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Application of Morphological and Phytochemical Markers for Polymorphism Studies in Some *Hibiscus sabdariffa* L. Accessions

Mansoor Saljooghianpour[1*] and Seyyed Mahdi Javadzadeh[1]

[1]*Department of Agriculture, Iranshahr Branch, Islamic Azad University, Iranshahr, Iran.*

Authors' contributions

This work was carried out in collaboration between both authors. Author MS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author SMJ managed the analyses of the study and the literature searches. Both authors read and approved the final manuscript.

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ABSTRACT

Fifteen Roselle (*Hibiscus sabdariffa L.*) accessions were collected from different geographical regions of Baluchestan in Iran and were grown in Randomized Complete Block Design (RCBD) with three replications. Data were recorded on days to 50% flowering (Calyces were harvested when they were tender and plump). When flowering reached 50%, data recording was started (Calyces were harvested when they were tender and plump). Morphological analysis indicated that all the studied characteristics have a significant difference at P< 0.05 or P< 0.01 among Roselle accessions. Cluster analysis indicated slight differences among accessions. Some of accessions performed better than others and were ranked higher. According to the results, the average of similarity among accessions was 97%. Thus, the observed morphological differences and similarity values of *Hibiscus sabdariffa* L. accessions maintained at Iran suggest that all the accessions possess a useable slight genetic diversity. Also, phenotypic correlations between various morphological characteristics indicated that there were highly significant correlations at P< 0.05 or P< 0.01 among the most studied characteristics. Phenotypic correlations indicated that some associated factors correlate with each other and contribute in the occurring of these characteristics.

Corresponding author: E-mail: m.saljooghian.p@gmail.com;

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1. INTRODUCTION

The genus Hibiscus (Malvaceae) includes more than 300 species of annual or perennial herbs, shrubs or trees [1]. Roselle *(Hibiscus sabdariffa* L.) is relatively a new crop in Iran. It was introduced into Iran in early 1850s. Roselle (*Hibiscus sabdariffa L.*) belongs to the family of Malvaceae, which is an auto-tetraploid species with 2n = 4x = 72 and therefore their segregating populations need longer time for purification compared to diploid species [2]. Furthermore, Roselle has cleistogamous flowers (a type of automatic self-pollination of certain plants that can propagate by using non-opening, self-pollinating flowers). Thus, crop improvement may be difficult through conventional hybridization [3,4]. Roselle is cultivated in tropical and subtropical regions for bast fibers, paper pulp or edible calyx, leaves and seeds [5,6,7]. Roselle is an ideal crop for developing countries as it is relatively easy to grow, can be grown as part of multi-cropping systems and can be used as food and fiber. In China the seeds are used for their oil and the plant is used for its medicinal properties, while in West Africa the leaves and powdered seeds are used in meals. Additionally, it is used in the pharmaceutical and food industries [8].

Hibiscus sabdariffa L. race ruber is an annual, erect, bushy, herbaceous subshrub that can grow up to 8 feet. (2.4 m) tall, with smooth or nearly smooth, cylindrical, typically red stems. The leaves are alternate, 3 to 5 in. (7.5–12.5 cm) long, green with reddish veins and long or short petioles. The leaves of young seedlings and upper leaves of older plants are simple; lower leaves are deeply 3 to 5 or even 7 lobed; the margins are toothed. Flowers, borne singly in the leaf axils, are up to 5 in. (12.5 cm) wide, yellow or buff with a rose or maroon eye, and turn pink as they wither at the end of the day. At this time, the typically red calyx, consisting of 5 large sepals with a collar (epicalyx) of 8 to 12 slim, pointed bracts (or bracteoles) around the base, begins to enlarge, becomes fleshy, crisp but juicy, 1 1/4 to 2 1/4 in. (3.2–5.7 cm) long and fully encloses the velvety capsule, 1/2 to 3/4 in. (1.25–2 cm) long, which is green when immature, 5-valved, with each valve containing 3 to 4 kidney-shaped, light-brown seeds, 1/8 to 3/16 in. (3–5 mm) long and minutely downy. The capsule turns brown and splits open when mature and dry. The calyx, stems and leaves are acid and closely resemble the cranberry (*Vaccinium* spp.) in flavor [9,10]. The main constituents of *Hibiscus sabdariffa* L. relevant in the context of its pharmacological are organic acids, anthocyanin, polysaccharides and flavonoids [11,12].

