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Full Length Research Paper

Morpho-agronomic Variation within local genetic resources of Roselle (*Hibiscus sabdariffa var sabdariffa L.*) in Sudan

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A total of 126 roselle (*Hibiscus sabdariffa* var *sabdariffa L.*) accessions collected from different parts of Sudan were morpho-agronomically characterized during the years 2006, 2007 and 2008. A descriptor list developed by the Plant Genetic Resources Unit of the Agricultural Research Corporation in Sudan was used in the absence of standard international descriptor list. A considerable variation was observed within and between accessions for both qualitative and quantitative characters such as growth habit, leaf, stem, flower and calyx characters. The different descriptor states recorded were found to occur at frequency levels ranging from very rare at 5% or less to abundant at more than 90%. The majority of accessions produced reddish enlarged medium sized calyces with different degrees of red colour. White, small and large calyces were also observed in the rest of accessions. The calyces produced had different four shapes. It was most interesting to note that some accessions flowered in about two months and harvested in less than four months, while others were as late as about four months to flowering and almost six months to harvesting. The variation recorded shows the potential of this germplasm for use in genetic improvement of roselle qualitatively and agronomically.

Key words: Roselle, Sudan, Characterization, Descriptors, Morpho-agronomical variation. Enlarged full ripe calyx.

INTRODUCTION

Roselle (*Hibiscus sabdariffa* var *sabdariffa* L.) is an important crop in the tropics and subtropics. It seems to be of an African origin, and might have been domesticated as 6000 years ago in Sudan (McClintock and El Tahir, 2004). Hibiscus section Furcaria (Malvaceae), to which Roselle belongs, is a morphologically distinct natural group of more than 100 known species, many of which are handsome ornamentals with large, showy, delicate flowers. This group includes a number of fibre, food, and medicinal plants such as kenaf (*H. cannabinus*) and roselle (*H. sabdariffa*). It also displays a remarkable amount of genome diversity in Sub-Saharan Africa, where the centre of genome diversity is found (Wilson-FD, 1994).

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In Africa roselle has two main uses: as a vegetable and for preparation of a beverage (McClintock and El Tahir, 2004). The dried red calyces are commonly used to prepare a tea, drunk hot or, more commonly, cold with sugar. It is a sour tasting, refreshing drink, very popular from Senegal to Sudan as well as in Egypt and other northern African countries where it is referred to as 'karkadé'. The commercial part of the plant for such type of use is the fleshy calyx (sepals) surrounding the fruit capsules. Fully developed fleshy calyx is peeled off from the fruit by hand and dried naturally under shade to give the dry calyces that are used to prepare either the cold or hot drinks. Roselle has many industrial and domestic uses. The crop is important in the manufacture of many industries such as cosmetics, sweets, sauces, jams, jellies, alternative for tea and as colouring material for food and wine (Watt *et al,* 1962). Young roselle shoots; leaves and calices are used as a cooked vegetable or

Table 1. Roselle accessions collected from Sudan and characterized up to 2008

Accessions characterized	Accessions collected	State
2	2	Bahr Elgabal (South Sudan)
14	14	Blue Nile
2	3	Gedarif
1	3	Gezira
2	2	Kassala
38	44	North Kordofan
4	5	Northern
0	4	Red Sea
7	12	River Nile
4	7	South Darfur
42	57	South Kordofan
2	3	West Kordofan
0	2	White Nile
8	8	Unknown
126	166	Total

finely cut and used in sauces. Roselle fibre is locally used in West Africa, but on a very small scale, as it is the situation in Asia. The colour of the calyx plays an important role in determining the quality of the Karkade. The crimson red colour is the characteristic most popular and desirable colour.

Sudan, among different African countries, produces large quantities of dried calyces of roselle that are used for preparation of beverage. The crop is an important cash crop in western Sudan particularly in North Kordofan state, where the largest grown area is found with an average annual planted area by a grower falls within the range of 0.25- 2.0 ha, while some few growers can have up to 20.0 ha. The crop is mostly produced under traditional small farmer growing conditions, depending on rainfall and natural soil fertility without using chemical fertilizers or insecticides. A portion of the roselle produced is used locally; however, the larger portion of the crop is exported.

