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Phenotypic variations among newly selected roselle (*Hibiscus sabdariffa* Linn.) genotypes in Nigeria.

*Daudu, O. A. Y¹, Falusi, O. A¹, Gana, A. S², Abubakar, A¹, Bello, I. M¹, Oluwajobi, A. O³ and D. J. Nwosu⁴. ¹ Department of Biological Sciences, School of Natural and Applied Sciences, Federal University of Technology, PMB, 65, Minna. ²Department of Crop Science, School of Agriculture and Agricultural Technology, Federal University of Technology, PMB, 65, Minna. ³ Department of Biology, Institute of Applied Sciences, Kwara State Polytechnic, Ilorin, Nigeria. ⁴National Centre for Genetic Resources and Biotechnology (NACGRAB), NCRI Sub-station, Badegi, Niger State.

Abstract

Phenotypic investigation was conducted on twenty newly selected genotypes of Nigerian Roselle (*Hibiscus sabdariffa* L) using visual character markers. These markers include: stem colours, stem hairiness, leaf colour, leaf hairiness, leaf shape, petiole colour, petiole hairiness, calyx colour, calyx hairiness and epicalyx colour. The experiment was conducted in the 2013 and 2014 growing seasons to ascertain the consistencies of the traits. Distinct variations were obtained among the new Roselle genotypes in terms of the parameters studied. Consistencies were observed in all the visual markers used except the flower colour; these consistencies indicate that the variations are not caused by environmental factors, such variations are good for selection and improvement. Whereas, the inconsistency in the flower morphology might be due to environmental influences. It is therefore concluded that Nigerian Roselle genotypes differ in some of their phenotypic (visual) characters which could be used for the improvement of the crop. Therefore, a scientific agro-metrical and molecular characterization is necessary to ascertain the genetic diversity that exist among these new selected Roselle genotypes in Nigeria. This will enhance the selection for improvement of the crop in the future.

Keywords: Visual Characters, Leaf shape, Calyx Colour, Selection, Improvement

Email address: dauduoladipupoyusuf@yahoo.com*; +2348062202142

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Introduction.

Roselle (*Hibiscus sabdariffa* Linn) is said to be native to India but was introduced to other parts of the world such as Central America, West Indies and Africa (Fasoyiro et. al., 2005). Crane (1949) opined that this species was probably brought to the western hemisphere by slaves from Africa and its use in Jamaica was noted as early as 1707; however, Gomez-Leyva et. al. (2008), said that it is native to Africa. Schippers (2000), also said the plant have originated from central Africa. It belongs to the Malvaceae (Gomez-Leyva et. al. 2008) which are characterized by typical showy and solitary flowers. It is grown as tropical and subtropical sub-shrubs (Fasoyiro et. al., 2005 and Atta et. al. 2011).

The plant can grow up to 3.5m tall with a deep penetrating taproot system. It has a smooth or nearly smooth, cylindrical dark-green to red stems (Amin et. al., 2008; Mahadevan et. al., 2009). The leaves are alternate, palmately lobed, with 3 to 5 or even 7 oblong, lanceolate serrated lobes (Mann et. al., 2003), sometimes with simple non-lobed leaves with long petioles. Flowers are borne singly on the inflorescence on the leaf axils; the flowers bear five (5) petals (Mann et. al., 2003) and are up to 12.5cm wide, yellow or buff with a rose or maroon eye, that turn pink as they wither at the end of the day. The typically red calyx, consist of 5 large sepals with a collar

(epicalyx) of 8-12 slim, pointed bracts (or bractioles) around the base (Rice et. al., 1993). The fruit is a velvety capsule, 2-5cm long, which is green when immature and light-brown nearly glabrous seed when dried, 5-valved, with each valve containing 3-4 seeds which usually contain high percentage of oil (Rice et. al., 1993; Mann et. al., 2003). The capsule turns brown and splits open when mature and dry. Seeds are kidney-shaped, light-brown, 3-5mm long and covered with minute, stout and stellate hairs (Julia, 1987).

Observational studies showed that diets high in plant foods are associated with a lower risk of chronic diseases, such as cardiovascular disease and some forms of cancer (Hertog et. al., 1995). The crop is used for many different purposes all over the world, the common of which are as a fibre crop, leafy vegetable, refreshing, beverage (Schippers, 2000); jam and preserves (Mahadevan et. al., 2009); in medicine (Qi et. al., 2005; Diane et. al., 2010; Ngamjarus et. al., 2010; Gurrola-Diaz et. al., 2010 Anjah et. al., 2012;). Amin et. al., (2008) stated that the fruit of roselle contains more Ascorbic acid (Vitamin C) than *Ribes nigrum* L (Black currant) and nine times more than Citrus (*Citrus sinensis* L.).

