Agro-morphological variability and prospects of genetic improvement of *Hibiscus sabdariffa* L. accessions in some central African countries

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Abstract

Objective: *Hibiscus sabdariffa* is a mallow plant with diverse economic and pharmacological potential that is underutilized in Cameroon. This study aimed to evaluate the genetic variability of 36 accessions from several countries of the Central African sub-region.

Methods: The experiment was conducted as an alpha lattice design (6 x 6) with two replications at Yaoundé I University. Data from five plants in each plot per replication were randomly selected from the middle row and harvested at the 50% flowering stage. Principal component analysis (PCA), hierarchical clustering analysis, and subsequent discriminant analysis were carried out to group the studied accessions.

Results: The results of the analysis of variance showed significant differences (p < 0.0001) among accessions for the studied agro-morphological traits. The height of the first branch (HFB) ranged from 2.62 to 14.89 cm and the plant height (PH) ranged from 20.66 to 83.68 cm. Chlorophyll content (CC) was negatively correlated with the date of initiation of the first flower bud (DIFB) (-0.6), date of the 50% of flower buds (D50F) (-0.4), and date of flowering (DF) (-0.3). DIFB was negatively correlated with PH (-0.4) but collar diameter (CD) was positively correlated with PH (0.6) and number of branches (NB) (0.7). In addition, D50F was positively correlated with DF (0.7), DIFB (0.5), NB (0.5), HFB (0.3), and CD (0.3). PH had a positive correlation with HFB (0.4). DIFB showed a positive correlation with NB (0.4). Cluster analysis grouped the accessions into three groups of 13, 13, and 10 entries. No differences were found between the accession origins for the characteristics examined. High heritability was observed for growth traits such as PH (0.88), HFB (0.87), NB (0.78), and CD (0.75). These

characteristics also showed very high genetic gain (as the percent of the mean). **Conclusion**: Large genetic diversity, high heritability, and high genetic gain for the studied traits indicate a great potential for selection and genetic improvement of these accessions in *H. sabdariffa*. The association of quantitative variables and accessions in the PCA indicated that 10 top accessions represent potential parents for breeding programs of *H. sabdariffa*.

Introduction

The Roselle (*Hibiscus sabdariffa*) with its attractive flower, is cultivated in many developing countries and belongs to the mallow family (Malvaceae) with a chromosome number of 72 (2n = 4x = 72) (Mohammad *et al.* 2020). *H. sabdariffa* plays an important role in people's lives as it provides basic needs such as food, clothing, shelter, and medicine. It is cultivated for its stems, leaves, calyxes, and seeds, which are used industrially, medicinally, and otherwise (Wright *et al.* 2007; Riaz and Chopra 2018). The most important aspects of Roselle, namely the nutritional composition including antioxidant properties, protein quality, and lipid profiles, together with dietary fiber, have been reported (Ismail *et al.* 2008; Karma and Chavan 2016). Research done by Shruthi *et al.* (2016) demonstrated that aqueous extracts of *H. sabdariffa* calyxes exhibited characteristic intense red coloration attributed to the presence of anthocyanins, suggesting their potential utilization as coloring agents in pharmaceutical products. According to Ali *et al.* (2017), *H. sabdariffa* and its anthocyanins could be considered as promising safe dietary agents that could be used to attenuate the progression of human chronic kidney disease. The antibacterial activity of Roselle extracts against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus* mutants, and *Pseudomonas aeruginosa*, showed varying degrees of inhibition on the tested organisms (Al-Hashimi 2012; Prasetyoputri *et al.* 2021).

Despite the undeniable role that plant genetic resources can play in sustainable development, some have not been receiving enough attention and have often been considered as neglected plants or orphan crops, and threatened with extinction. For these species, a considerable amount of information is lacking, preventing any breeding activities that can help to enhance their value. According to our knowledge, there has been a notable absence of scientific studies regarding the genetic diversity of *H. sabdariffa* in Cameroon. The assessment of phenotypic and genotypic

variability is of great importance for using any efficient selection method to improve a population (Ibrahim *et al.* 2013). Genetic diversity is a dynamic property of germplasm and its estimation may be based on morphological evaluation, biochemical, or molecular assessment (Bhandari *et al.* 2017). In any crop improvement program, genetic diversity plays a crucial role, as it aids in selecting the appropriate parents for hybridization programs, leading to the development of superior hybrids and desirable recombinants (Ravali *et al.* 2017). Diversity in plant genetic resources provides an opportunity for plant breeders to develop new and improved cultivars with desirable characteristics, which include both farmer-preferred traits (yield potential and large seed, etc.) and breeders-preferred traits (pest and disease resistance and photosensitivity, etc.) (Govindaraj *et al.* 2015). A better understanding of genetic diversity and its distribution for a crop is essential for its conservation and use (Rao and Hodgkin 2002). This study aimed to assess the genetic variability of 36 accessions from three countries in the central African sub-region to use them later for improvement of grain yield.

Materials and Methods

Experimental site

This study was carried out from February 2023 to April 2023 at the University of Yaoundé I. Yaoundé is located in the central region of Cameroon. The climate is equatorial Guinean with four seasons including two dry seasons (December-February and July-August) and two rainy seasons (March-June and September-November). It is located in agro-ecological zone V (forest zone with bimodal rainfall/bi-modal forest zone), with rainfall generally ranging from 1500 to 2000 mm/year and the average temperature is 23 °C at an altitude of around 797 m (Yengoh *et al.* 2010).

Plant material

The plant material consisted of 36 accessions of *H. sabdariffa* collected from Cameroon, Chad, and the Central African Republic. The information about accessions is reported in Table 1.

Experimental design

The trial was carried out in an alpha lattice design with two replications. Each replication consisted of 36 units of 1.5 m long and 0.70 m wide, separated from each other by a 0.40 m furrow. The two blocks were separated from each other by a distance of 1.20 m giving a total surface area of 149.6 m^2.

