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Genetic Parameters Evaluation among Some Selected Lines of Sudanese Roselle Variety in Egypt, Using Morpho-agronomic Traits and ISSR Markers.

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ABSTRACT

Roselle (*Hibiscus sabdariffa* L.) is an economically important plant in Egypt and used in various applications including foods and medicine. Fifteen lines of Sudanese Roselle variety were evaluated in Egypt during two seasons 2011-2012.Field experiment was conducted in a randomized complete block design with three replications. Data were collected on seven quantitative characters. Analysis of variance indicated significant differences among the Roselle lines in single and combined data in most studied characters. In general, the highest genotypic and phenotypic coefficients of variation were exhibited by seed yield/plant followed by fresh capsule weight / plant and No. of capsules / plant in both seasons. The highest genetic advance, (112.04 %) and (128.04.13 %) were obtained for fresh sepal's weight / plant in the first and second seasons, respectively. Meanwhile, the lowest genetic advance were obtained by No. of total branches /plant. High values of heritability estimates (> 0.90) were recorded for most characters. On the other hand, the results of phenotypic correlation showed that fresh capsule weight/plant had a highly significant positive association with dry seed yield/plant in both seasons and fresh sepals weight/plant with No. of total branches . The high, moderate and low sepals yield selected lines (L1, L8 and L15) of Sudanese Roselle were screened using ISSR molecular markers technique. ISSR results, from using five primers, revealed 26 amplified fragments, 8 of them were polymorphic (30.77 %). The primer HB-13 showed the highest fragments,7 with polymorphism71.43%, while the lowest polymorphism 0.00% was produced by the primers HB-08 and HB-10. The highest similarity (92.0%) was found between the high (L1) and moderate (L8)lines. Thus, these ISSR markers have the potential for identification and characterization of lines /varieties variations. This is also helpful in Roselle breeding programs and provides a major input into conservation biology.

Key words: Genetic parameters, Roselle (*Hibiscus sabdariffa* L), ISSR Marker, Correlation coefficient

Introduction

Roselle (*Hibiscus sabdariffal*. L) Family *Malvaceae*, Known commonly as "Karkade" is cultivated in the tropical and subtropical countries. It is considered as one of the most important and popular medicinal and industrial plants. The part used is the dried, fleshly calyces which is widely used for producing drinks or tea because of its high content of anthocyanins and organic acids (Gomez-Leyva *et al*., 2008 and Cissé *et al*., 2009). Roselle has been cultivated extensively in India, Sudan, Egypt, Senegal and Thailand for its red calyxes (Mohamed *et al.*, 2012). In Egypt, 'Karkadeh' is considered a very popular beverage and valuable medicinal plant due to its effect on lowering and/or adjusting the blood pressure without producing any side effect (Faraji and Tarkhani, 1999).Recently the sepal extract has been used as an effective treatment against leukemia due to its high content in polyphenols, particularly protocatechuic acid (Tseng *et al*., 2000). Moreover, it has many applications used in folk medicine for pyrexia and liver damage in China (Ibrahim and Hussein 2006; Louis *et al*., 2013). The sepals are the most important economic parts of the plant which is the source of raw material for drinks, wine, beverages jams, jelly, color and flavor ingredients in Europe countries (Egharevba and Lawogbomo 2007; Ismail *et al*., 2008 and Louis *et al*., 2013). Also, it has effect on stomach function, and can resist various infections of intestinal disease (Owolabi *et al.*, 1995).Obiefuna *et al*.,(1994) added that Roselle flowers can be used to relax the pain muscles of uterus and intestine. It has highly antibacterial properties and considered as cardio tonic. It is useful as laxatives (Hayat 2007).

Crop improvement through successful selection programme essentially depends on nature, magnitude of genetic variability, genetic advance, characters association, direct and indirect effects on yield and yield attributes (Ibrahim *et al*., 2013a,b) Several studies on Roselle have been carried out but there is limited information regarding its genetics, breeding and production. Moreover, to improve the yield of Roselle, plant breeders should have a better understanding of the genetic variability of yield and its components (Sanoussi *et al*., 2011).

Sepal's yield in Roselle is a complex character which depends on many components. Therefore improvement of sepal's yield requires consideration of all yield components in breeding programs and knowledge of associations between these plant attributes is very essential to determine the most efficient breeding procedure. Understanding of relationships among these components lead to the choice of elite lines,

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authenticates the benefits of a selection pattern and highlights real-time increase in yield through interrelated characters. The traditional methods of breeding are now being complemented by molecular techniques, enabling breeders to make better decisions when choosing the germplasm used in breeding programs (Jain *et al.,* 1999)

DNA fingerprinting has become an important tool for cultivar identification in plant breeding and for germplasm management. ISSR markers are inherited in Mendelian mode and segregated as dominant markers. This technique has been widely used in the studies of cultivar identification, genetic mapping, genetic diversity, evolution and molecular ecology (Zietziewicz *et al.*, 1994 and Abdel-tawab *et al*., 2007).