Roselle extracts contain a high percentage of organic acids, including citric acid, hydroxycitric acid, hibiscus acid, malic and tartaric acids as major compounds, and oxalic and ascorbic acid as minor compounds. Based on previous studies, the percentage of organic acids in ‘‘hibisciflos’’ varies; hibiscus acid accounts for 13–24%, citric acid 12–20%, malic acid 2–9%, tartaric acid 8% and 0.02– 0.05% of ascorbic acid (vitamin C) [11]. In the late 1930s, citric and malic acids were first reported in aqueous extracts of the calyx [13, 14,15] and also in five different accessions (from Egypt, Senegal, India, Thailand and Central America) of Roselle var. *sabdariffa* [16]. Ascorbic acid is also present in *H. sabdariffa* L. but its content varies dramatically between fresh (6.7–14 mg/100 g) [9,17] and dried calyces (260–280 mg/100 g) [17]. The amount of ascorbic acid in the latter report being much higher than the ones previously reported in the literature. The differences observed might be due to different varieties, genetics, ecology, environment and harvest conditions.

Development of any crop improvement program essentially depends on nature, magnitude of genetic variability, genetic advance, characteristics association, direct and indirect effects on yield and yield attributes [18]. Several studies on Roselle has been carried out but there is limited of information regarding its genetics, breeding and production, especially genetic improvement under rain-fed conditions [19,20,21]. Moreover, to improve the yield of Roselle, plant breeders should have a better understanding of the genetic variability of yield and it components [22].

High genotypic and phenotypic coefficient of variation and heritability for dry weight, dry calyx and seed weight coupled with high genetic advance values [21]. Similar conclusion was reported by [18,23].The variation in the composition of individual cluster with regard to the number of genotypes indicated the presence of large amount of diversity in the population. The clustering pattern indicated that there was no relationship between distribution and genetic diversity as the genotypes from different origins were grouped into the same clusters. It is an indication for the absence of relationship between genetic diversity and geographic diversity. This suggests that there are forces other than geographical separation such as natural and artificial selection, exchange of breeding materials, genetic drift and environmental variation. The results are in accordance with the findings of [24,25,26,27,28, 29]. Information about the genetic diversity of Roselle germplasm in Iran is particularly important for variety identification, to enhance the classification of germplasm collections and exploiting them in breeding programs and for the development and introduction of new accessions. Thus, the present study was taken to characterize the Roselle germplasm accessions collected from different geographical regions of Iranshahr and maintained in Islamic Azad University for investigating genetic diversity in some *Hibiscus sabdariffa* L. accessions using morphological and phytochemical markers.

2. MATERIALS AND METHODS

Fifteen Roselle (*Hibiscus sabdariffa L.*) accessions were collected from different geographical regions of Baluchestanin Iran and were transferred and planted in the farmland of Iranshahr Islamic Azad University in September 2015. All of the Roselle accessions were grown in Randomized Complete Block Design (RCBD) with three replications. Plants began to bloom as the days shorten (in 4-5 months) and the calyces were ready for harvest when they were tender and plump; they stayed fresh for about a week after picking. When flowering reached 50%, data recording was started. Harvesting encourages more flower buds to develop.

The selective Roselle accessions were washed with distilled water and were kept it in-room temperature for air dried. Dried fruits were crushed to the small pieces and were powdered and kept it in polythene bags for further uses. Aqueous extract of studied samples were used to carry out the qualitative and quantitative analysis using standard procedures to identify the Bioactive Phytochemical Components as described by Sofowara, Trease and Evans [30,31]. Morphological characteristics such as plant height (cm), canopy diameter(cm), number of branches per plant, weight of calyx per plant(g), fruit size(cm), Percent of calyx per fruit(%), number of capsules per plant, dry weight (kg), stem diameter(cm), mean of internode length(cm) and number of node and phytochemical characteristics such as vitamin C(mg/100 g dry calyx), anthocyanin(mg/100 g dry calyx) and hydroxycitric acid (HCA) (mg/100 g dry calyx) contents were recorded in all Roselle accessions for comparative studies. Morphological and phytochemical data were analyzed by SPSS version 22, MSTATC and SAS version 9.4 softwares.