Roselle planted by traditional farmers in Sudan is usually of different types and cultivars. Several local strains can be identified on the basis of the calyx shape and colour as well as other plant characteristics. Such diverse genetic resources of Roselle in Sudan are threatened by different factors including environmental and production factors. The drought spells in the traditional areas of crop production especially in Kordofan region western to the White Nile River is one of these factors. According to local farmers a number of Roselle cultivars have disappeared due to replacement by other crops, lack of production inputs and difficulty of harvesting especially for some spiny cultivars. The Plant Genetic Resources (PGR) Unit of the Agricultural Research Corporation (ARC) in Sudan has, therefore, been targeting Roselle for collecting and conserving its local genetic resources. By 2008 a total of 162 accessions have been collected from 13 states in the country, with the majority from Kordofan region, where Roselle has been traditionally produced for commercial purposes. These accessions are conserved under long term conditions in the seed genebank of the PGR Unit in Wad Medani, Sudan. A number of these accessions have been planted during the years 2006, 2007 and 2008 for characterization and seed multiplication purposes. This paper highlights the main results of such an activity and documents the main features of variability recorded within the material studied.

MATERIALS AND METHODS

A total of 126 accessions of roselle that were collected from different parts of Sudan were grown during the years 2006, 2007 and 2008 for characterization and seed multiplication purposes (Table 1). They were grown in Elobeid Agricultural Research Station in North Kordofan state of western Sudan, which is located at latitude 13° N and longitude 30° E. The material was grown during the three years on the land of the research station's nursery under rains with supplemented sprinkler irrigation, while a copy of the material was grown in the research farm of the station under complete rain-fed conditions during the year 2008. A total of 120 accessions were repeatedly grown during the three years, while the rest were grown either in two seasons or in one season for reasons such as failure to be established in some years or being newly collected during or after 2006.

The land was prepared manually around mid-June in the three consecutive years, and then cleaned from weeds after the first rain showers. Seed sowing took place during the period between the 10th of July and the 3rd of August depending on the time and amount of rainfall during the three years. Each accession was planted in one row including eight plants under supplemented irrigation in the nursery, while during the year 2008 a copy of each accession represented by 20 plants was grown in one row under full rain within the research farm.

Table 2. Descriptor list used for characterization of roselle genetic resources

Descriptor states	Descriptor name
Erect, Compact, Prostrate	Growth habit
Green, Red, Green with red patches, Other	Stem colour
Smooth, Prickly	Stem texture
Shape 1: Deeply incised narrowly lobed	Leaf shape
Shape 2: Shallowly incised broad-lobed	
Shape 3: Obscurely lobed (non-lobed)	
3, 4, 5	Leaf lobes number
Green, Reddish green, Red, Other	Leaf colour
Small (≤5cm), Medium (>5-10 cm), Large (>10 cm)	Leaf size (leaf mid rib length)
	Number of days to 50% flowering
Pink, Yellowish white, Other	Flower colour
Linear, Toothed	Epicalyx segment shape
≤ 10, > 10	Epicalyx segments number
Shape 1: Completely closed round calyx	Shape of enlarged full ripe calyx
Shape 2: Slightly open calyx	
Shape 3: Moderately open calyx with lobes bending inwards	
Shape 4: Completely open calyx with lobes pointing upwards	
Shape 5: Completely open calyx with lobes curving outwards	
Descriptor states	Descriptor name
Dark red, Red, Light red, White, Other	Colour of enlarged full ripe calyx
Small (≤2 cm), Medium (>2-4 cm), Large (>4 cm)	Size of enlarged full ripe calyx (calyx length)
	Number of days to harvest
	100 seed weight

As there is no internationally recommended descriptor list for Roselle, a descriptor list has been developed by the Plant Genetic Resources Unit for characterization purposes. It included a number of descriptors covering different growth aspects and plant parts such as growth habit and stem, leaf, inflorescence, calyx and seed characters (Table 2). Plants were described while in the field for all vegetative and inflorescence descriptors. Calyx characters were described upon full ripening of fruit capsules that are enclosed within the enlarged calyces. Seeds were characterized after harvest in the laboratory. Characterization of vegetative and inflorescence descriptors was started after 6-8 weeks from sowing, while characterization of the enlarged calyx and seed descriptors was started after 12 weeks from sowing.

Controlled self-pollination was practiced on the flowers of different plants within each accession. It was conducted by closing the flower buds using metal clips at a stage just prior to anthesis preventing flower opening and hence avoiding natural cross pollination. Flower buds were closed when fully mature with top edges start to show emerging coloured corolla indicating that the flower would be opening the following day.

Frequency of occurrence was calculated for different qualitative descriptor states observed. Accordingly, the occurrence levels of each descriptor state were categorized as very rare (< 5%), rare (> 5-20%), medium (> 20-60%), common (> 60-90%) or abundant (>90%). Mean value, range, standard deviation and coefficient of variation were also calculated for the quantitative characters in order to obtain an indicative estimate for the level of phenotypic variation among the characters described.