Table 1. Code, name, and origin of the *Hibiscus sabdariffa* accessions used in the study.

No.	Code	Accession name	Origin
01	HS-A01	Yaoundé (A) Rouge	Yaoundé (Cameroon)
02	HS-A02	CAR III (bimbo)	CAR
03	HS-A03	Chad II	Chad
04	HS-A04	Garoua II (2)	Garoua (Cameroon)
05	HS-A05	Mokolo rouge	Mokolo (Cameroon)
06	HS-A06	Guider I	Guider (Cameroon)
07	HS-A07	Yaoundé blanc	Yaoundé (Cameroon)
08	HS-A08	Obala III blanc	Obala ((Cameroon)
09	HS-A09	CAR I rouge	CAR
10	HS-A10	Maroua I (A)	Maroua (Cameroon)
11	HS-A11	Garoua IV (B) rouge	Garoua (Cameroon)
12	HS-A12	Garoua IV (A) rouge	Garoua (Cameroon)
13	HS-A13	Garoua (I) blanc	Garoua (Cameroon)
14	HS-A14	Ngaoundal (I) blanc	Ngaoundal (Cameroon)
15	HS-A15	Garoua (III) rouge	Garoua (Cameroon)
16	HS-A16	Yaoundé (B) blanc	Yaoundé (Cameroon)
17	HS-A17	Yaoundé (A) blanc	Yaoundé (Cameroon)
18	HS-A18	Guider (A) blanc	Guider (Cameroon)
19	HS-A19	Garoua II (1) rouge	Garoua (Cameroon)
20	HS-A20	Chad I (B)	Chad
21	HS-A21	Guider II	Guider (Cameroon)
22	HS-A22	Bibemi II (A)	Bibemi (Cameroon)
23	HS-A23	Garoua II (B) rouge	Garoua (Cameroon)
24	HS-A24	Foumbot B	Foumbot (Cameroon)
25	HS-A25	Ngaoundal (A) blanc	Ngaoundal (Cameroon)
26	HS-A26	Chad IV	Chad
27	HS-A27	Bibemi II (B)	Bibemi (Cameroon)
28	HS-A28	Garoua IV (C)	Garoua (Cameroon)
28	HS-A29	Guider II (A)	Guider (Cameroon)
30	HS-A30	Bibemi (A) blanc	Bibemi (Cameroon)
21	HS-A31	Chad I (A)	Chad
32	HS-A32	Garoua II (C)	Garoua (Cameroon)
33	HS-A33	Chad III	Chad
34	HS-A34	Foumbot A	Foumbot (Cameroon)
35	HS-A35	Ngaoundal II blanc	Ngaoundal (Cameroon)
36	HS-A36	CAR III	CAR

Phenotypic assessment

The data for morphological traits were collected based on previous studies on this species (Satyanarayana *et al*. 2015; Sharma *et al.* 2017). The data were collected from five plants in each plot of four rows of five plants, which were randomly selected from the middle rows, excluding the border plants, and harvested at the stage of 50% flowering as reported by Bandi and Appalaswamy (2014). The study focused on 10 quantitative traits including plant height (PH), height to the first branch (HFB), collar diameter (CD), number of branches (NB), flowering date (FD) (i.e. date of the first bloom appearance), date of initiation of the first flower bud (DIFB), date of the 50% of the plants with flower buds (D50F), number of separate lobes (NSL), petiole length (PL), and chlorophyll content (CC), assessed 60 days after sowing (Ouangraoua *et al.* 2022). The 11 qualitative traits concerning morphotype, leaf color, leaf type, shape of leaf base, apex shape, petiole color, petal color, stigma color, sepal color, calyx color, and capsule shape were evaluated.

Assessment of diversity

The estimation of the parameters related to diversity, covered environmental variance, genotypic variance, phenotypic variance on an entry mean basis, environmental coefficients of variation, genotypic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), broad sense heritability, genetic gain, and the genetic gain as a percentage of the mean (Hajam *et al.*2018; Tessema *et al.*2022). The corresponding formulae for estimating these parameters are presented in Table 2. The magnitude of genetic advance, which is estimated as a percent of the mean, was classified as low (<10%), moderate (10-20%), and high (>20%) according to Ferdoush *et al.* (2017). Heritability was also categorized as low <30%, moderate 30–60%; high > 60% (Medagam *et al.* 2015; Singha and Ullah 2020).

Statistical analysis

The data were subjected to analysis of variance with R software (version 3.5.1) and subsequent multiple comparisons of the means were carried out by the least significant difference (LSD) method at the 5% probability level. Principal component analysis (PCA), hierarchical clustering analysis, and subsequent discriminant analysis were performed using Variability Package of R software. PCA is generally used to describe genotypes based on several variables (Maia *et al.* 2019).

The potential accessions for later genetic improvement programs were selected based on the several quantitative characteristics including PH, HFB, CD, and NB, that showed significant differences.

Table 2. Parameters and formulas for their estimation in *Hibiscus sabdariffa* accessions.

Parameter	Formula
Phenotypic variance (PV)	$VP = VG + (MSE/r) = MSG/r$
Genotypic variance (GV)	$VG = (MSG - MSE)/r$
Broad sense heritability on an entry mean basis (h^2)	$h^2\ (\%) = (VG/VP)*100$
Genotypic coefficient of variation (GCV)	$GCV\ (\%) = (\sqrt{VG}/X)*100$
Phenotypic coefficient of variation (PCV)	$PCV\ (\%) = (\sqrt{VP}/X)*100$
Expected genetic gain (GA)	$GA = h^2\sqrt{VP}*I$
Expected genetic gain as a percent of the mean	$GAM = (GA/X)*100$

MSE: Error mean square; MSG: Mean square of genotypes; r: Number of replications; $\sqrt{VG}$: Standard deviation of genotypic variance; $\sqrt{VP}$: Standard deviation of phenotypic variance; I: Standardized selection differential; I = 2.06 for a selection intensity of 5%; X: Trait mean.