H. sabdariffa has shown *in vitro* antimicrobial activities against *E. coli* (Fullerton et. al., 2011). A recent review stated that specific extracts of *H. sabdariffa* exhibit activities

against atherosclerosis, liver disease, cancer (Chang *et al.*, 2006), diabetes, and other metabolic syndromes (Lin *et al.*, 2011). Diane *et al.* (2010), also said that daily consumption of three servings of *H. sabdariffa* tea, an amount readily incorporated into the diet, effectively lowered Blood pressure in pre- and mildly hypertensive adults. The Roselle seeds until now do not have any commercial applications though they are a valuable food resource on account of their protein, calorie and substantial amount of fiber and valuable micro-nutrient (Akanbi *et al.*, 2009). The seeds contain an edible fixed oil (17-20 %) that is similar to cotton seed oil properties (Ottai *et al.* 2004, Ottai and Abd-El-Khair 2004 and Hussein *et al.* 2010). The calyces are used in the production of refreshing drinks due to their high contents of vitamin C, anthocyanins, amino acids and mineral salts (Babajide *et al.* 2004 and Cisse *et al.* 2009). The calyces colour extract is a natural colorant which replaces red synthetic coloring agents in nutritional, drinks, pharmaceutical and cosmetic industries according to the world return to nature (Shalan *et al.* 2001, Ottai *et al.* 2004, Hussein *et al.* 2010 and Falusi *et al.* 2014).

Genetic variability, which is a heritable difference among cultivars, is required in an appreciable level within a population to facilitate and sustain an effective long-term plant breeding programme (Salami *et al.*, 2007). Progress from selection has been reported to be directly related to the magnitude has been reported to be directly related to the magnitude of genetic variance in the population (Tabanao and Bernardo, 2005). Large amount of genetic variability has been observed to occur in the original accessions and races among sampled population representing different climatic, geographical regions (Ilarsam *et al.*, 2002; Abayi *et al.*, 2004).

It is on this background that this study is aimed at determining the genetic variability among the Nigerian roselle genotypes using some phenotypic characters. This will give an insight to the variations existing among the crop and the basis for selection and improvement in the future.

Materials and methods

Description of the Study Area.

The study was carried out at the Experimental Garden of the Department of Biological Sciences, Federal University of Technology, Minna, Niger State, Nigeria.

Collection and Labeling of Plastic Flower Pots.

A total of five (5) 15 litres plastic flower pots were allocated for each accession; they were properly labeled using masking tape and permanent marker.

Experimental Design, Sowing of Seeds and Thinning of the Roselle Seedlings.

All the different accessions collected were grown in a Complete Randomize Block Design (CRBD). Each of the accessions were sown in five replicates after the viability of the seeds had been verified using floatation method. Five seeds were sown at the depth of 1-2 cm for each of the accession in the planting pots. At 30 days after sowing, the emerging seeds (seedlings) were thinned to two seedlings per plant in the planting pots. The seedlings were thinned to about 15 cm plant-to-plant spacing.

Visual Phenotypic Characterization

In order to arrange the available accessions into distinct groups (genotypes), a number of phenotype-based genetic markers were used. These markers include: stem colours, stem hairiness, leaf colour, leaf hairiness, leaf shape, petiole colour, petiole hairiness, calyx colour, calyx hairiness and epicalyx colour.

Results

It was observed that all the genotypes showed distinct features as regards these morphological markers. Using these phenotype-based genetic markers, twenty distinct genotypes were obtained, these are: NGR-HS-001, NGR-HS-002, NGR-HS-003, NGR-HS-004, NGR-HS-005, NGR-HS-006, NGR-HS-007, NGR-HS-008, NGR-HS-009, NGR-HS-010, NGR-HS-011, NGR-HS-012, NGR-HS-013, NGR-HS-014, NGR-HS-015, NGR-HS-016, NGR-HS-017, NGR-HS-018, NGR-HS-019, and NGR-HS-020. There were distinct variations among the accessions as regards these visual characteristics (Table 1).