Multivariate analysis was run as Cluster analysis for the characterization data of 126 accessions. The data matrix of numerical codes for qualitative and quantitative characters was used to generate similarity indices based on Euclidian distances. Cluster analysis was carried out using hierarchical analysis and complete link method using the GenStat Discovery Edition 4.

RESULTS

Morpho-agronomic variation was observed within and between the characterized roselle accessions for almost all descriptors studied. Only four accessions (3%) were observed to be homogeneous with regard to all characters recorded, while the majority of the accessions (97%) were found to be heterogeneous in all or some of

Table 3. Variation in qualitative characters among the characterized roselle accessions

Descriptor states occurrence level* and frequency						Descriptor
Heterogeneous	**Very rare**	**Rare**	**Moderate**	**Common**	**Abundant**	
40.4%		Erect (10.7%)	Compact (48.9%)			Growth habit
7.6%	Green (4.6%), Green with red patches (1.5%)			Red (86.3%)		Stem colour
1.6%		Prickly (9.5%)		Smooth (88.9%)		Stem texture
44.3%	Shape 3 (0.8%)	Shape 1 (19.8%)	Shape 2 (35.1%)			Leaf shape
	4 (1.6%)		3 (42.1%), 5 (56.3%)			Leaf lobes number
6.1%					Green (93.9%)	Leaf colour
	Small (3.2%)		Large (25.4%)	Medium (71.4%)		Leaf size
19.8%	Yellowish white (4.6%)			Pink (75.6%)		Flower colour
32%	Toothed (3.1%)			Linear (64.9%)		Epicalyx segment shape
31.3%	>10 (1.5%)			≤10 (67.2%)		Epicalyx segments number
55%		Shape 1 (6.1%), Shape 2 (17.6%), Shape 3 (16%), Shape 5 (5.3%)				Shape of enlarged full ripe calyx
41.5%	Dark red (3.1%), White (3.1%)	Light red (8.5%)	Red (43.8%)			Colour of enlarged full ripe calyx
Moderate		Small (17.5%)	Large (26.2%), Medium (56.3%)			Size of enlarged full ripe calyx

* Abundant (>90%), Common (>60-90%), Moderate (>20-60%), Rare (>5-20%), Very rare (≤ 5%).

the characters described. The level of heterogeneity varied when considered for each of the descriptors studied. The highest heterogeneity level was observed for the shape of enlarged full ripe calyx, which was heterogeneous within 55% of the accessions, while the lowest level of heterogeneity was recorded for the stem texture being heterogeneous within less than 2% of the accessions. Otherwise, the rest of accessions were observed to be homogeneous with respect to each of the specific descriptor states recorded.

Different descriptor states at different frequency levels were recorded for the qualitative descriptors studied. They frequently occurred at levels ranging between very rare (≤5%), rare (>5-20%), moderate (>20-60%), common (>60-90%) and abundant (>90%) among those accessions showing homogeneity for the specific descriptor state recorded (Table 3). The character that was occurring more abundantly was the green colour of leaves, which was observed in almost 94% of the accessions, followed by the smooth stems, in about 89% of the accessions, and the red stems in 86% of them, both classified as commonly occurring characters. At the same time, the non-lobed leaves (shape 3), occurring in less than 1% of the accessions, was the least and very rarely recorded character, followed by stems showing green colour with red patches and epicalyx segments counting to more than ten, each of them occurring in less than 2% of the accessions. A number of descriptor states were found to exist in the majority of the material at frequency levels of more than 50% of the accessions. They were, therefore, abundantly, commonly or moderately occurring. These included characters such as green leaves (94%), smooth stems (89%), red stems (86%), pink flower colour (76%), medium leaves (71%), linear epicalyx segments (65%), epicalyx segments counting to ten or less (61%), and leaves that are five-lobed (56%). On the other hand some characters were found to rarely or very rarely exist in less than 20% or even in 5% or less of accessions respectively. These included descriptor states such as erect growth habit

Figure 1. Variation in calyx characteristics among Roselle accessions in Sudan

Table 4. Variation in quantitative characters among the characterized roselle accessions

CV%*	SD*	Mean	Maximum	Minimum	Descriptor
9	8.3	92.4	114	66	Number of days to 50% flowering
7	8.5	122	181	110	Number of days to harvest
15	0.5	3.3	4.5	1.4	100 seed weight (g)