Results

Phenotypic assessment

***Qualitative traits*:** Regarding the color of the plant, the results of the qualitative variables showed that the 36 accessions are grouped into white or green morphotypes (55.56%) and red morphotypes (44.44%). Leaves were green (47.22%), light green (22.22%), red (19.44%), and purple (11.11%) (Table 3). For the lobe traits, there were lobeless accessions (38.89%) and accessions with two separate lobes (8.33%), three separate lobes (19.44%), four separate lobes (2.78%), five separate lobes (19.44%), and six separate lobes (11.11%) (Table 3, Figure 1). The lobes were either light (11.11%), moderate (13.89%), deep (25%), or very deep (11.11%). The base of the leaf was cordate (25%), rounded or obtuse (5.56%), truncate (19.44%), acute (30.56%) or linear (19.44%). The apex was either acute (47.22%), very acute (22.22%), obtuse (25%), or pointed (5.56%). Roselle accessions were all characterized by the presence of a short petiole except Obala_I which had an intermediate petiole. The color of the petioles was green (33.33%), dark red (16.67%), light green (19.44%), red (11.11%), and light red (19.44%) (Table 3). The petals in our accessions varied in color, including pink (77.78%), light pink (2.78%), and yellowish (19.44%) (Table 3, Figure 2). The stigma color was purple (72.22%) and white (27.78%) (Table 3, Figure 3). Calyces were green (13.89%), light green (27.78%), purple (44.44%) and pigmented (13.89%). They are either ovoid (66.67%) or round (33.33%) (Table 3).

***Quantitative traits*:** Analysis of variance showed significant differences among the accessions for all characteristics assessed. The average heights of the plants ranged from 20.66 cm (for HS-A18) to 83.68 cm (HS-A15), and HFB in the accessions varied from 2.62 cm (HS-A33) to 14.89 cm (HS-

A10). The CD of the accessions ranged between 0.50 cm (HS-A29) and 1.41 cm (HS-A03). NB ranged from 1 (HS-A18 and HS-A20) to 10.85 (HS-A25). The CC ranged from 38.46 Meq (HS-A16) to 49.67 Meq (HS-A15) (Table 4).

Estimates of genetic parameters

The phenotypic variance ranged from 0.16 to 466.36 for the CD and PH, respectively, while the genotypic variance ranged from 0.12 to 412.69 for the same traits, respectively (Table 5). GCV ranged from 8.32% (CC) to 67.23% (NB), while PCV ranged from 10.02% (CC) to 76.24% (NB). The PCV was relatively higher than the GCV for all traits. The environmental coefficient of variation ranged from 0.00 (DF and DIFB) to 50.81 (NB). Heritability values ranged from 69% (CC) to 100% (DF and DIFB). All traits expressed high heritability values ($h^2 \geq 60\%$). Expected genetic gains as a percentage of the mean, ranged from 65.74% for CD to 119.55% for FD (Table 4). According to Ferdoush *et al.* (2017), all gains were regarded as high (>20%).
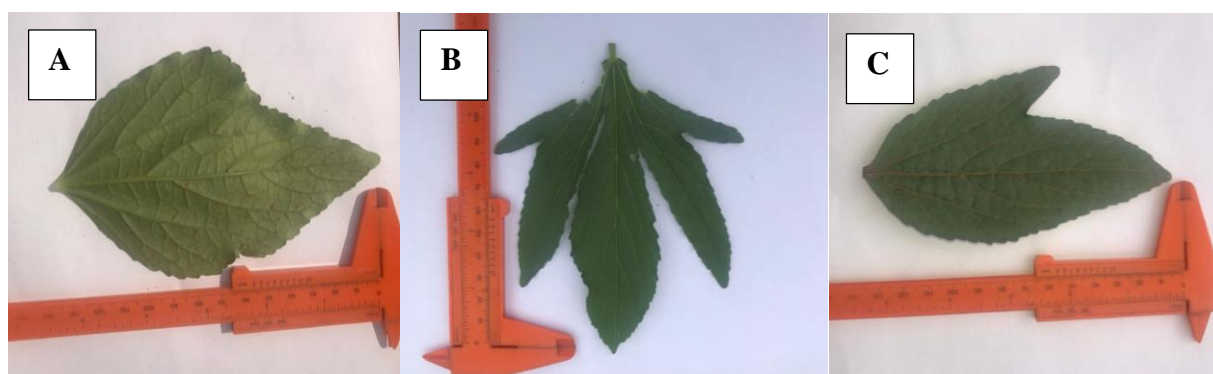
Figure 1. Characterization of *Hibiscus sabdariffa* leaves based on the number of lobes; A= Leaf without lobes, B= Leaf with deep lobes, C= Leaf with two lobes.

Figure 2. Characterization of *Hibiscus sabdariffa* accessions based on color of petals; A= Light pink, B= Yellowish, C= Pink.
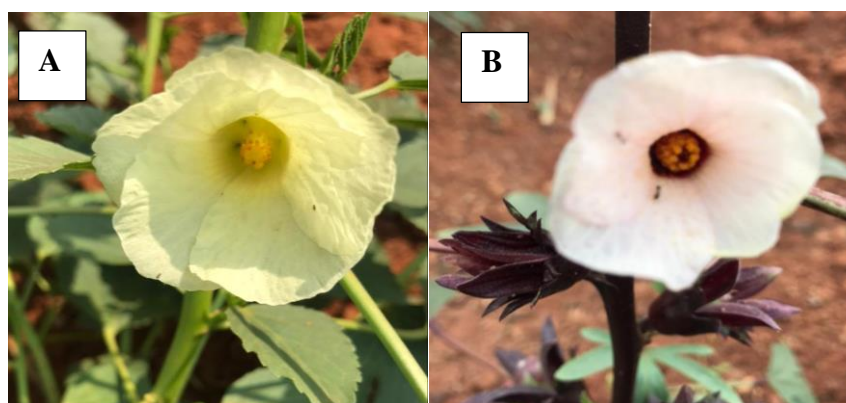
Figure 3. Characterization of *Hibiscus sabdariffa* accessions based on the stigma color: A= White, B= Purple.