In spite of its potential economic importance, Karkade has received little attention and information regarding breeding, genetics and production of karkade is meager. (Ibrahim *et al*., 2013a).

The aim of this study is to evaluate the nature of genetic variability, heritability and character association of some quantitative traits in some selected lines of Sudanese Roselle beside DNA ISSR (Inter Simple Sequence Repeats) markers as a tool to distinguish between some selected lines of Roselle in Egypt.

Materials and methods

1- Field selection experiments:

The plant materials used in this study consist of fifteen selected inbred lines of Sudanese Roselle (*Hibiscus sabdariffa* var. *sabdariffa) variety,* which were derived by single plant selection from imported Sudanese population. These lines differ mainly in quantitative characters.

This investigation was carried out at El-Barka Experimental Station of El-Barka Company, Sohag governorate, Egypt, during two successive seasons (2011 and 2012). Seeds of 15selected lines of Sudanese Roselle variety were selected from the big population and planted on1st April in both seasons. The experimental design was randomized complete blocks with three replications, each replicate consisted of five lines 3.5m long and 0.6m in between, thus the plot was $10.5m^2$ (1/400 Fadden). Hills were 50cm apart with 4-5 seeds per hill. After three weeks plants were thinned to one plant per hill. All agricultural practices were carried out under organic fertilization without any additional nutrient chemicals. At full ripen five plants of each replicate per each entry of different seasons were harvested and the plant records were considered as already mentioned.

2-Plant records:

Plant records were considered on individual plant basis. They included:
1-Plant height (cm) (PH). 2- Number of total branches/plant (NTB).
3- Number of capsules/plant (NC). 4- Fresh sepals yield/plant (g) (FSPY).
5- Dry sepals yield/plant (g) (DSPY). 6- Fresh capsules yield/plant (g) (FCY).
7-Dry seeds yield/plant (g) (DSEY).

2- Statistical procedures:

The general statistical procedures were practiced using version 11 of SPSS software 2001. The analysis of variance and broad sense heritability (h^2_b) were generally assigned according to Robinson *et al*., 1951. Genetic advance GA was computed according to Johnson *et al*., 1955. The phenotypic and genotypic coefficients of variance (P.C.V. and G.C.V %) were computed according to Burton 1953. Phenotypic correlation coefficient was estimated according to Steel &Torrie 1984.

4-ISSR molecular markers

Plant Materials and DNA Extraction:

Young growing leaves of three selected Roselle (*Hibiscus Sabdariffal* L) lines (L1, L8 and L 15) were selected according to high , medium and low sepals yield and collected to isolate the total DNA . The extraction method was described by Dellaporta *et al*., (1983).

ISSR Amplification:

In order to obtain clear reproducible amplification products, different preliminary experiments were carried out in which a number of factors were optimized. These factors included PCR temperature cycle profile and concentration of each of the template DNA, primer, MgCl2 and Taq polymerase. A total of fifteen random DNA oligonucleotide primers were independently used according to Williams *et al*., (1990). In the PCR reaction, five primers succeeded to generate reproducible polymorphic DNA products.

The PCR amplification was performed in a 25 µl reaction volume containing the following: 2.5 µl of dNTPs (2.5 mM), 1.5µl of Mg Cl2 (25 mM), 2.5 µl of 10x buffer, 2.0 µl of primer (2.5 µM), 2.0 µl of template DNA (50 ng/µl), 0.3 µl of Taq polymerase (5 U/µl) and 14.7 µl of sterile ddH2O. Amplification was carried out in Techni TC-512 PCR System. The reaction was subjected to one cycle at 95 ºC for 5 minutes, followed by 35 cycles at 94 ºC for 30 seconds, 57 ºC for 30 seconds, and 72 ºC for 30 seconds, then a final cycle of 72 ºC for 12 minutes. PCR products were run at 100 V for one 30 min on 1.5 % agarose gels to detect polymorphism between virus strains under study. After electrophoresis, the ISSR patterns were visualized with UVTec. Documentation system. ISSR markers were scored from the gels as DNA fragments present or absent in all lanes.