SD = Standard Deviation, CV = Coefficient of Variation

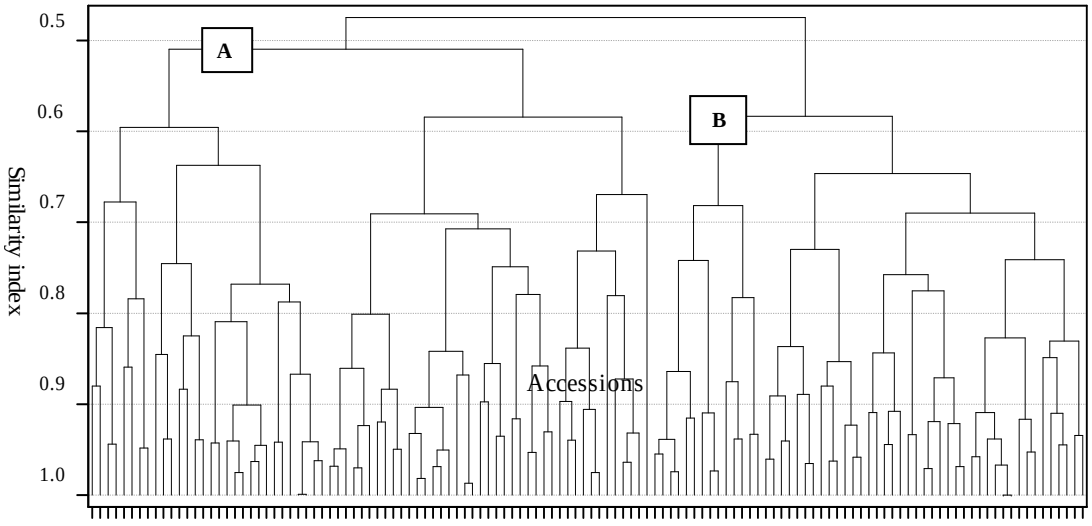

Figure 2. Dendogram of similarity among Roselle accessions

(11%), prickly stems (10%), yellowish white flowers (5%); dark red (3%), light red (9%) or white calyces (3%), and small sized calyces (18%).

The enlarged calyces, which are the consumable commercial part of roselle, showed a considerable level of variation among and within the characterized accessions (Figure 1). They varied considerably in shape, colour and size. Four calyx shapes were observed within the germplasm studied. These were the completely closed round calyx (shape 1), slightly open calyx (shape 2), moderately open calyx with lobes curving inwards (shape 3) and completely open calyx with lobes curving outwards (shape 5). The level of heterogeneity for calyx shape within those accessions was relatively high in 55% of the accessions. In the rest of accessions, which were homogeneous for such descriptor, the different shapes were rarely occurring at frequency levels of less than 20% of accessions (Table 3). However, the majority of

Table 5. Distribution of accessions and sites of collections within each cluster and sub-clusters

Cluster	Sub-cluster	Total number of accession per sub-cluster	State	Number of accessions per state
A	A1	4	North Kordofan	3
			South Kordofan	1
	A2	4	South Kordofan	2
			North Kordofan	1
			Unknown	1
	A3	7	South Kordofan	4
			South Darfur	1
			River Nile	1
			Unknown	1
	A4	15	North Kordofan	8
			South Kordofan	4
			Blue Nile	2
			South Darfur	1
	A5	10	Blue Nile	3
			South Kordofan	2
			North Kordofan	2
			West Kordofan	1
			Bahr Elgabal (South Sudan)	1
			Unknown	1
	A6	19	North Kordofan	10
			South Kordofan	5
			West Kordofan	1
			Kassala	1
			Unknown	2
	A7	14	South Kordofan	4
			Blue Nile	4
			River Nile	3
			Kassala	1
			Gezira	1
			Northern	1
	A8	1	South Darfur	1
B	B1	6	North Kordofan	3
			Blue Nile	1
			Northern	1
			Unknown	1
	B2	5	South Kordofan	2
			North Kordofan	1
			River Nile	1
			Northern	1

such accessions produced calyces of either shape 2 or shape 3; both are either slightly or moderately open from above. Red calyces were homogeneously produced by

more than 40% of accessions, while each of the other calyx colours, including dark red, light red and white, was produced by less than 10% of the accessions, and, therefore, regarded as being rarely or very rarely occurring. However, calyces with either degree of red colour were produced by the majority of accessions including about 55% which were homogeneous for such character and 41% which were heterogeneous and included plants that carried at least one degree of red coloured calyces. The majority of the accessions (56%) produced medium sized calyces with lengths ranging between >2 and 4 cm, while larger or smaller calyces were produced on less than 30% of accessions each.