3. RESULTS AND DISCUSSION

3.1 Variance Analysis

In the present study, fifteen Roselle accessions were studied with some morphological traits. Some of these accessions were easily separated from others by their distinct phenotypic characteristics of stem and flower. Morphological analysis indicated that some of the studied characteristics have a significant difference at $P < 0.05$ or $P < 0.01$ among Roselle accessions (Table 1). Similar conclusion was reported by [21,29].

Results from Duncan's test shown in Table 2 indicated that Dalgan-1 accession was found to be the tallest (126 cm) with long internodes and high number of node. Minimum canopy diameter (112 cm) and number of branches per plant (6.3) were recorded for zehkalot and Chahshor-3 accessions. Therefore, these accessions contained a lesser amount of dry weight. Maximum amounts for weight of calyx per plant, fruit size and number of capsules per plant characteristics were recorded for Chahshor-2, Bampoor-1 and Dalgan-1. Maximum stem diameter for zehkalot accession and Maximum Percent of calyx per fruit in the Iranshahr-2 accessions were recorded. Furthermore, maximum anthocyanin and vitamin C were found in the Iranshahr-1 accession and maximum hydroxycitric acid and Minimum anthocyanin were recorded in the Dalgan-4 accession.

3.2 Cluster Analysis

Genetic diversity was studied based on various morphological characteristics using analysis of the cluster among Roselle accessions. Dendrogram using average linkage (between groups) indicated that the accessions were divided to two major groups (Fig. 1), the first group divided into four clusters (4, 2, 1 and 5 genotypes, respectively) and the second group contained one cluster (3 genotypes). There were Iranshahr-1, Iranshahr-3, Bampoor-2, Bampoor-3, Dalgan-4, Zehkalot, Iranshahr-2, Chahshor-1, Dalgan-2, Dalgan-3, Chahshor-3 and Zehkalot-1 accessions in the first group. The most accessions were in this group. Also there were Chahshor-2, Bampoor-1 and Dalgan-1 accessions in the second group. The mean of studied characteristics for the first group were lower than one of the second group and the best accessions were in the second group. This clustering indicated that there were differences among accessions. Some of accessions performed better than others and were ranked higher; whereas the worse performance of accessions were in the first group than ones in the second group and were ranked lower. According to obtained results, the average of similarity among accessions was 97%. Thus, the observed morphological and phytochemical differences and similarity values of *Hibiscus sabdariffa* L. accessions maintained at Iran suggest that they possess a useable slight genetic diversity.

3.3 Correlation Studies

Phenotypic correlations between various morphological characteristics indicated that there were significant correlations at $P< 0.05$ or $P< 0.01$ among the most studied characteristics. Similar conclusion were reported by [18,21]. For example, highly significant positive correlation at $P<0.01$ among the plant height and all the studied characteristics except number of branches per plant, vitamin C, anthocyanin and HCA were observed. Moreover, a highly significant negative correlation at $P< 0.05$ or $P< 0.01$ among the number of branches per plant with plant height, fruit size, percent of calyx per fruit, number of node and mean of internode length were observed (Table 3). Phenotypic correlations among characteristics indicated that some associated factors correlate with each other and contribute in the occurring of these characteristics [32]. Roselle accessions were more separated with the help of their fruit morphology. Although, common Roselle accessions exhibited some variations in height, fruit size, number of capsules per plant and etc., these could be attributed to the adaptations of their original geographical and environmental conditions. Complete phenotypic expression of vegetative characteristics that show variations, makes the identification more difficult. Moreover, traditional morphological observations and chemical characteristics cannot alone determine the roles of phenotypic plasticity and genetic differentiation on population variation and adaptation. Hence, they lack the resolving power needed to identify individual genotypes [33]. There are other Roselle accessions, which resemble *Hibiscus sabdariffa L.* accessions in growth habits and morphological traits. Moreover, identifying Roselle accessions at its early stage is also sometimes ambiguous.