Statistical analysis:

The DNA bands generated by each primer were counted and their molecular sizes were compared with those of the DNA markers. The bands scored from DNA profiles generated by each primer were pooled together. Then the presence or absence of each DNA band was treated as a binary character in a data matrix (coded 1 and 0, respectively) to calculate genetic similarity and to construct dendrogram tree among the studied three virus strains. Calculation was achieved using Dice similarity coefficients **(Dice 1945)** as implemented in the computer program SPSS-10.

Results:

A- Morpho- agronomic characterizations variability:

1-Analysis of variance:

Results of the analysis of variance for single and combined analysis of seven studied characters for fifteen lines of Roselle in two seasons are presented in Tables (1 and 2).Highly Significance differences in mean squares of lines were observed in single and combined data in both seasons for all traits. However, the interaction between lines x season of mean squares was also highly significant for all traits under study (Table 2). It is worth to note that significant differences observed in single and combined analysis for the replicates in fresh and dry sepals weight may be due to environmental genotype interaction in the second season

Table 1: Analysis of variance (mean squares) of seven quantitative characters of Roselle lines grown in two separated seasons (2011 and 2012).

SOVA	Df	Plant height (PH)	No. of total branches/plant (NTB)	No. of capsules/plant (NC)	Fresh sepals weight/plant (FSW)	Dry sepals weight/plant (DSW)	Fresh capsules weight/plant (FCW)	Dry seed yield/plant (DSY)
First season								
Lines	14	1999.26**	49.38**	6086.07**	12449.6**	2111.38**	208592.69**	1953.50**
Replicates	2	39.80	4.07	9.89	2100.55**	16.25	469.40	18.07
Error	28	50.23	4.78	20.51	223.77	8.32	113.11	8.07
Second season								
Lines	14	1709.95**	26.46**	1813.53**	9230.32**	2879.81**	181242.38**	1402.26**
Replicates	2	29.85	2.94	13.04	135.56	35.29*	93.17	3.42
Error	28	5.97	4.47	8.30	59.36	9.08	200.95	8.63

Table 2: Combined analysis of variance for seven quantitative characters of Roselle lines, seasons and the interaction between them (2011 and 2012).

SOVA	Df	Plant height (PH)	No. of total branches/ plant (NTB)	No. of capsules/ plant (NC)	Fresh sepals weight/ plant (FSW)	Dry sepals weight/ plant (DSW)	Fresh capsules weight/ plant (FCW)	Dry seed yield/plant (DSY)
Lines (L)	14	1188.04**	22.14**	3820.99**	21186.56**	1635.69**	127018.29**	1088.35**
Seasons (S)	1	1950.68**	117.88**	2646.04**	5522.11**	2200.78**	12984.01**	71.11**
L x S	14	48.37**	3.14**	21.24*	493.36**	28.04**	2926.77**	30.23**
Replicates	2	11.99	1.02	14.77	1410.09**	9.19	89.13	4.07
Errors	58	11.50	0.81	10.31	165.37	7.43	63.42	2.18

2-Lines performances:

Mean performances of the fifteen investigated Roselle lines are presented in Table (3). Line 1 had the highest mean values in four characters (number of total branches, number of capsules, fresh sepals weight/ plant and dry sepals weight/ plant in both seasons (22.70 & 24.00, 203.30 & 215.00, 810.00 & 855.00 and 192.7 &

205.00), respectively. Line 2 had the highest values in two characters (number of total branches and dry sepal's weight per pant) in both seasons (20.3 &21.3 and 172.0 &190.0), respectively. Dry sepals weight per plant had the highest values (161.0 and 174.0), respectively in line 3 in both seasons. In case of lines 14 and 15 the characters fresh capsule weight per plant and dry seeds yield per plant gave the highest values (855.0&793.3, 79.3 & 70.0 and 790.0 & 790.0, 74.3 &73.3) in both seasons, respectively. While, in same lines the lowest values (142.7 & 145.7and 134.0& 142.7), were recorded in dry sepals weight per plant in both seasons, respectively**.**

3-Genetic parameters:

Estimates from genotypic and phenotypic coefficient of variations, broad sense heritability % and expected genetic advance % for seven characters from data collected in 15 lines of selected Sudanese Roselle variety are given in Table (4). They are discussed separately foreach character.

a- Plant height (cm) (PH):

The phenotypic coefficient of variability (P.C.V) and genotypic coefficient of variability (G.C.V) were higher in the first season than the second season. Also, heritability estimates (h^2b %)were high (92.8 % and 99.0%) in the first and second seasons, respectively. Expected genetic advance in the first and the second seasons estimates were high (50.59% and 48.84%), respectively.

b-No. of total branches/plant (NTB):

The results obtained for number of branches per plant showed that PCV and GCV were greater in the first season than the second season and heritability estimates ranged from medium to low values in both seasons (75.7 %and 62.1%). In addition genetic advance showed low values in both seasons too.

