Assessment of chemical composition of flower, variability, heritability and genetic advance in *Hibiscus sabdariffa*

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Abstract
Calyx of three genotypes of Hibiscus sabdariffa collected from Nigeria were analyzed for proximate, minerals, vitamins and phytochemical composition in a completely randomized design replicated 3 times. The aim was to determine variations among them and the proportions of heritable and non heritable components, and expected genetic advance. Analysis of variance showed that proximate, minerals, vitamins and phytochemical were significant ($P < 0.05$). The result showed that H. sabdariffa calyx is a good source of essential nutrients, minerals (phosphorus and calcium), vitamins C and B_3, β carotene and phytochemical (alkaloids and flavonoids). Considerable variations were observed among the genotypes as shown by the genetic component analysis for those characters. Hence, room exist for improvement of nutritional, vitamins C and B_3, phosphorus, calcium, alkaloids and flavonoids composition of H. sabdariffa calyx and increase its availability for consumption by average Nigerians.

Keywords: *Hibiscus sabdariffa, chemical composition, phenotypic variability, heritability.*

Introduction

Roselle (*Hibiscus sabdariffa*), originated from India and has become wide spread in the tropics and subtropics of both hemispheres and assumed its current importance as a vegetable crop during the twentieth century (Okereke *et al*., 2015) .It is sensitive to frost but does best in tropical and subtropical regions from sea levels up to 9000m with a rainfall of about 182cm during its growing season. It prefers deep, fairly fertile sandy loamy soil. It is an excellent source of fibre and finds various uses as food. The leaves, tender shoots, succulent calyx and immature fruits are chopped and added to sauces. The leaves and tender shoots are sometimes eaten raw in salads. Roselle has antihypertensive properties, the seeds are a good source of lipid-soluble antioxidants, particularly gammatocopherol, (Mohammed *et al*. 2007). Extracts from leaves are used to treat colds, toothache, urinary and tract infections (Haji and Haji, 1999). Heated leaves are applied on cracks in the feet, on boils and ulcers to speed up maturation. It is very rich in anthocyanins, protocatechuic acid and flavonoids. The decorative red stalks with ripe red fruits are exported to Europe where they are used in flower arrangements (Barbara, 2004). In Nigeria, the calyx is used to make a very common and refreshing drink known as Zobo which is a mild diuretic (Nwafor, 2012).In Western Nigeria, it is also used in cooking vegetable soup together with 'egusi-melon. The most exploited part of Roselle plant is its calyces which may be green, red or dark red (Schippers, 2000). The green calyx is used in making vegetable stew while red and dark red ones are used in producing drinks, jellies, sauces, chutneys, wines and tea (Barbara, 2004).The calyx drink, which is receiving industrial attention internationally is a readily available and inexpensive source of vitamin C. It contains nine times more vitamin C than *Citrus sinensis* (Amin *et al*., 2008). In Nigeria, the colour of the calyx plays important role in determining the quality of the crop. The dark red calyx is the most desireable colour and is increasingly exported to America and Europe where they are used as food colourants (El-Naim and Ahmed, 2010). Plant breeders should have a good understanding of the genetic variability within the Roselle genotypes. Hence, this study was conducted to determine the phenotypic variations of three *H. sabdariffa* genotypes with respect to nutrients, minerals, vitamins, and phytochemical compositions, estimate the heritable and non heritable components, broad sense heritability and genetic advance.

Materials and method

Calyx of 3genotypes of *H. sabdariffa* collected from three states in Nigeria namely, Garki in Federal Capital Territory Abuja, Sabon-Garri in Kano State in Northern Nigeria and Awka in Anambra State in Eastern Nigeria were analyzed for proximate, mineral, vitamin and phytochemical compositions in a completely randomized design with 3 replicates at the research laboratory of National Root Crops Research Institute, Umudike in 2015.

Proximate analysis

Moisture content was determined by the method of Pearson (1996). Crude protein (Nx6.25) was determined by the Kjeldahl method (1983). The recommended method of Association of Official Analytical Chemist was used to determine the ash, fibre and lipid contents. The carbohydrate content was obtained by the differences as nitrogen free extract.