Table 3. Diversity of qualitative characteristics in the *Hibiscus sabdariffa* accessions.

Trait	Phenotypic form	Score	%	Trait	Phenotypic form	Score	%
MPT	White	20	55.56	LC	Green	17	47.22
	Red	16	44.44		Light green	8	22.22
PL	Short	35	97.22		Purple	4	11.11
	Intermediate	1	2.78		Red	7	19.44
NSL	No	14	38.89	CaC	Green	5	13.89
	Two	3	8.33		Light green	10	27.78
	Three	7	19.44		Purple	16	44.44
	Four	1	2.78		Pigmented	5	13.89
	Five	7	19.44	LT	No lobe	14	38.89
	Six	4	11.11		Light lobe	4	11.11
PC	Green	12	33.33		Moderate lobe	5	13.89
	Light green	7	19.44		Deep lobe	9	25.00
	Red	4	11.11		Very deep lobe	4	11.11
	Light red	7	19.44	ShLB	Linear	7	19.44
	Dark red	6	16.67		Cordate	9	25.00
StC	White	10	27.78		Truncated	7	19.44
	Purple	26	72.22		Sharp	11	30.56
SC	Green	7	19.44		Rounded/obtuse	2	5.56
	Light green	11	30.56	ASh	Acute	17	47.22
	Purple green	13	36.11		Pointed	2	5.56
	Purple-green	1	2.78		Obtuse	9	25.00
	Pink	4	11.11		Very sharp	8	22.22
PCo	Light pink	1	2.78	CSh	Round	12	33.33
	Yellowish	7	19.44		Ovoid	24	66.67
	Pink	28	77.78				

MPT: Morphotype; PL: Petiole length; NSL: Number of separate lobes; PC: Petiole color; StC: Stigma color; SC: Sepal color; PCo: Petal color; LC: Leaf color; CaC: Calyx color; LT: Leaf type; ShLB: Shape of leaf base; ASh: Apex shape; CSh: calyx shape.

Table 4. Quantitative characteristics of the *Hibiscus sabdariffa* accessions.

Accession	PH (cm)	HFB (cm)	CD (cm)	NB	CC (Meq)
HS-A01	48.68 ± 12.12	10.70 ± 2.50	1.20 ± 0.44	8.45 ± 5.63	45.47 ± 4.29
HS-A02	35.65 ± 14.93	5.18 ± 1.16	0.97 ± 0.29	7.28 ± 2.87	40.80 ± 4.73
HS-A03	48.14 ± 15.00	3.53 ± 1.64	1.41 ± 0.50	9.55 ± 3.50	42.29 ± 1.50
HS-A04	65.34 ± 10.38	5.48 ± 1.16	1.27 ± 0.45	6.57 ± 3.64	48.78 ± 3.12
HS-A05	59.27 ± 11.87	9.51 ± 3.40	0.97 ± 0.29	4.22 ± 2.82	49.13 ± 6.05
HS-A06	50.25 ± 16.55	8.12 ± 3.38	1.06 ± 0.32	4.50 ± 2.83	45.30 ± 2.09
HS-A07	53.31 ± 10.20	7.47 ± 1.56	1.29 ± 0.32	10.90 ± 3.14	44.83 ± 4.98
HS-A08	45.46 ± 11.53	7.77 ± 2.93	0.87 ± 0.36	6.84 ± 3.31	44.32 ± 2.56
HS-A09	35.58 ± 12.40	7.44 ± 1.98	0.71 ± 0.51	5.77 ± 4.49	41.66 ± 5.37
HS-A10	63.46 ± 8.80	14.89 ± 2.76	0.99 ± 0.37	6.54 ± 3.29	42.81 ± 0.69
HS-A11	62.28 ± 9.54	10.56 ± 3.94	0.81 ± 0.18	4.50 ± 1.93	42.04 ± 2.18
HS-A12	53.5 ± 18.03	12.70 ± 3.55	0.93 ± 0.20	3.33 ± 2.52	49.60 ± 3.89
HS-A13	48.93 ± 20.45	8.53 ± 3.95	0.98 ± 0.27	6.25 ± 2.66	46.66 ± 4.70
HS-A14	46.85 ± 7.53	12.78 ± 3.08	0.78 ± 0.34	4.70 ± 2.45	41.48 ± 4.75
HS-A15	83.68 ± 4.09	11.13 ± 1.02	1.03 ± 0.09	3.75 ± 3.09	49.67 ± 4.22
HS-A16	33.90 ± 6.97	9.58 ± 1.87	0.73 ± 0.35	6.83 ± 1.27	38.46 ± 3.52
HS-A17	29.20 ± 5.93	7.08 ± 2.72	0.64 ± 0.25	6.41 ± 3.05	40.24 ± 3.16
HS-A18	20.66 ± 12.40	3.93 ± 1.83	0.60 ± 0.26	1.00 ± 1.00	47.29 ± 3.48
HS-A19	83.04 ± 11.73	8.30 ± 3.20	1.27 ± 0.28	9.75 ± 2.59	49.54 ± 2.83
HS-A20	32.15 ± 9.54	10.05 ± 2.75	0.70 ± 0.42	1.00 ± 1.00	49.53 ± 2.85
HS-A21	60.69 ± 13.03	6.61 ± 2.23	0.93 ± 0.19	4.25 ± 2.76	46.69 ± 2.90
HS-A22	54.92 ± 8.07	9.12 ± 2.39	0.73 ± 0.27	4.50 ± 3.67	43.75 ± 4.19
HS-A23	55.80 ± 3.20	5.80 ± 2.64	1.36 ± 0.53	8.80 ± 4.76	44.33 ± 1.33
HS-A24	35.25 ± 13.68	4.08 ± 1.33	0.79 ± 0.20	9.55 ± 4.06	45.16 ± 2.34
HS-A25	56.8 ± 15.77	8.64± 2.53	1.31 ± 0.17	10.85 ± 2.85	43.99 ± 2.64
HS-A26	58.24 ± 6.51	11.56 ± 2.04	1.11 ± 0.19	10.00 ± 2.11	46.97 ± 2.69
HS-A27	32.01 ± 5.20	7.48± 3.11	0.83 ± 0.15	2.75 ± 1.16	46.01 ± 3.33
HS-A28	37.75 ± 14.48	6.87 ± 0.92	0.95 ± 0.41	8.67 ± 3.87	42.87± 3.22
HS-A29	40.25 ± 11.12	3.21 ± 2.43	0.50 ± 0.27	2.13 ± 1.77	46.32 ± 0.67
HS-A30	52.48 ± 7.69	8.78 ± 1.74	0.95 ± 0.26	4.80 ± 1.62	46.95 ± 2.49
HS-A31	69.88 ± 8.26	8.60 ± 2.96	0.74 ± 0.20	4.00 ± 2.21	46.62 ± 3.54
HS-A32	55.35 ± 11.93	9.46 ± 2.85	0.81 ± 0.25	4.10 ± 2.18	45.31 ± 2.96
HS-A33	36.16 ± 1.29	2.62 ± 2.51	0.84 ± 0.13	3.60 ± 0.89	43.28 ± 1.71
HS-A34	48.17 ± 16.91	10.05 ± 1.82	0.90 ± 0.37	6.77 ± 2.77	46.49 ± 6.014
HS-A35	51.15 ± 10.27	10.75 ± 2.34	1.09 ± 0.40	7.90 ± 2.96	46.43 ± 2.89
HS-A36	56.33 ± 18.47	5.84 ± 2.47	0.77 ± 0.30	3.00 ± 1.93	46.47 ± 2.93
LSD5%	21.34	4.91	0.55	6.22	7.36