c -Number of capsules per plant (NC):

Phenotypic and genotypic coefficients of variability (PCV and GCV) values were higher in the first season than in the second season. Heritability and genetic advance estimates were also high (99.0% and 98.6%) and (92.16% and 50.19 %) in both seasons.

d-Dry sepals weight per plant (DSPW):

In the first season the phenotypic and genotypic coefficient of variability (P.C.V) and (G.C.V) were lower (17.93 and 17.82) than in the second season (19.62 and 19.52), respectively. In both seasons the heritability estimates gave high values (98.8% and 99.1%), respectively. Also genetic advance (GA) estimate sexhibited high values (54.22% and 63.42%) in the first and second season, respectively.

Table 3: Mean values of seven quantitative studied characters of Roselle in two seasons (2011 and 2012).

Code No.	Plant height (cm.) (PH)		No. of total branches/Plant (NTB)		No. of capsules/plant (NC)		Fresh sepals weight/plant (gm.) (FSW)	
	1st	2nd	1st	2nd	1st	2nd	1st	2nd
1	154.3±2.3	163.0±1.7	22.7±0.7	24.0±0.6	203.3±3.3	215.0±2.9	810.0±5.8	855.0±5.0
2	151.7±0.9	162.0±1.2	20.3±0.3	21.3±0.7	168.0±1.2	185.0±2.9	805.0±2.9	855.0±2.9
3	172.3±1.5	187.3±1.5	15.7±0.3	20.7±0.7	153.3±0.9	162.7±1.5	845.0±2.9	840.0±5.8
4	178.0±1.2	187.3±1.5	17.0±0.6	21.0±0.6	138.7±0.9	152.7±1.5	833.3±8.8	841.7±8.3
5	170.7±1.2	182.7±1.5	19.0±0.6	19.7±0.3	140.0±1.7	151.0±1.0	701.7±1.7	795.0±2.9
6	195.0±2.9	201.7±1.7	18.7±0.6	20.7±0.7	205.0±2.9	205.0±1.5	705.0±2.9	803.3±3.3
7	152.7±1.5	160.3±1.5	15.0±0.0	17.3±0.3	192.7±1.5	201.7±1.7	540.0±2.9	605.0±2.9
8	173.3±0.9	182.3±1.5	15.3±0.3	17.7±0.3	181.3±0.9	194.0±2.1	701.7±1.7	701.7±1.7
9	179.3±0.7	193.0±1.7	15.7±0.3	18.3±0.3	178.3±0.9	187.3±1.5	798.3±4.4	705.0±2.9
10	152.7±1.5	164.0±2.1	18.3±0.3	20.7±0.7	149.3±0.7	162.7±1.5	693.3±6.7	755.0±2.9
11	155.0±2.9	162.7±1.5	15.3±0.3	18.7±0.7	137.7±1.5	150.0±2.9	701.7±1.7	753.3±3.3
12	152.0±1.2	161.3±1.3	20.7±0.7	19.7±0.3	152.7±1.5	161.0±1.0	645.0±2.9	696.7±3.3
13	190.0±5.8	182.7±1.5	18.3±0.3	21.3±0.7	142.0±1.2	155.0±2.9	790.0±5.8	803.3±3.3
14	155.0±2.9	162.3±1.5	19.0±0.6	21.0±1.0	141.7±0.9	153.0±1.7	745.0±2.9	805.0±2.9
15	155.0±1.2	174.0±2.1	15.7±0.3	19.0±0.6	122.3±1.5	133.0±1.7	803.3±3.3	790.0±5.8
General	165.8±2.2	175.1±2.0	17.8±0.4	20.1±0.3	160.4±3.8	171.3±3.6	741.2±12.1	773.7±10.4