Table 1. Variance analysis of studied characteristics in Roselle accessions

Characteristics	Mean of square	F_c value
Plant height	134.07	11.421^{**}
Number of branches per plant	10.54	5.406^{*}
Canopy diameter	19.23	7.224^{*}
Weight of calyx per plant	65.96	3.275^{**}
Fruit size	87.55	9.683^{**}
Percent of calyx per fruit	65.03	25.312^{**}
Number of capsules per plant	98.09	34.089^{**}
Stem diameter	38.56	3.765^{*}
Dry weight	43.11	28.631^{**}
Number of node	46.77	5.154^{**}
Mean of internode length	114.34	12.536^{**}
Vitamin C	3.02	0.947^{ns}
Anthocyanin	2.76	0.638^{ns}
HCA (hydroxycitric acid)	2.01	0.135^{ns}

Table 2. Means comparison of characteristics using Duncan's test

Accessions	Plant height	Number of branches per plant	Canopy diameter	Weight of calyx per plant	Fruit size	Percent of calyx per fruit	Number of capsules per plant	Stem diameter	dry weight	Number of node	Mean of internode length	Vitamin C	Anthocyanin	HCA
Zehkalot	72.33b	6.267c	112.85b	4.913bc	4.764bc	0.4517cd	10.42b	5.4a	19.81e	3.264f	10.89ab	13.78ab	59.35a	20.85a
Zehkalot-1	99ab	7.5bc	144.56ab	4.88bc	5.079bc	0.7132ab	11.39b	3.87abc	30.12b	8.959bcde	10.62ab	14.67a	63.14a	23.26a
Bampoor-3	69.53b	7.7bc	142.43ab	4.363bc	4.436bc	0.4542cd	11.81b	4.013ab	27.3bc	3.262f	10.57ab	14.13ab	65.45a	21.52a
Dalgan-1	126a	9.867ab	141.18ab	6.34ab	9.233a	0.4517cd	31.92a	4.643ab	35ab	12.03ab	14a	15.0a	63.28a	19.07a
Dalgan-2	119.2a	8.533abc	146.97ab	4.793bc	3.973bc	0.4257cd	22.1ab	2.667bcd	20.95cd	5.980def	13.4a	12.69ab	70.33a	21.75a
Dalgan-3	105.6ab	9.6ab	132.19ab	3.947bc	3.48c	0.3447cd	18.17ab	1.267d	21.31cd	6.213cdef	12ab	15.09a	67.01a	23.36a
Dalgan-4	71.33b	7.4bc	131.95b	4.413bc	4.527bc	0.4607cd	10.42b	4.733ab	19.55de	3.367f	10.8ab	13.33ab	81.5a	24.06a
Iranshahr-1	100ab	5.867c	145.56ab	4.38bc	4.987bc	0.7455ab	32.54a	3.667abc	40a	8.967bcde	10.73ab	16.47a	67.07a	23.61a
Iranshahr-2	72.53b	7.39bc	143.63ab	4.763bc	4.437bc	0.9327a	11.81b	4.213ab	20.15de	3.1267f	10.32ab	14.33a	65.25a	20.42a
Iranshahr-3	104.7ab	9ab	142.83ab	5.693abc	3.693c	0.3414d	18.1ab	2.267cd	19.43de	4.633f	10.67ab	13.33ab	59.75a	22.46a
Bampoor-2	69.2b	7.986abc	155.66a	2.1c	5.633bc	0.6969b	18.42ab	1.667cd	26.2bcd	9.353bcd	10.33ab	15.22a	60.26a	19.51a
Bampoor-1	95.7ab	9.467ab	142.01ab	9.24a	8.787a	0.9305a	31.24a	2.867bcd	41.6a	17a	13.8a	16.22a	58.62a	22.39a
Chahshor-2	101.5ab	7.333bc	140.5ab	4.58bc	6.547ab	0.5359bcd	32.54a	3.133bcd	20.04de	10.27bc	11.27ab	14.21ab	61.7a	21.29a
Chahshor-3	96ab	6.261c	112.89b	2.873bc	3.553c	0.5263bcd	25.07ab	2.2cd	18.15e	9.667bcd	8.467b	13.57ab	60.15a	20.83a
Chahshor-1	78.8b	10.47a	132.64ab	1.707c	5.227bc	0.5681bc	12.31b	1.267d	20.69cde	5.133ef	10.87ab	10.53ab	48.68ab	19.60a