PH: Plant Height; HFB: Height to the first branch; CD: Collar diameter; NB: Number of branches; CC: Chlorophyll content

Table 5. Estimates of the different parameters for quantitative traits in *Hibiscus sabdariffa* accessions.

| Parameters	PH	HFB	CD	DIFB	DF	CC	NB
Maximum	83.68	14.89	1.41	60.00	47.00	49.67	10.85
Minimum	20.66	2.62	0.50	29.00	7.00	38.46	1.00
Overall mean	50.02	7.96	0.94	39.06	24.11	45.21	5.94
Standard error of the mean	8.86	1.68	0.19	0.00	0.00	2.52	2.13
Environmental variance	107.34	5.69	0.07	0.00	0.00	12.75	9.11
Genotypic variance	412.69	18.57	0.12	182.73	195.76	14.16	15.95
Phenotypic variance	466.36	21.42	0.16	182.73	195.76	20.54	20.51
Environmental coefficient of variation	20.71	29.97	28.15	0.00	0.00	7.90	50.81
Genotypic coefficient of variation	40.61	54.14	36.85	34.60	58.03	8.32	67.23
Phenotypic coefficient of variation	43.17	58.14	42.55	34.60	58.03	10.02	76.24
Broad sense heritability	0.88	0.87	0.75	1.00	1.00	0.69	0.78
Genetic gain	39.37	8.27	0.62	27.85	28.82	6.44	7.26
Genetic gain as a percentage of the mean	78.70	103.84	65.74	71.29	119.55	14.24	122.14

PH: Plant height; HFB: Height to the first branch; CD: Collar diameter; DIFB: Date of initiation of the first flower bud; DF: Date of flowering; CC: Chlorophyll content; NB: Number of branches.

Correlation between traits

Twenty-eight correlation coefficients were generated from eight quantitative traits. Eighteen were significant and 10 of them were not significant. Among the significant correlations, five were negative and 13 were positive. CC was negatively correlated with DIFB (-0.6), D50F (-0.4), DF (-0.3), and NB (-0.3), and positively correlated with PH (0.4). DIFB was negatively correlated with PH (-0.4) and positively correlated with D50F (0.5) and NB (0.4). CD was positively correlated with NB (0.7), PH (0.6), DF (0.4), and D50F (0.3). In addition, D50F was positively correlated with DF (0.7), NB (0.5), and HFB (0.3). HFB was positively correlated with PH (0.4) and DF (0.3) was positively correlated with PH (0.4) with NB (0.5). (Figure 4).