Table 1: Phenotypic Traits of the New Twenty Nigerian Roselle Genotypes

S/No	Accession Number	STEM		LEAVES			PETIOLE		CALYX		
		Colour	Hairiness	Colour	Hairiness	Shape	Colour	Hairiness	Colour	Hairiness	Epalalyx
1.	NGR-HS-001	Deep red	Glutinous	purple with purplish veins	Smooth	mixture of lobed and non-lobed leaves	deep red	smooth hairs	deep red	hairy	deep red
2.	NGR-HS-002	Red	Glutinous	green with light red veins	Smooth	non-lobed leaves and lobed leaves (deeper lobes)	light red	smooth hairs	Pink	hairy	reddish-green
3.	NGR-HS-003	Red	Smooth	green with light red veins	Hairy (smooth hairs)	non-lobed leaves and lobed leaves (deeper lobes)	red	sharp hairs	Red	hairy	red
4.	NGR-HS-004	Deep red	hairy (sharp hairs)	green with red veins	Smooth hairs	non-lobed leaves and lobed leaves (deeper lobes)	red	sharp hairs	deep red	hairy	deep red
5.	NGR-HS-005	Deep red or brown	hairy (scattered hairs)	green with red vein or green vein	Smooth	non-lobed leaves	deep red with green patch	Hairy	deep red	hairy	deep red
6.	NGR-HS-006	Deep red or brown	glutinous	green with brown patches; green veins	Smooth	lobed leaves and non-lobed leaves present	deep red with green patch	smooth hairs	deep red	hairy	deep red
7.	NGR-HS-007	Deep red	hairy and glutinous	green with brown patches; red veins	Smooth	non-lobed leaves	deep red with red patches	smooth hairs	deep red	hairy	deep red
8.	NGR-HS-008	Deep red or brown	Smooth	green with brown patches; red veins	Smooth	lobed leaves (2-4) mostly 2	deep red with red patch	smooth hairs	deep red	hairy	deep red
9.	NGR-HS-009	Deep red or brown	hairy (scattered hairs)	purple, veins are deep red	Smooth	lobed leaves (2) and non-lobed leaves	deep red	smooth hairs	deep red	hairy	deep red
10.	NGR-HS-010	deep red (branches are red)	smooth hairs	purplish or deep red veins are deep red	Smooth	2-4 lobed leaves (mostly 2)	deep red	smooth hairs	deep red	hairy	deep red
11.	NGR-HS-011	Red	smooth hairs + glutinous	green, veins are green	Smooth	simple without lobes	red	smooth hairs	Red	hairy	red
12.	NGR-HS-012	Light red	smooth hairs + glutinous	green, veins are light red	Smooth	non-lobed and 2 lobed leaves	light red	smooth hairs	green	hairy	green
13.	NGR-HS-013	Red	Hairy	green with brown patches	Smooth	lobed leaves (maximum of 4 lobed)	red with green patches	smooth hairs	Red	hairy	red
14.	NGR-HS-014	Deep red	glutinous	purple with purplish veins	Smooth	mixture of lobed and non-lobed leaves	deep red	smooth hairs	deep red	hairy	deep red
16.	NGR-HS-016	Green	glutinous	green, midrib and veins green	Smooth	lobed leaves (2-5 lobes)	green	sharp hairs	green	hairy	green
17.	NGR-HS-017	Green	Glutinous	green, midrib and veins green	Smooth	larger lobed leaves (maximum of 2 lobes) and simple leaves	green	smooth hairs	green	hairy	green
18.	NGR-HS-018	Green	glutinous + tiny short hairs	green, midrib and veins green	Smooth	Maximum of 4 lobed tiny leaves	green	smooth hairs	green	hairy	green
19.	NGR-HS-019	Green	Glutinous	green, midrib and veins green	Smooth	1-4 lobed leaves and non-lobed leaves	green	smooth hairs	green	hairy	green
20.	NGR-HS-020	Green	Glutinous	green, midrib and veins green	Smooth	Tiny leaves with 6 lobes	green	smooth hairs	green	hairy	green

Within all the accessions, six distinct stem colours were observed; these are light red, deep red, pink, pinkish-green and green (Plate I).



Plate I: Variations in the stem colours among the Roselle accessions collected. A (Red stem), B (Deep-Red or Brown stem), C (Green stem), D (light-red stem), E (Pink stem), F (Pinkish-green stem).

Distinct variations exist among the accessions in terms of the hairiness nature of the stem,

they varied from hairy, thorough smooth to glutinous (i.e. sticky). NGR-HS-003, NGR-HS-004, NGR-HS-013 have sharp hairs on the stem, although NGR-HS-009 has sharp hairs, the hairs are scattered on the stem. NGR-HS-015 (green accession) has sharp hairs which are long and difficult to touch along the body. NGR-HS-007 is hairy but the stem is also glutinous, which is similar to what was observed in some of the accession. However, NGR-HS-008 neither possesses glutinous stem nor hairy stem, but smooth stem (Table 1).

The morphological variations in the leaf, among all the accessions showed variations exist among them. Variations were observed in leaf colour (blade and veins), shape and hairiness (Table 1); on the leaf blades, presence or absence of hair was observed. Apart from NGR-HS-003, NGR-HS-004 and NGR-HS-015 which are pubescent, the remaining accessions were glabrous. However, where NGR-HS-004 has smooth hairs, NGR-HS-003 and NGR-HS-015 have sharp hairs.