Mineral and Vitamins Analysis

Determination of calcium, potassium and phosphorus content of the samples was carried out by AOAC method No 968.08 using atomic absorption spectrophotometer (Mgaya, *et al*., 2014). Vitamins B_1, B_2, B_3 were determined by spectrophotometric method (Okwu, 2004),and vitamin C was determined as outlined by Kirk and Sawyer (1998).

Phytochemical analysis

Alkaloids were estimated by the alkaline precipitation gravimetric method, flavonoids were determined by acidification and ethyl acetate extraction (Harborne, 1973). Tannin was determined using spectrophometric method (Hang and Lantzal, 1983). Hydrogen cyanide was estimated according to AOAC (1990). All the determinants were done in triplicates.

Data analysis

Data were analyzed by one way analysis of variance and significant means separated with the least significant difference (Snedeco and Cochran, 1989).The gross variability was partitioned into genetic and non- genetic components. Phenotypic, Genotypic and error variances were estimated using the method of Wricke and Weber (1986).

$\sigma^2 P = \frac{MSG,}{r}, \sigma^2 G = \frac{MSG-MSE}{r}, \sigma^2 E = \frac{MSE}{r}$ MSG, MSE and r are the mean squares genotypes, mean square error and number of replication respectively. $PCV = \frac{\sigma P}{mean} x \frac{100}{1}$, $GCV = \frac{\sigma G}{mean} x \frac{100}{1}$

PCV, GCV and ECV are phenotypic, genotypic and environmental coefficients of variations respectively. Broad sense heritability (h^2B) was expressed as the ratio of genotypic (σ^2G) to the phenotypic (σ^2P) variances as described by Allard (1991). Genetic advance (GA) was estimated with the method of Fehr (1987), using the formula, GA = K(Sp)h^2B. K is the standardized selection differential at 5% (K=2.063), sp is the phenotypic standard deviation σP; h^2B is the broad sense heritability. Genetic advance (G.A) as $\%\ of\ the\ mean = \frac{\delta^2 g}{\delta^2 P}$ x $\frac{100}{mean}$

Result and discussion

The *H. sabdariffa* genotypes significantly differed (P<0.05) in proximate, mineral, vitamin and phytochemical composition (Table1). These results confirmed variations in colour and nutrient contents in calyces among Roselle genotypes as reported by Amin *et al*., (2008) and Babalola *et al*., (2001).From Table 2, the colour of the genotypes from Kano, Abuja and Awka were dark red, red and green respectively. Genotypes obtained from Awka (89.070%) and Kano (84.60%) States had the highest and lowest carbohydrate content, respectively. Similarly, genotypes from Kano (5.907%) and Awka (5.143%) had the highest and lowest protein content. The genotypes from Kano (4.8770%), Abuja (1.9540%) and Awka (1.807%) had the highest moisture, ash and crude fibre contents respectively. The carbohydrate content of the calyx of the genotypes was higher than that reported by Nwofia and Adikibe (2012)in *Ocimum gratisseum*. Moisture content of the calyx of the genotypes was lower than that reported by Dike (2010) and Nwofia *et al*., (2013) in *P. guineense*, and *P. nigrum* respectively. Moisture content is an index of water activity of crops indicating if the crop will have a short shelf life, since micro-organisms that cause spoilage thrive in food with high moisture (Emebu and Anyika, 2011). This result showed that *H. sabdariffa* calyx is a rich source of energy in diets with a relative long shelf life due to very low moisture content. Fibre content of these genotypes were lower than that reported by Dike (2010) in Poaceae, Portulaceae, and Cucurbitaceae leaves, indicating that *H. sabdariffa* calyx is not a good source of dietary fibre. Protein content of the genotypes was higher than that reported by Dike (2010) on Portulacaceae (2.4%) and Cucurbitaceae (4.20) leaves. Mgaya *et al*., (2014) and Cissé *et al*., (2009) reported higher protein content in the leaves and seeds of Roselle than in Millet and Sorghum. With the increasing population in Saharan Africa and the problems of nutrition and health, Roselle may serve as source of nutrition mainly for cheap protein.