Principal component analysis

The biplot for the 10 most contributed *H. sabdariffa* accessions based on quantitative variables is shown in Figure 5. Only the first two axes of the PCA were taken into account because they explained about 64.7% of the variation observed in the accessions. Dimension 1 contrasts individuals such as HS-A07, HS-A35, HS-A02, HS-A16, HS-A09, and HS-A19 (on the right of the graph, characterized by a positive coordinate on the axis) with individuals such as HS-A18, HS-A29, HS-A15, and HS-A17 (on the left of the graph, characterized by a negative coordinate on the axis). Dimension 2 contrasts individuals such as HS-A19, HS-A15, HS-A07, and HS-A35 (at the top of the graph,
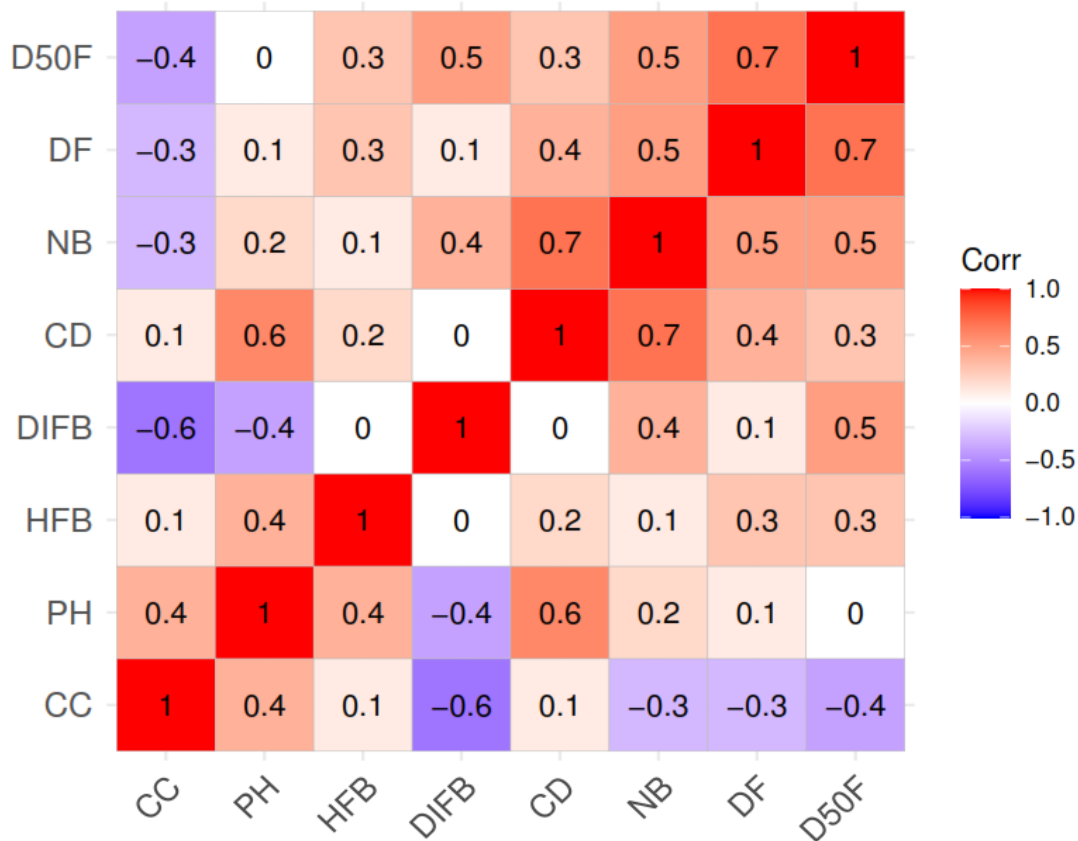
Figure 4. Correlation coefficients between some characteristics of *Hibiscus sabdariffa* accessions studied. PH: Plant height; HFB: Height to the first branch; CD: Collar diameter; DIFB: Date of initiation of the first flower bud; DF: Date of flowering; NB: Number of branches; CC: Chlorophyll content; D50F: Date of the 50% of the plants with flower buds. The correlations higher than or equal to 0.30 and 0.424 are significant at 0.05 and 0.01 probability levels, respectively.
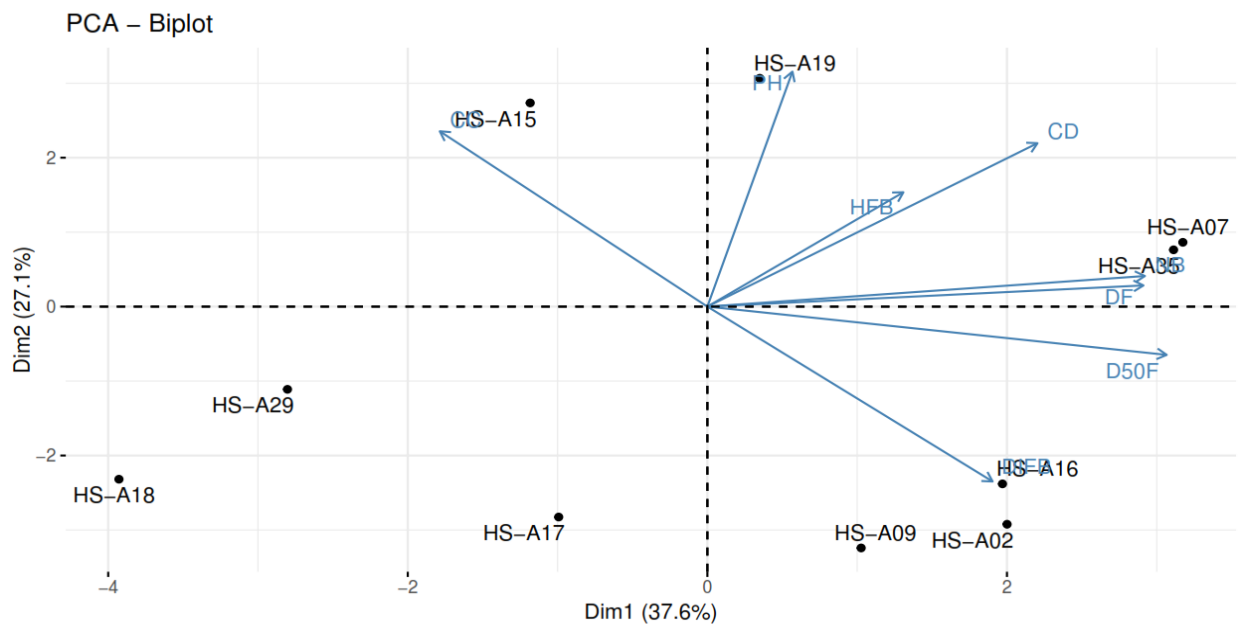
Figure 5. Biplot for the 10 most contributed *Hibiscus sabdariffa* accessions based on quantitative variables studied. PH: Plant height; D50F: Date of the 50% of the plants with flower buds; CD: Collar diameter; DIFB: Date of initiation of the first flower bud; CC: Chlorophyll content; NB: Number of branches; DF: Date of flowering; HFB: Height to the first branch.