Table 3: Continue

Code No.	Dry sepals weight/plant (gm.) (DSW)		Fresh capsules weight/plant (gm.) (FCW)		Dry seed yield/plant (gm.) (DSY)	
	1st	2nd	1st	2nd	1st	2nd
1	192.7±1.5	205.0±2.9	705.0±2.9	790.0±5.8	68.0±1.2	69.3±0.7
2	172.0±1.2	190.0±2.9	696.7±3.3	783.3±8.8	67.0±0.6	70.3±1.5
3	161.0±1.0	174.0±2.1	701.7±1.7	745.0±10.4	66.7±0.9	68.3±1.7
4	147.0±1.5	162.7±1.5	733.3±8.8	760.0±5.8	69.0±0.6	67.7±1.5
5	144.7±1.5	157.3±1.5	575.0±2.9	613.3±6.7	53.3±0.9	59.3±0.7
6	142.7±1.5	152.7±1.5	611.7±4.4	655.0±2.9	58.7±0.9	63.7±0.7
7	141.3±0.9	152.3±1.5	557.3±1.5	603.3±3.3	49.0±0.6	59.3±0.7
8	140.7±0.7	153.3±2.0	704.0±2.1	755.0±2.9	66.3±0.3	65.7±0.3
9	142.7±1.5	152.3±1.5	756.7±3.3	730.0±2.9	67.3±1.5	65.7±0.7
10	141.0±1.0	147.3±1.5	425.0±2.9	455.0±2.9	36.0±0.6	40.7±0.7
11	142.3±1.5	147.3±1.5	305.0±2.9	355.0±2.9	34.0±0.6	34.7±0.9
12	141.7±0.9	146.7±1.7	455.0±2.9	455.0±2.9	36.0±0.6	40.7±0.7
13	142.0±1.2	147.3±1.5	756.7±3.3	705.0±2.9	46.3±0.9	49.3±0.7
14	142.7±1.5	145.7±1.2	855.0±2.9	793.3±3.3	79.3±0.7	70.0±0.7
15	134.0±2.1	142.7±1.5	790.0±5.8	790.0±5.8	74.3±0.7	73.3±0.9
General	148.6±2.3	158.4±2.6	641.9±22.2	655.9±20.7	58.1±2.2	59.9±1.8

e-Dry seeds yield per plant (DSEY):

The results obtained for dry seeds yield per plant showed that Phenotypic and genotypic coefficients of variability (PCV and GCV) values were greater in the first season than in the second season., (44.11, 43.84) and (36.33, 36.00), respectively. The highest heritability (h^2b) estimates values were exhibited by dry seeds yield per plant (98.8% and 98.2%) in the first and second seasons, respectively. The high genetic advances 52.14% and 61.23 % were obtained also for this character in the first and second seasons, respectively.

On the basis of obtained results on genetic parameters, the remained traits, fresh sepal's weight / plant(FSPW) showed the highest values for genetic advance (112.03, 128.04) and heritability (> 0.90 %) estimates in both seasons. On the other hand, the highest values for PCV, GCV and h^2bwere recorded for fresh capsule weight per plant (FCY) in both seasons.

Table 4: Estimates of genotypic and phenotypic coefficients of variation (GCV and PCV), broad sense heritability (h^2b) and genetic advance (GA) for seven quantitative characters of Roselle lines grown in two separated seasons (2011 and 2012).

parameter	seasons	Plant height (cm.) (PH)	No. of total branches/plant (NTB)	No. of capsules plant (NC)	Fresh sepals weight/ plant (gm.) (FSW)	Dry sepals weight/ plant (gm.) (DSW)	Fresh capsules weight/plant (gm.) (FCW)	Dry seed yield/plant (gm.) (DSY)
GCV	S1	15.37	21.69	28.03	12.23	17.82	41.07	43.84
	S2	13.61	13.49	14.66	13.69	19.52	36.89	36.00
PCV	S1	15.96	24.93	28.17	12.39	17.93	41.10	44.11
	S2	13.68	17.12	14.76	14.06	19.62	36.95	36.33
h^2b	S1	0.928	0.757	0.990	0.974	0.988	0.998	0.988
	S2	0.990	0.621	0.986	0.948	0.991	0.997	0.982
GA	S1	50.59	6.91	92.16	112.03	54.22	-	52.14
	S2	48.84	4.40	50.19	128.04	63.42	-	61.23

4-Phenotypic correlation coefficients:

The results presented in Table 5 revealed varying degrees of association among the seven studied characters of Roselle in the two seasons. It was observed that Roselle traits associate in both negative and positive manner with each other. The correlations among studied characters showed positive and highly significant association between fresh sepal's weight/ plant with dry sepal's weight /plant, fresh capsules weight / plant with dry seed yield / plant in both seasons (Table 5). It was also observed that fresh capsule weight /plant was correlated with dry seeds yield / plant in both seasons while dry sepals weight was correlated with each of fresh capsules weight / plant and dry seeds yield / plant in the second season only.

B-Molecular characterization for selected lines of Roselle using ISSR markers:

Five primers were representative of most types of repeated sequence as ISSR markers using ISSR-PCR amplification, to assess the level of polymorphism in the three selected Roselle lines, L1 (the highest values of sepals yield), L8 (the medium values of sepals yield) and L15 (the Lowest sepals yield). The banding pattern of ISSR fragmenent and level of polymorphism are presented in Table 6 and fig 1.