The accessions also varied considerably in terms of leaf shapes, leaves varied in presence or absence of lobes, number of lobes, depth of lobes and nature of the lobes (Plate II). Some of the accessions like: NGR-HS-001, NGR-HS-002, NGR-HS-003, NGR-HS-004, NGR-HS-006, NGR-HS-009, NGR-HS-010, NGR-HS-012, NGR-HS-017, NGR-HS-018 and NGR-HS-020, all possess mixture of lobed and non-lobed (simple) leaves; however, lobed leaves are more than non-lobed leaves.

Two markers were considered for the petiole, these are colour and hairiness and accessions varied in petiole colours considerably. In terms of presence or absence of hairs, all accessions possess hairs. Apart from NGR-HS-015 that possesses sharp hairs on the petioles, all other accessions bear petioles that have fine and smooth hairs (Plate II).

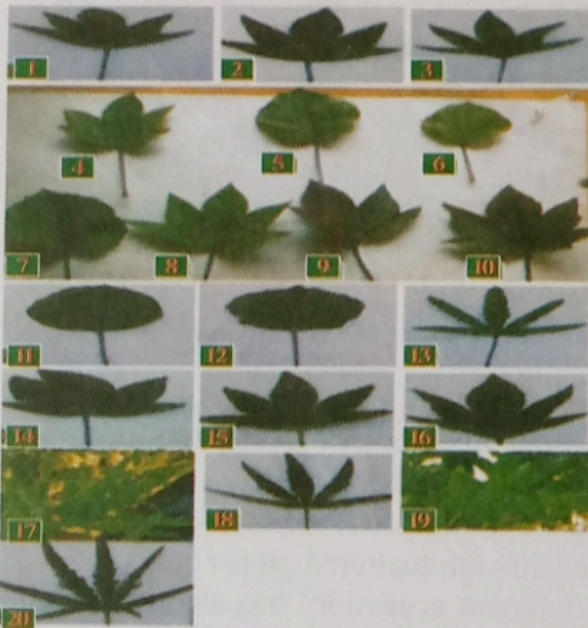


Plate II: Variations in the Leaves of the Roselle Accessions Collected (001-020 represent the accession numbers, i.e. NGR-HS-001-NGR-HS-020).

The flower colours of all the roselle accessions vary distinctly from one another as shown in Plate III.



Plate III: Variation in flower colours and shapes of the New Twenty Roselle Genotypes

NGR-HS-009 and NGR-HS-010 have pink coloured petals; dark-red or maroon eye and golden yellow pollen grains. NGR-HS-007 and NGR-HS-008 have pink colour on the upper part and white colour on the lower part of the petals, with dark-red eye and golden-yellow pollen grains. NGR-HS-004, NGR-HS-005 and NGR-HS-006 have pinkish-white petals, light red-eye and golden yellow pollen grains.

Calyces and epicalyces varied considerably in terms of colour and hairiness some of the accessions possess the same colour for calyces and epicalyces, whereas some others tend to differ in their colours. NGR-HS-001, NGR-HS-004, NGR-HS-005, NGR-HS-006, NGR-HS-007, NGR-HS-008, NGR-HS-009, NGR-HS-010, NGR-HS-013 and NGR-HS-014, produced deep red calyces and epicalyces, NGR-HS-002 showed pink calyces and pinkish green epicalyces, whereas, NGR-HS-003 and NGR-HS-011 showed red coloured calyces and epicalyces. Others include NGR-HS-015 with light-green calyces but deep-green epicalyces. NGR-HS-016, NGR-HS-017, NGR-HS-018, NGR-HS-019, and NGR-HS-020 all possess green calyces and green epicalyces (Plate IV).



Plate IV: Variations in Calyces and Epicalyces Colours as well as shapes of the New Roselle Genotypes.

Discussion

The differences observed among the accessions of Roselle collected in visual characters are indications of significant difference in their genetic bases and high genetic variability of the plant in Nigeria. The results of the morphological groups according to stem, leaf shapes and calyx colours agree with the work of Yandong *et al.* (2012) and Futuless *et al.* (2010) on Roselle. These variations might be due to different ecological regions as well as different planting seasons as reported by Anonymous (2012). The variations in terms of calyx colours among the accessions is totally different from the report of Udom *et al.*, (2001), who reported that there are three common varieties of Roselle grown in Nigeria. This might be due partly to evolution of new varieties due to natural hybridization that could have occurred among closely related group. Although distinct variation occurred among the accessions in terms of flower colours and shapes, this character might not serve as a good morphological marker in characterizing these accessions. This is so because of the similarities that exist among the flowers of the different roselle types. In addition, flower colour may be greatly influenced by certain environmental conditions.

It is therefore concluded that Nigerian Roselle genotypes differ in some of their phenotypic (visual) characters which could be used for the improvement of the crop. Therefore, a scientific agro-metrical and molecular

characterization is necessary to ascertain the genetic diversity that exists among these new selected Roselle genotypes in Nigeria. This will enhance the selection for improvement of the crop in the future.

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