Table 3. Genotypic correlation of studied characteristics in Roselle accessions

	Plant height	Number of branches per plant	Canopy diameter	Weight of calyx per plan	Fruit size	Percent of calyx per fruit	Number of capsules per plant	Stem diameter	Dry weight	Number of node	Mean of internode length	Vitamin C	Anthocyanin	HCA
Plant height	1													
Number of branches per plant	-.427*	1												
Canopy diameter	.465**	.635**	1											
Weight of calyx per plan	.277**	.541**	.182*	1										
Fruit size	.693**	-.329**	.656**	.723**	1									
Percent of calyx per fruit	.697**	-.173*	.670**	.759**	.657**	1								
Number of capsules per plant	.661**	.631**	.571**	.781**	-.481**	-.213*	1							
Stem diameter	.371**	.398**	.436**	.241**	.0620**	.423**	.209**	1						
Dry weight	.774**	.497**	.642**	.208*	.243*	.180*	.575**	.786**	1					
Number of node	.879**	-.134*	.239*	.349**	.312**	.464**	.365**	.156**	.551**	1				
Mean of internode length	.722**	-.378**	.531**	.632**	.784**	.480**	.725**	.244*	.837**	-.291**	1			
Vitamin C	-.127*	.336**	.426**	.612**	-.286*	.648**	-.580**	.150*	.614**	-.235*	.160^{ns}	1		
Anthocyanin	-.225*	.219**	.334**	.233**	-.348**	.543**	-.411**	.104*	.465**	-.361*	.115^{ns}	.255**	1	
HCA	-.214**	.272*	.548**	.569**	-.302**	.417**	-.310**	.290**	.312**	-.352*	.082^{ns}	-.157*	-.174*	1

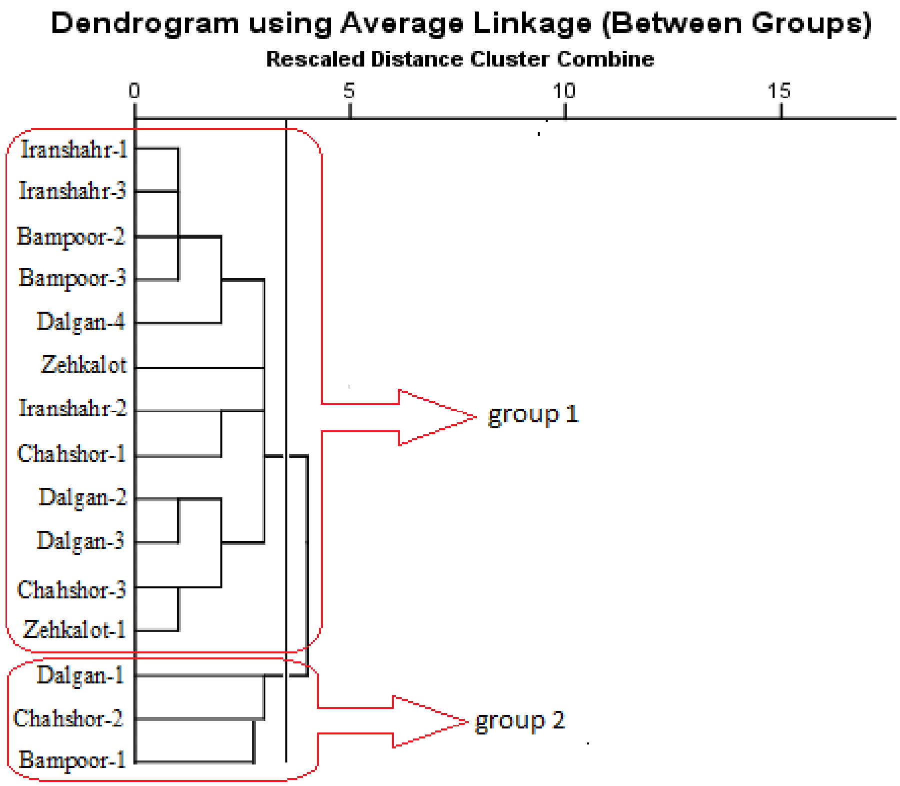

Fig. 1. Dendrogram cluster analysis of Roselle accessions based on morphological characteristics. The big rectangle indicates the first group and the small one indicates the second group

