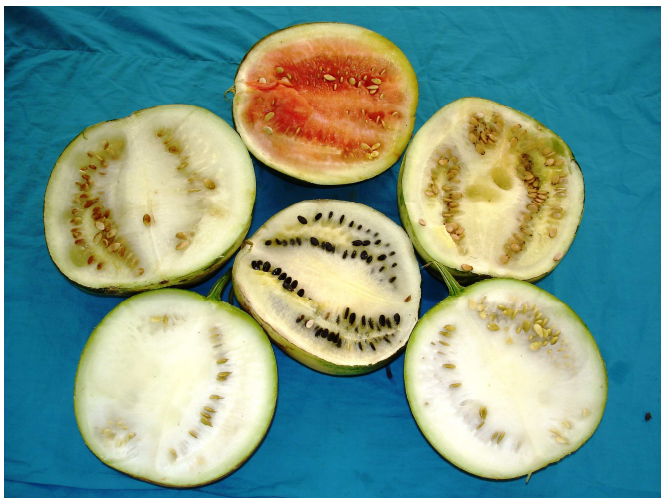
Table (6) between accessions distribution. Acc No (28)

Descriptor	**Descriptor state**					
	Homogeneous					**Heterogeneous**
	Very rare ≤5%	**Rare >5-20%**	**Medium >20-60%**	**common >60-90%**	**Abundant >90%**	
Leaf shape		Shape1 (7%) Shape 3 (7%)		Shape 2 (67%)		19%
Leaf size	Medium 3%			Large 90%		7%
Leaf color		Light green 10% Dark green 7%	Green 60%			23%
Leaf pubescence density		Medium 10% Dense 7%		Slight 70%		13%
Leaf pubescence texture	Soft 3%	Coarse 7%		Intermediate 87%		3%
Internode length			Short 27% Intermediate 40%			33%
Stem pubescence density		Dense 7%	Slight 47% Medium 23%			23%
Stem pubescence texture		Coarse 7%	Soft 50% Medium 27%			16%
Plant canopy coverage		Poor 13%	Fair 23% Good 43%			21%
Sex type			Monoecious 13%	Andromonoecious 83%		4%
Number of female flower per axil					100%	0%
Flower color					Sulphary yellow 100%	0%
Flower color intensity						
Female flower size		Small 10%	Medium 47% Large 27%			16%
Male flower size		Small 10% Large 13%	Medium 60%			17%
Ovary length	Long 3%	Medium 10%		Short 70%		17%

Ovary pubescence density		Slight 10%	Medium 30% Dense 33%			27%
Fruit shape	Elongate 3%		Globular 57%			40%
Primary skin color	Dark green 3%	Green 10% Light green 20% Orange 7%				70%
Secondary skin color	No secondary color 3%	Green 7%	Dark green 40%			50%
Design produced by secondary color	No design 3% Streak 3%		Stripes 43%			51%
Flesh color	Granium lake 20/3 , 3%	White 7%				90%
Flesh texture		Watery 7% Hard 7% Spongy 13%				73%
Seed size		Large 13% Medium 7% Small 10%				70%
Dominant seed color	Brown 3% Dark brown 3% Red 3%		Black 10% Tan 17%			64%
Other seed color	Brown 3% Light brown 3% Tan 3% Yellow 3%		No other seed color 13%			75%

Appendix 2. Variation in watermelon germplasm

Different fruit skin designs



Different flesh and seed color

Different leaf shape and color