Analysis of mineral and vitamin composition of the genotypes showed high phosphorus and moderate calcium with Kano genotype having the phosphorus (55.80mg/100g) and calcium (2.843mg/100g) contents. Fasoyiro *et al*., (2005) reported higher contents of calcium and phosphorus in Roselle than in Orange, Pineapple and Apple, Orange and Pineapple respectively. High calcium is useful in blood clotting, maintenance of electrical conductivity in nerve cells, enzymes activation in metabolic processes and in the absorption of vitamin B (Ekeanyanwu *et* *al*., 2010). Calcium is also major mineral constituent of the skeleton, lack of it in the body results in the breakdown of bones. Phosphorus is necessary for the innumerable biochemical reactions in which P serves as substrates or products such as ATP and as an integral part of phos-pholipids, phosphoproteins and phosphosugars (Taiz and Zeiger, 1998).This showed that Roselle calyx can supplement the daily calcium and phosphorus requirements of an average Nigerian. High content of vitamin C and vitamin $B_{3,}$ were recorded for the genotypes, Kano genotype had the highest composition of each (Table 3). The vitamin C content of these genotypes compared favourably with vitamin C content of *Citrus* spp and *Piper nigrum* (Fasoyiro *et al*., 2005, Nwofia *et al*., 2013).The inclusion of Vitamin B complex and vitamin C in diets is a good source of antioxidants and enough vitamins for the formation of enzymes that are essential for optimum health. Vitamin C is the primary soluble anti-oxidant which prevents damage in the aqueous environment in and outside the cells. Vitamin C, calcium and proteins are involved in bone formation (Ekeanyanwu *et al* 2010). These underline the importance of Roselle calyx for the good health of average Nigerians.

Alkaloids, oxalates and phenol content of the calyx (Table 4) were lower than earlier reports of Dike, (2010) for *Piper guineense* leaves, and Okereke, *et al*., (2015) in *H. sabdariffa*. Tannins and flavonoids concentrations were slightly higher in these genotypes. Tannins were also slightly higher than those reported by Echo *et al*., (2012) in *Afraomomum melegueta*. Flavonoids act as antioxidants and have been implicated in anti-microbial, anti-inflammatory, anti-allegic, anti-viral and anti-neoplastic activities. High tannin content has been reported to reduce feed intake, growth rate and protein digestibility in animals. The tannin content of *H. sabdariffa* calyx could be reduced much more during processing. High level of cyanide is associated with serious health problem and plants with more than 200mg/100g of fresh weight are considered dangerous (Betaneur- Ancona *et al*., 2008).The very minute levels of cyanide and oxalate showed that the nutrient quality of these genotypes is high and could improve the diet of Nigerians. The moderate levels of flavonoids indicate the usefulness of *H. sabdariffa* in the diets of average Nigerian. Apart from tannin, *H. sabdariffa* compared favourably with phytochemical compositions of *Piper nigrum* (Nwofia *et al*., 2013) and *Piper guineense* (Dike, 2010). Low phytochemical contents lead to better bioavailability and absorption of minerals. Hence, calyx of these *H. sabdariffa* genotypes could improve the diet of Nigerians who eat meals from cereals mostly.

The estimates of the variance components for all the traits showed that the differences between genotypic and phenotypic components were very small, indicating that genotypic componenet contributed most to the total variance for these traits (Table5). The variability observed in the proximate, minerals, vitamins and phytochemical compositions may be attributed to genetic factor, offering considerable opportunity for selection among the genotypes. Similar results were

Table1: Variance ratio of proximate, mineral, vitamins and phytochemical composition of calyx of genotypes of *H.sabdariffa* from Nigeria.