4. CONCLUSION

In this paper, genetic diversity was studied based on morphological and phytochemical various characteristics. Although, common Roselle accessions exhibited some variations in height, fruit size, number of capsules per plant and etc., these could be attributed to the adaptations of their original geographical and environmental conditions. However, cluster analysis indicated that there were differences among accessions. Some of accessions performed better than others and were ranked higher; whereas the worse performance of accessions were in the first group than ones in the second group and were ranked lower. Also Phenotypic correlations among characteristics indicated that some associated factors correlate with each other and contribute in the occurring of these characteristics. Thus, the observed morphological and phytochemical differences and similarity values of *Hibiscus sabdariffa* L. accessions maintained at Iran suggest that they possess a useable slight genetic diversity.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Wang ML, Morris B, Tonnis B, Davis J, Pederson GA. Assessment of oil content and fatty acid composition variability in two economically important Hibiscus species. J of Agri and Food Chem. 2012; 60(26):6620–6626.
2. Hiron N, Alam N, Ahmed FA, Begum R, Alam SS. Differential fluorescent banding and isozyme assay of *Hibiscus cannabinus* L. and *H. sabdariffa* L. (Malvaceae). Cytologia. 2006;71(2):175–180.
3. Vaidya KR. Natural cross-pollination in Roselle, *Hibiscus sabdariffa* L. (Malvaceae). Genetic and Mole Biol. 2000; 23(3):667–669.
4. Takebayashi N, Morrell Peter L. Is self-fertilization an evolutionary dead end?