characterized by a positive coordinate on the axis) with individuals such as HS-A09, HS-A02, HS-A17, HS-A18, HS-A16, and HS-A29 (at the bottom of the graph, characterized by a negative coordinate on the axis). Accessions HS-A16, HS-A02, and HS-A09 belonged, shared higher values for the variables DIFB and D50F and lower values for the variable CC. Accessions HS-A07 and HS-A35 shared higher values for the variables NB, DF, D50F, CD, and HFB, and lower values for CC. Accessions HS-A18, HS-A29, and HS-A17 shared lower values for the variables PH, CD, NB, HFB, and DF. Accessions HS-A19 and HS-A15 shared higher values for the variables PH and CC, respectively, and lower values for the variables D50F and DIFB (Figure 5).

Classification of H. sabdariffa accessions

The hierarchical ascending classification allowed classifying or structuring the 36 accessions of *H. sabdariffa* into three clusters based on eight quantitative characteristics of *H. sabdariffa* accessions. Figure 6 shows the cluster members after a discriminant analysis for the three clusters. The discriminant analysis separated the three clusters obtained from the cluster analysis. Cluster 1 included 13 accessions HS-A01, HS-A02, HS-A07, HS-A09, HS-A10, HS-A14, HS-A16, HS-A24, HS-A25, HS-A26, HS-A30, HS-A34, and HS-A35. This group was characterized by higher values for the variables D50F, DF, DIBF, and NB (variables were sorted from the highest value) and low values for CC. Cluster 2 contained 13 accessions and made of individuals including HS-A03, HS-A06, HS-A08, HS-A13, HS-A17, HS-A18, HS-A20, HS-A22, HS-A23, HS-A27, HS-A28, HS-A29, and HS-A33. This group was characterized by lower values for the variables D50F, PH, HFB, and DF (variables were sorted from the lowest). The cluster 3 had 10 accessions which included HS-A04, HS-A05, HS-A11, HS-A12, HS-A15, HS-A19, HS-A21, HS-A31, HS-A32, and HS-A36. This group was characterized by higher values for the variables PH and CC (variables were sorted from the highest) and lower values for the variables DIBF and D50F (variables were sorted from the lowest). Accessions from the three countries (Cameroon, CAR, and Chad) appeared in all three groups without any sharp distinction.

Discussion

The results obtained indicate significant differences among the accessions for all the morphological traits evaluated. This finding aligns with the study conducted by Atta *et al.* (2011) who reported substantial variability among ecotypes for the majority of the yield-related characteristics in *H. sabdariffa* L. Our results showed a positive correlation among CD, NB, and DF. The CD also had a positive correlation with PH. Selection for such traits may optimize yield through their simultaneous
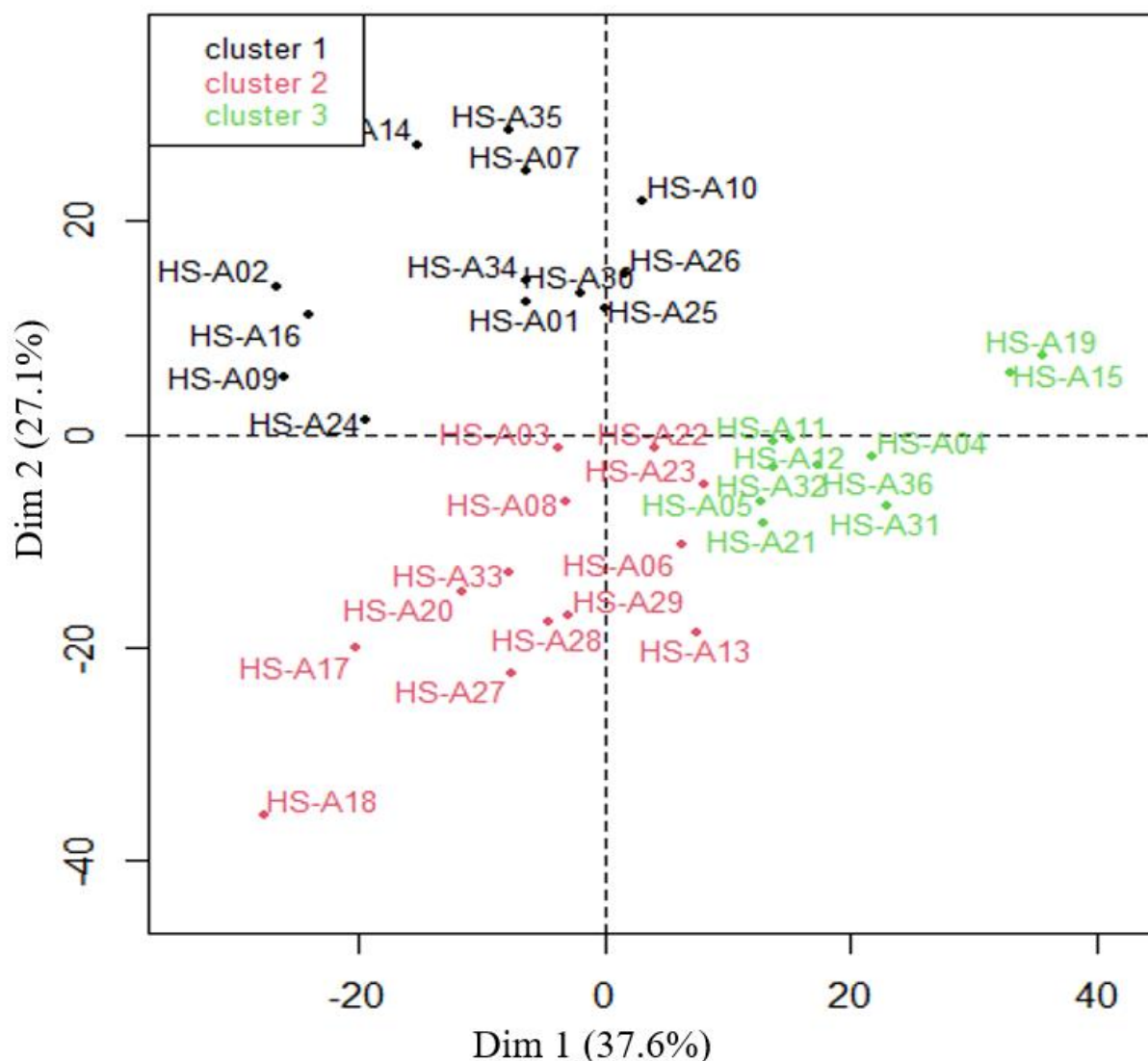
Figure 6. Grouping of different *Hibiscus sabdariffa* accessions by discriminant analysis using eight quantitative morphological characters.