Parameter	Mean square Genotype	Error	Variance ratio
Proximate			
Ash	0.7203	0.0213	33.8000***
Carbohydrate	15.3230	1.4820	10.3400**
Crude Fibre	0.2938	0.0177	16.6000**
Fat	0.3321	0.0158	21.0200**
Moisture	2.0922	0.0307	68.2200***

Protein	113.6440	1.3650	83.2600**
Minerals			
Calcium	0.88522	0.0379	22.49**
Potassium	1.7071	0.2740	6.2300*
Phosphorus	26.154	1.5950	16.4000**
Vitamins			
B carotene	0.0368	0.0003	122.6780***
Vitamin C	3.5505	0.0621	57.1500***
Vitamin B3	2.1929	0.0579	37.8700***
Vitamin B2	0.0043	0.0005	8.6000*
Vitamin B1	0.0131	0.0002	65.5000***
Phytochemical			
Alkaloids	0.1390	0.0002	736.0600***
Cyanide	0.0610	0.0009	67.7800***
Flavenoid	0.4549	0.0035	129.9700***
Oxalate	0.0141	0.0002	70.7000***
Saponin	0.0128	0.0026	48.0400***
Tannin	0.6860	0.0005	1,372.000***
Phenol	0.0034	0.0001	33.7800***

*****=Significant at 5% probability, ******= Significant at 1% probability, *******= Significant at 0.1% probability

Table 2: Proximate composition of the calyx of some *H. sabdariffa* genotypes from Nigeria

Genotype	%Ash	% Carbohydrate	% Crude Fibre	%Moisture	% Protein	% Fat
Kano genotype (Dark red calyx)	1.7270	84.600	1.2770	4.8770	5.9070	1.5770
Abuja genotype (Red calyx)	1.9540	86.230	1.2530	4.0200	5.3800	1.1800
Awka genotype (Green calyx)	1.1630	89.070	1.8070	3.2070	5.1430	0.9160
LSD0.05	0.2916	2.432	0.2658	0.3499	0.2593	0.2511

Table 3: Minerals and Vitamins composition of calyx of *H. sabdariffa* genotypes from Nigeria

Genotype	Calcium (	K mg/100	P)	Vitamin C (	Vitamin B3	Vitamin B2 mg/g	Vitamin B1	BCarotene)
Kano	2.843	1.800	55.80	5.217	4.080	0.3356	0.1636	0.4103
Abuja	1.807	1.810	50.33	7.377	2.713	0.2324	0.1207	0.2453
Awka	2.110	3.110	51.13	6.523	2.507	0.2128	0.0882	0.1998
Average	2.253	2.240	52.420	6.372	3.100	0.2603	0.1242	0.2851

$LSD_{0.05}$	0.3893	1.046	2.523	0.4980	0.4808	0.0296	0.0440	0.0378

Table 4: Phytochemical composition of calyx of *H. sabdariffa* genotypes from Nigeria

Genotype	Alkaloid (	Cyanide	Flavonoids	Oxalate Mg/g	Saponin	Tanninmg/g	Phenol)
Kano	1.8367	0.1533	3.4430	0.3733	0.8233	2.7733	0.2433
Abuja	1.7833	0.1233	3.3130	0.4133	0.7467	2.3667	0.2033
Awka	1.4400	0.0647	2.7130	0.2800	0.6933	1.8300	0.1767
Average	1.6867	0.1138	3.0187	0.3555	0.7544	2.3133	0.4045
$LSD_{0.05}$	0.0275	0.0196	0.1182	0.0298	0.0326	0.0441	0.0199

Table 5: Phenotypic, genotypic and error variances for proximate, minerals, vitamins and phytochemical composition of calyx of *H. sabdariffa* genotypes from Nigeria.

Parameters	**σ^2ph**	**σ^2g**	**σ^2e**
Proximate			
Ash	0.2401	0.2188	0.0213
Carbohydrate	5.1077	4.6137	1.4820
Crude Fibre	0.0979	0.0920	0.0177
Fat	0.1107	0.1054	0.0158
Moisture	0.6974	0.6872	0.0307
Protein	37.8813	37.4263	1.3650
Minerals			
Calcium	0.2951	0.2824	0.0379
Potassium	0.5690	0.4777	0.2740
Phosphorus	8.7180	8.1863	1.5950
Vitamins			
B carotene	0.0123	0.0122	0.0003
Vitamin C	1.1835	1.1628	0.0621
Vitamin B3	0.7310	0.7117	0.0579
Vitamin B2	0.0020	0.0015	0.0005
Vitamin B1	0.0044	0.0043	0.0002
Phytochemical			
Alkaloids	0.0464	0.0463	0.0002
Cyanide	0.0203	0.0200	0.0009
Flavonoids	0.1516	0.1505	0.0035
Oxalate	0.0141	0.0139	0.0002
Saponin	0.0043	0.0034	0.0026
Tannin	0.2287	0.2285	0.0005
Phenol	0.0012	0.0011	0.0199