Revisiting an old hypothesis with genetic theories and a macroevolutionary approach. American J of Bot. 2001;88(7): 1143-1150.
5. Satyanarayana NH, Visalakshmi V, Mukherjee S, Priya B, Sarkar KK. Genetic diversity in Roselle (*Hibiscus sabdariffa* L.) for fibre yield. Elec J of Plant Breeding. 2015;6(3):826-30.
6. Chandramohan D, Marimuthu K. A review on natural fibers. Inter J of Res and Reviews in Applied Sciences. 2011; 8(2):194-206.
7. Satyanarayana NH, Mukherjee S, Priya B, Roy S. Genetic Variability and Inter-relationship between Fibre Yield and its Components in Roselle (*Hibiscus sabdariffa* L.) in Terai zone of West Bengal. Vegetos-An Inter J of Plant Res. 2015;28(3):135-40.
8. Da-Costa-Rocha I, Bonnlaender B, Sievers H, Pischel I, Heinrich M. *Hibiscus sabdariffa* L. – A phytochemical and pharmacological review. Food Chem. 2014;165:424–443.
9. Morton FJ. Roselle (*Hibiscus sabdariffa* L). Fruit of warm climates. Creative resources systems Inc. Miami, Florida; 1987.
10. Ross IA. Medicinal plants of the world: Chemical constituents, traditional and modern medicinal uses. Humana Press Inc. 2003;1.
11. Zhen J, Villani TS, Guo Y, Qi Y, Chin K, Pan MH, Ho CT, Simon JE, Wu Q. Phytochemistry, antioxidant capacity, total phenolic content and anti-inflammatory activity of *Hibiscus sabdariffa* leaves. Food chem. 2016;190:673-80.
12. Wong PK, Yusof S, Ghazali HM, Che Man YB. Physico-chemical characteristics of roselle (*Hibiscus sabdariffa* L.). Nutrition & Food Science. 2002;32(2):68-73.
13. Fasoyiro SB, Babalola SO, Owosibo T. Chemical composition and sensory quality of fruit-flavoured roselle (*Hibiscus sabdariffa*) drinks. World J of Agri Sci. 2005;1(2):161-4.
14. Ali BH, Wabel NA, Blunden G. Phytochemical, pharmacological and toxicological aspects of *Hibiscus sabdariffa* L.: A review. Phytotherapy Res. 2005; 19(5):369-75.
15. Parkouda C, Diawara B, Ouoba LI. Technology and physico-chemical characteristics of Bikalga, alkaline fermented seeds of *Hibiscus sabdariffa*. African J of Biotech. 2008;7:7.
16. Khafaga ER, Koch H, El Afry MMF, Prinz D. Stage of maturity and quality of karkadeh (*Hibiscus sabdariffa* L. var. *sabdariffa*). 1. organic acids. 2. anthocyanins. 3. mucilage, pectin and carbohydrates. 4. improved drying and harvesting systems. Angewandte Botanik. 1980;54(5/6):287–318.
17. Ismail A, Ikram EHK, Nazri HSM. Roselle (*Hibiscus sabdariffa* L.) seeds nutritional composition protein quality and health benefits. Food. 2008;2(1):1–16.
18. Ibrahim MM, Hussein RM. Variability, heritability and genetic advance in some genotypes of Roselle (*Hibiscus sabdariffa* L.). World J Agri Sci. 2006;2(3):340-345.
19. Ahmed AAS, Abdelrahman MK, Abuelgasim E. Some genotypic and phenotypic traits of Roselle (*Hibiscus sabdariffa* var. *Sabdariffa*) and their practical implications. J Sci Tech. 2009; 10:2.
20. Louis SJ, Kadams AM, Simon SY, Mohammed SG. Combining ability in Roselle cultivars for agronomic traits in Yola, Nigeria. Greener J of Agri Sci. 2013; 3(2):145-149.
21. Mohamed BA, Sulaiman AA, Dahab AA. Roselle (*Hibiscus sabdariffa* L.) in Sudan, Cultivation and their uses. Bulletin Envir Pharmac Life Sci. 2012;1(6):48–54.
22. Sanoussi A, Hadiara HS, Yacoubou B, Benoit S, Issaka L, Mahamane S. Yield character variability in Roselle (*Hibiscus sabdariffa* L.). Afri J of Agri Res. 2011; 6(6):1371-1377.
23. Osman M, Golam F, Saberi S, Majid NA, Nagoor NH, Zulqarnain M. Morpho-agronomic analysis of three roselle (*Hibiscus sabdariffa* L.) mutants in tropical Malaysia. Australian Journal of Crop Science. 2011;5(10):1150.
24. Dudhane AS, Mukherjee S, Das B, Sarkar KK. Studies on Divergence for Fibre Yield and its Contributing Traits in Tossa Jute (*Corchorus olitorius* L.). Int J Bio-resource and Stress Manage. 2013;4(2):372-374.
25. Hariram Kumar B, Appalaswamy A, Durga Prasad AVS, Ismail S. Genetic divergence studies in roselle (*Hibiscus sabdariffa* L.). Madras Agri J. 2013;100(1-3):20-22.
26. Nayak BK, Baisakh B, Bastia D. Genetic divergence among genotypes of tossa jute (*Corchorus olitorius* L.). Envir and Ecol. 2009;27(1):86-88.
27. Roy SK, Das B, Kale VA, Haque S. Genetic divergence study for yield and

quality traits in tossa jute. J Crop Weed. 2011;7(1):130-132.
28. Shaikh MD, Soyab Akhil Mohd AB, Mazid Mohrir MN, Jadhav RS. Genetic divergence in okra (*Abelmoschus esculentus* L. Moench.). Electron J Plant Breed. 2013;4(3):1258-1260.
29. Hari Satyanarayana N, Visalakshmi V, Mukherjee S, Priya B, Sarkar KK. Genetic diversity in Roselle (*Hibiscus sabdariffa* L.) for fibre yield. Electron J of Plant Breed. 2015;6(3):826-830.
30. Sofowara A. Medicinal plants and traditional medicine is Africa spectrum books LTD. Ibadan, Nigeria. 1993;289.
31. Trease GE, Evans WC. Pharmacognosy. 11th edn Brailliar Tiridel can Macmillian Publishers; 1989.
32. Nayanakantha NMC, Singh BR, Gupta AK. Assessment of genetic diversity in Aloe germplasm accession from India using RAPD and morphological markers. Ceylon J of Sci. (Biol Sci). 2010;39(1):1–9.
33. Saljooghianpour M. Genetic diversity in different accessions of *Aloe* sp. using morphological and AFLP markers. Indian J of Agri Sci. 2013; 83(12):1396–1401.

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