The results revealed 26 amplified fragments; 8 of them were polymorphic (30.77%). The total number of amplified and polymorphic fragments obtained with each primer is found in Table (6). ISSR data revealed that

Primer HB -13 led to the greatest number of polymorphic bands, thus having a high polymorphic/amplified bands ratio, equal to 71.43 %. But, primers HB - 8, HB -10 , HB-11 and HB-12 led to similar results of number of monomorphic bands (4), however these primers amplified bands ratio, ranged from 0.00 to 30.77. %.The ranges of amplicones were 95 to 575bp in all studied primers (Table 6). The genetic similarity matrices depended on all possible pairs of three selected linesof Roselle are illustrated in table 7 and fig2. The highest genetic similarity indices were found between Roselle lines L1 and L8 (0.92%). Followed by equal similarity matrices 0.85 for each of (L1, L15) and (L8, L15).

Table 5: Correlation coefficients of seven quantitative characters of Roselle lines for two separated seasons (2011 and 2012).

Characters		Plant height (PH)	No. of total branches/plant (NTB)	No. of capsules/plant (NC)	Fresh sepals weight/plant (FSW)	Dry sepals weight/plant (DSW)	Fresh capsules weight/plant (FCW)	Dry seed yield/plant (DSY)
First season	X1	1.00						
	X2	-0.149	1.00					
	X3	0.171	0.208	1.00				
	X4	0.279	0.137	-0.246	1.00			
	X5	-0.203	0.586**	0.430**	0.420**	1.00		
	X6	0.306*	0.036	0.021	0.593**	0.163	1.00	
	X7	0.137	-0.006	0.099	0.585**	0.279	0.886**	1.00
Second season	X1	1.00						
	X2	-0.014	1.00					
	X3	-0.158	0.102	1.00				
	X4	0.192	0.738**	-0.187	1.00			
	X5	-0.102	0.607**	0.446**	0.538**	1.00		
	X6	0.315*	0.286	0.155	0.448**	0.418**	1.00	
	X7	0.312*	0.172	0.148	0.376*	0.423**	0.934**	1.00

The dendrogram based on the similarity matrices of ISSR-PCR banding patterns separated the three selected lines of Roselle into two major groups, one of them formed by L15, while the other one only had individuals L1 and L8 (Fig2).

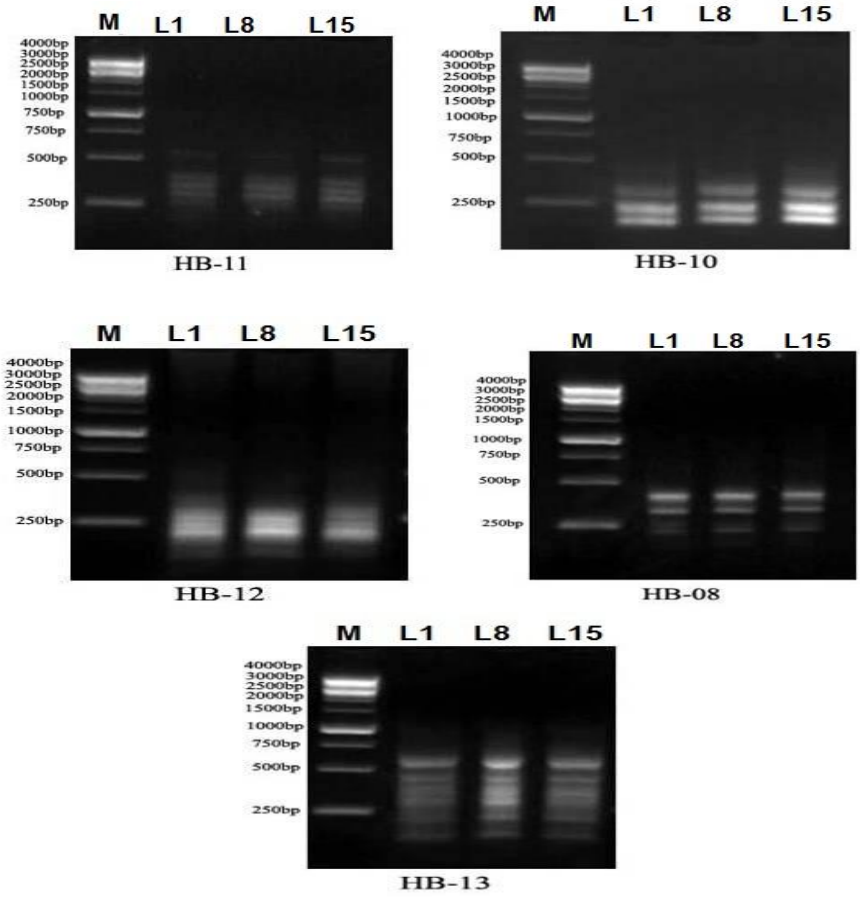

Fig. 1: ISSR banding patterns in selected three lines of Roselle (*Hibiscus sabdariffa* L.) (M= marker, L1, L8 and L15) with five primers.