improvement (Tchio *et al*. 2020).

The high coefficients of variation and the large gap between the minimum and maximum values of the characteristics also reflect the variability that exists in the collection of *H. sabdariffa* accessions studied. According to Torres-Morán *et al.* (2011), genetic diversity was observed by using both agro-morphological and molecular traits among the studied *H. sabdariffa* genotypes. Ouangraoua *et al.* (2022) also observed genetic variability among the *H. sabdariffa* accessions of Burkina Faso.

The PH, as well as HFB, which can be considered indicators of plant biomass, varied from 20.66 *to* 83.67 cm and from 2.62 to 14.89 cm, respectively. In a similar work on *H. sabdariffa*, Tetteh *et al.* (2019) obtained the plant height of *H. sabdariffa* from 184.83 cm to 283.50 cm. Their results were different from the results obtained during this experiment. Also, our CD values (0.5 to 1.36 cm) were

lower than those obtained by Tetteh *et al.* (2019). These differences could be due to the plant materials used and/or environmental conditions.

In our research, for all variables, PCVs were higher than the GCVs, suggesting the role of the environment in the expression of the observed traits (Annigeri and Hiremath 2022). According to Ibrahim *et al.* (2013), estimates of environmental variance for the number of capsules/main stem, number of capsules/branch, calyx weight, fruit yield/plant, seed yield/plant, calyx yield/plant, and calyx yield/unit area in *H. sabdariffa* were greater than their respective genotypic variances in two seasons.

We showed that all traits had PCVs and GCVs greater than 20%, except for the CC, which appeared to have low values. The highest GCV (67.23) and PCV (76.24) were obtained for the number of branches. These high values indicate a high potential for selecting these traits effectively (Sattar *et al.* 2007).

The 36 accessions studied were subdivided into three groups or clusters. Ouangraoua *et al.* (2022) could also classify 48 accessions of *H. sabdariffa* collected from eight provinces located in two different climatic zones of Burkina Faso, Soudano-Sahelian, and Soudanian zones. Cisse *et al.* (2009) identified two variants of *H. sabdariffa* in Senegal, namely, the green and red varieties.

Morphological diversity assessed by PCA, cluster analysis, and subsequent discriminant analysis revealed a strong contribution of eight traits to the diversity within the accessions studied. This diversity can be justified by the method through which farmers select seeds, prioritizing main traits based on phenotypic characteristics such as biomass, earliness, and yield (Danbe *et al.* 2019), or based on the effect of environmental factors on the morphology of individuals.

The biplot graph drawn after PCA, in the present study showed that it could be possible to discriminate the 36 accessions of *H. sabdariffa* by the agro-morphological characteristics. Other studies have also shown that PCA could potentially discriminate accessions based on physicochemical and biochemical characteristics (Sanou *et al.* 2022). Torres-Morán *et al.* (2011) divided 12 genotypes of Roselle into four groups.

Estimated heritability values ranged from 0.0.69 (CC) to 1.0 (DIFB and DF). High heritability and genetic gain were observed for PH, HFB, CD, DF, DIFB, and NB. Similar results have been found by Bandi and Appalaswamy (2014). The CC index showed high heritability but moderate genetic gain. Traits with high genetic gain and heritability imply that selection for these traits will give a good response. According to Hajam *et al.* (2018), selection efficiency is achieved more quickly for traits with high heritability and genetic gain, which was the case for traits such as PH, HFB, CD, and NB.

Conclusion

This study aimed to evaluate the genetic variability of 36 accessions of *H. sabdariffa* from several countries of the central African sub-region. The average HFB varied from 2.62 to 14.89 cm and the PH ranged from 20.66 to 83.68 cm. The CD of the accessions ranged from 0.50 to 1.36 cm. The DIFB was negatively correlated with CC (-0.6) and PH (-0.4), and positively correlated with NB (0.4) and D50F (0.5). The negative correlation between CC and D50F (-0.4) was also observed. Also, the CD was positively correlated with PH (0.6) and NB (0.7). In addition, D50F was positively correlated with NB (0.5), DF (0.7), and DIFB (0.5). High heritability was found for growth characteristics such as PH (0.88), HFB (0.87), NB (0.78), and CD (0.75). Also, except for CC, high genetic gains (as % of the mean) were observed for the agro-morphological traits under study (ranging from 65.74 to 122.14%). Large genetic diversity high heritability, and very high genetic gains for these traits in the studied accessions of *H. sabdariffa* indicate a strong potential for selection and genetic improvement of the accessions under study. Using the hierarchical ascending classification, the different accessions were divided into groups of 13, 13, and 10 accessions. However, there were no differences among accessions' origins for any of the traits studied. The association of quantitative variables and accessions shows that 10 top accessions, which actively contributed to the construction of axes 1 and 2 in PCA, are potential parents for use in the breeding programs.

Acknowledgments

Conflict of Interest

On behalf of all authors, the corresponding author states that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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