reported by Baye (2002) on *Venonia galamensis*, Nwofia and Adikibe (2012) on *Osimum gratissimum*, as well as Chinatu (2015) on *Abelmoschus caillei*. Phenotypic and genotypic coefficients of variability, broad sense heritability and genetic advance were estimated for proximate, mineral, phytochemical and vitamin compositions (Table 6). PCV was slightly higher than GCV. This confirmed that heritable variation is present in quantitative traits of the genotypes. The amount of heritable variations and improvement efficiency is related to the magnitude of

**Table 6: Phenotypic coefficient, genotype coefficient and environmental coefficient of variations,
broad sense heritability and genetic advance of proximate, mineral, vitamins and phytochemical composition of the leaves of *H. sabdariffa* genotypes.**

Parameter	Mean	PCV	GCV	H_2B	GA
Proximate					
Ash	1.390	35.2518	33.6518	0.95	0.4950
Carbohydrate	90.630	2.4937	2.3700	0.95	10.5357
Crude fibre	0.446	70.1547	68.0079	0.97	0.6066
Fat	1.224	27.1827	26.5240	0.98	40.6504
Moisture	5.035	16.5860	16.4643	0.99	19.5705
Protein	1.477	416.7081	414.1980	0.98	48.5450
Mineral					
Calcium	2.253	24.1115	23.5869	0.96	1.0725
Potassium	2.240	33.6750	30.8553	0.92	1.3064
Phosphorus	52.42	5.6326	5.4582	0.97	16.8881
Vitamin					
B Carotene	0.285	38.9142	38.7556	0.99	0.2271
A.Acid	6.372	17.0723	16.9230	0.99	15.4190
Niacin	3.100	27.5802	27.2136	0.98	31.4063
Thiamin	0.124	36.0656	31.2337	0.75	0.0692
Riboflavin	0.260	25.5124	25.2209	0.98	0.1337
Phytochemical					
Alkaloids	1.6867	12.7709	12.7571	0.99	49.8922
HCN	0.3556	40.0669	39.7698	0.99	0.2896
Flavonoids	3.1570	12.3332	12.2883	0.99	32.0915
Oxalate	0.3556	33.3924	33.1547	0.99	0.2415
Saponin	0.7544	8.6922	7.7293	0.90	0.1069
Tannins	2.3233	20.5839	20.5749	0.99	43.0040
Phenol	0.2078	16.6704	15.9607	0.99	0.0569

GCV, h2B and GA (Johnson *et al.*,1955). Traits with high GCV, h2B and GA could be improved through selection, since they are considered to be products of additive gene effect (Iwo and Ekaette, 2010). These were observed on carbohydrate, protein, phosphorus and vitamin C content of the genotypes. On the other hand, traits with high heritability and very low genetic advance are less likely to facilitate an effective selection due to influence of non-additive gene action, i.e., dominance and epistasis (Iwo and Ekaette, 2010). This was observed on ash, βcarotene, vitamins B_2 and B_1, alkaloids. The environmental effect appears to be more prominent on the performance of these traits.

Conclusion

From the overall results, *H. sabdariffa* calyx is a good source of essential nutrients, mineral (phosphorus), vitamins C , vitamin B3, βcarotene, alkaloids and flavonoids. Considerable variations in proximate, mineral, vitamins and phytochemical composition were observed among the genotypes as shown by the genetic component analysis for those characters. Hence, rooms exist for improvement of its nutritional, phosphorus and phytochemical composition and increase its availability to Nigerians for consumption.

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