Table 6: Primer names, sequences, Range of amplicones and polymorphism degree expressed as polymorphic /amplified bands ratio in three selected Roselle lines (L1 , L8 and L15).

Primer code	Sequence	Range of amplicones (bp)	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism %
HB-08	5 GAG AGA GAG AGA GG 3	215-400	4	4	0	0.00
HB-10	5 GAG AGA GAG AGA CC 3	145-325	4	4	0	0.00
HB-11	5 GTG TGT GTG TGT TGT CC 3	235-510	5	4	1	20.00
HB-12	5 CAC CACCAC GC 3	95-300	6	4	2	33.33
HB-13	5` GAG GAGGAG GC 3`	95-575	7	2	5	71.43
Total primers			26	18	8	30.77

Table 7: Similarity indices between three Roselle genotypes (L1, L8and L15) with ISSR markers based on Jaccard's coefficients.

Selected lines	L1	L8	L15
L1	1.00		
L8	0.92	1.00	
L15	0.85	0.85	1.00

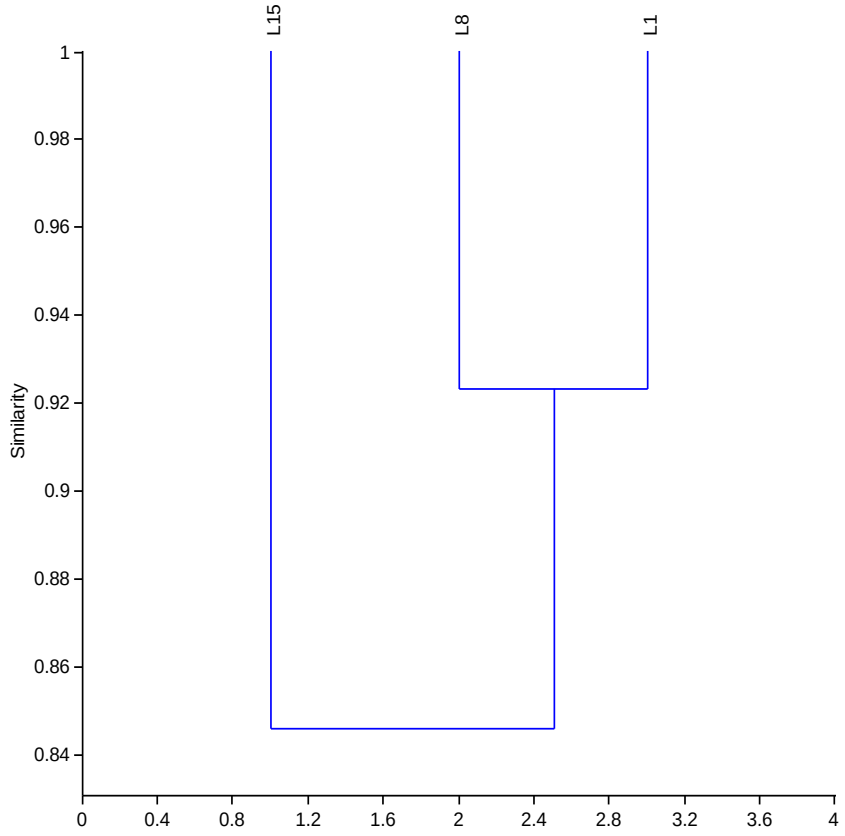

Fig. 2: Dendrogram represented the genetic relationships among the three selected Roselle lines (L1, L8 and L15) using UPGMA cluster analysis of Jaccard genetic similarity coefficients.

Discussions:

Genetic variability is a very important component of plant breeding which is a major tool being used to cope with the ever-increasing pressure of an expanding world population on food production (Ariyo 1990).

Conventional analysis of variance and genetic parameters like phenotypic and genotypic coefficients of variability, heritability and genetic advance have been used to assess the nature and magnitude of variation and improvement of Roselle lines in breeding program.

The results of the present study on the analysis of variance for all the traits in single and combined showed highly significant difference among the lines. It is worth to note that significant differences in the replicates in plant height and total number of branches which may be due to environmental genotype interaction in the second season. The interaction of lines and seasons (LXS) also exhibited highly significances differences in both seasons, similar pattern of variability also reported by (Ibrahim and Hussein2006; Sanoussi *et al*., 2011 and Louis *et al*., 2013).