Variation in quantitative characters was also recorded between the characterized accessions. The highest level of variation among the descriptors studied was observed for 100 seed weight with a coefficient of variation (CV) of 15% and a range between a minimum weight of 1.4 g and a maximum weight of 4.5 g. (Table 4). The other quantitative characters, which were days to 50% flowering and days to harvest, showed closer levels of variation with CVs of 9% and 7% respectively. However, the recorded observations showed a quite considerable variation in either of the times to flowering or to harvesting. Some accessions were as early as about two

Table 5. Contd.

Cluster	Sub-cluster	Total number of accession per sub-cluster	State	Number of accessions per state
B	B3	13	South Kordofan	8
			North Kordofan	3
			Blue Nile	2
	B4	13	South Kordofan	9
			North Kordofan	2
			Blue Nile	2
	B5	15	North Kordofan	5
			Gedarif	2
			River Nile	2
			Northern	1
			Bahr Elgabal (South Sudan)	1
			South Kordofan	1
			Unknown	3
Total		126		126

months up to flowering and less than four months up to harvesting compared with other accessions that lately flowered in about four months and be ready for harvesting in almost six months.

The cluster analysis showed that all roselle accessions grouped into two main clusters (A and B) at similarity index of 50% (Figure 2). Each of these clusters sub-divided into more sub-clusters totaling to 13 with similarity levels of more than 70%, including eight sub-clusters under A and five sub-clusters under B. Sub-clusters consisted of variable numbers of accessions ranging from one accession as in sub-cluster A8 to 19 accessions as in sub-cluster A6 (Table 5). Each sub-cluster composed of accessions collected from different geographical sites. However, it was noted that accessions collected from North Kordofan state were distributed among 10 sub-clusters out of 13.

DISCUSSION

The local germplasm of roselle in Sudan seems to be quite variable morphologically and agronomically, with high level of heterogeneity within the studied accessions. The heterogeneity in roselle germplasm could be attributed to the fact that the studied germplasm represented local farmers' cultivars that are used for commercial production in Sudan, rather than improved advanced varieties. Moreover, roselle is a crop species, although highly self-pollinated, some limited level of natural cross pollination could occur (Vaidya, 2000), a situation that may lead, through years, to heterogeneity in the absence of controlled seed production processes within farmers' fields.

The cultivated material studied is rich with calyx related traits that favour the commercial use of roselle as beverage rather than as vegetable or fibre producing crop. Different shapes, sizes and colours of enlarged calyx, which are mostly reddish, provide a wide opportunity for further genetic improvement in this crop on the basis of calyx characters for commercial purposes. The variation recorded in respect to maturity time is indicative to possible wider range for ecological adaptation of this crop under different growth conditions, especially during this era of climate change.

The presence of red colour on stems and calyces of Sudanese roselle germplasm has positive indications in respect to ecological adaptability and human health. According to Stevels (1990), roselle plants with anthocyanin pigmentation are able to withstand the harsh Sahelian environment better than plants with a yellow-green colour. Hence red types are more drought tolerant than green types. The anthocyanins have also a long history as part of the human diet, and these and other flavonoids are receiving renewed attention for their positive health attributes (Bridle and Timberlake, 1997). Therefore, the dominance of red coloured stems and calyces in the studied germplasm, which is attributed to the anthocyanin pigment, is an indication that such germplasm is greatly adapted to the harsh Sahelian environment as well as being rich with material that could be used for purposes related to improving human health either as food or medicine.

The clustering patterns of the accessions under study showed that they tended to group under different clusters irrespective of their collection sites since each group could include accessions that were collected originally from different regions. This is an indicator that the diversity in roselle germplasm is spreading throughout the different regions of Sudan where the crop is grown. However, twelve out of the thirteen subgroups contained accessions that were collected from Kordofan region including ten from North Kordofan state. This can also be considered as an indication that while diversified roselle

genetic resources are available in different regions, they are well concentrated in Kordofan region, and more specifically in the North Kordofan state, where the crop has been traditionally cultivated in large areas for commercial purposes for long time. It is from there the crop has been introduced into the other regions with the movement of people.

In conclusion, the variation recorded within the Sudanese roselle germplasm shows its potential for use in the genetic improvement of the crop agronomically and qualitatively. Due to the relatively high level of heterogeneity within different roselle accessions, splitting and purification of such germplasm into pure lines is a necessary step towards enhancing its future use.

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