Means No. of capsules / plant showed that lines no 1, 6,7 and 8 had the highest means values in both seasons . However, the means of drysepal's weight / plant revealed that lines 1, 2, 3, and 4gave the highest mean values also in both seasons

All genotypes expressed significant variations for mean performance for studied characters. The differences observed between selected lines of Roselle collected in morphological and yield components are indications of significant difference in their genetic bases. The overall means of some traits showed differences between the two seasons, this change in the overall mean for these traits was due to the interaction of the lines with the environment. This reveals that the observed differences among lines in these traits can be attributed to genetic causes as well as their interactions with the environment (Koorsa1987, Thirthamallappa and Sherif 1991 and Zayed *et al*., 1996).

The phenotypic coefficient of variation (PCV) was slightly higher than corresponding genotypic coefficient of variation (GCV) for most of the characters in both seasons,which indicates that environmental influence on the characters expression.

The coefficient of variability was higher for dry seed yield /plant followed by fresh capsule weight /per plant, No. of capsules /plant and No. of total branches/plant, While it was low for fresh sepals /plant and plant height. Identification of components of genetic variances facilitates the selection of a desirable breeding method. Heritability and genetic advance are important selection parameters. Heritability estimates along with genetic advance are normally more helpful in predicting the gain under selection than heritability estimates alone (Abou El-Nasr *et al*., 2004 andBisne *et al*., 2009).

The present study showed higher heritability values ranging from 62.1 to 99.8 %. Maximum heritability(above 90.00 %)was observed for most studied characters except in the character No. of total branches which gave medium value in both seasons. Similar conclusion was reported by Ibrahim and Hussein 2006 and Sabiel etal.,2014.

The results suggested that selection based on these characters will be meaningful in predicting for calyx yield in Roselle. The high heritability indicates that this character is highly genetically controlled and less affected by environment.

In a selection programme where the primary objective is character improvement, a study of genetic gain is more advantageous than heritability studies. In the present study genetic advance was maximum for fresh sepal's weight followed by No of capsules, dry sepals weight, dry seed yield per plant and plant height. High heritability coupled with high genetic gain and coefficient of variability was observed for fresh sepal's weight, No of capsules and plant height. (Sanoussi *et al*., 2011 and Falusi *et al*., 2014).The results of variability on the morphological characters according to yield and yield components agree with the work of Futuless *et al*., 2010 and Yandong *et al.* 2012on Roselle.

The association among yield components in the seven quantitative characters of Russell lines showed different patterns at phenotypic levels in this study. Phenotypic correlation coefficients indicated that fresh capsules weight / plant showed highest significance and positive association with dry seed weight , per plant followed by no of total branches with fresh sepals weight and dry sepals weight in both seasons . However, dry sepal's weight was higher with fresh capsules weight and dry seed yield in the second season only. The fluctuation which appeared in the estimates of the phenotypic correlation coefficients between the two seasons can be attributed to the fact that estimates of the phenotypic correlation depends on the environmental correlation. (Atta *et al*., 2011and Ibrahim *et al.*,2013b).

The significant phenotypic association among yield components had been attributed to linkage or may be due to developmentally induced relationships between these components. Gasim and Khidir 1998 suggested that such associations might be caused by competition between these components for assimilation during their development. Similar results have been drawn by many workers in different crops (Adams 1967, Laota1990 and Gasim1994).

The use of molecular markers has become a common practice in studies of population structure, genetic diversity for pre-breeding and breeding germplasm and in distinguishing one individual genotype to preserve the property of breeding rights (Langridge and Chalmers 2004). In addition, ISSR markers are useful in areas of genetic diversity, phylogenetic studies, gene tagging, genome mapping and evolutionary biology in a wide range of plant species (Christopoulos *et al.*, 2010; Jabbarzadeh *et al*., 2010).

The results obtained in this study, revealed total fragments of 26 amplified DNA fragments with different sizes ranged from 95-575bp. Whereas 18 from them were monomorphic bands and the other 8 fragments were polymorphic bands with 30.77 % polymorphism.The possibility of characterization of every individual examined in Roselle lines, offers a promising perspective as a molecular tool for varietal identification and breeding program applications.Similar results were reported by Hanboonsong *et al.* 2000,Omalsaad*et al.* 2014. And Khafaga 2013. On the other hand, the dendrogram constructed by the UPGMA method revealed the genetic relationship detected between the studied lines and divided them into two clusters; the first (L1 and L8) contained the high and the medium lines and the second cluster (L1) included only the high line. Higher

similarity index (0.85-0.92) indices suggest that the tested lines have closer genetic relation and slightly differences. Similar results were detected by Cleveland *et al.* 2000 and Khafaga 2013.

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