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Phenotypic and Molecular Characterization of New Selected Genotypes of Roselle in Egypt

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ABSTRACT

Roselle (*Hibiscus sabdariffa* L.) is an important medicinal plant, belongs to family Malvaceae. Its cultivated area is increasing gradually in Egypt for local utilization and exports of calyces. The calyces (sepals) extract is a natural colorant replacing the red synthetic coloring agents in many nutritional, medicinal and industrial purposes due to their high contents of vitamin C, anthocyanins, amino acids and mineral salts. Roselle seeds have a fixed oil, similar with cotton seed oil properties and can participate to cover the need of edible oils in Egypt. However, Roselle yields of the local variety from sepals and seeds until now do not reach the expected income, so their is need to insert new germplasms that improve the Roselle economically. Therefore, 15 new genotypes were selected from the local Roselle plantations in Qena and Aswan Governorates. They were bred for two seasons to study their genetic diversity on the levels of yield component characters, genetic parameters and molecular markers RAPD-PCR to confirm their efficacy as new germplasms to enhance the Roselle income. The genotypes and their interactions with the seasons appeared significant differences in the seven studied traits for both seasons separately and combined. Genotype 3 had the highest dry sepals yield/plant (DSPY) value in the first season S1 while, the genotype 1 had the highest value in the second season S2. On the other hand, values of all the studied genetic parameters were higher in S1 for all characters than in S2 except the heritability broad sense of four characters and genetic advance for DSPY. Genotypic and phenotypic coefficients of variation exhibited the lowest values of plant height PH trait in both seasons. Number of total branches/plant (NTB) had a highly significant positive association with both number of capsules/plant NC and DSPY in both seasons. Concerning the molecular DNA fingerprinting of high, moderate and low DSPY genotypes, the PCR products revealed 94 amplified DNA fragments. Whereas 77 from them were monomorphic bands and 17 fragments were polymorphic bands with 18.1 % polymorphism. The primer A16 showed the highest fragments (19 fragments) while 14 fragments were the lowest fragments produced by primers A09 and A20. The primer A04 revealed the highest polymorphism 37.5%, while the lowest polymorphism 6.25% was produced by the primer A01. The highest similarity (98%) was found between the moderate and low genotypes. All the studied levels confirmed the genetic diversity among the studied genotypes which can be exploited as new germplasms enhancing the Roselle income in Egypt.

Key words: Roselle, Yield component characters, Heritability, Genetic advance and RAPD-PCR.

Introduction

Roselle (*Hibiscus sabdariffa* L.) is an important and popular medicinal and industrial plant, belongs to Malvaceae family and native to Africa (Gomez-Leyva *et al*. 2008 and Sie *et al*. 2011). It is grown in tropical and subtropical regions (Fasoyiro *et al*., 2005 and Atta *et al*. 2011). The cultivated area of Roselle is increasing gradually in Egypt for local utilization and exports of seeds and calyces (Ibrahim and Hussein 2006). The plant young leaves are prepared like spinach, rich in phosphorus, calcium, magnesium and potassium (Delgado-Vargas and Parcedes-Lopez 2003 and Atta *et al*. 2010). The Roselle seeds until now do not have any commercial applications though they are a valuable food resource on account of their protein, calorie and substantial amount of fiber and valuable micro-nutrient (Akanbi *et al*. 2009). The seeds contain an edible fixed oil (17-20 %) similar with cotton seed oil properties (Ottai *et al*. 2004, Ottai and Abd-El-Khair 2004 and Hussein *et al*. 2010). Meanwhile, the calyces (sepals) are used for producing refreshing drinks due to their high contents of vitamin C, anthocyanins, amino acids and mineral salts (Babajide *et al*. 2004 and Cisse *et al*. 2009). The calyces color extract is a natural colorant replaces red synthetic coloring agents in nutritional, drinks, pharmaceutical and cosmetic industries according to the world return to nature (Shalan *et al.* 2001, Ottai *et al*. 2004, Hussein *et al*. 2010 and Falusi *et al*. 2014). Roselle is also cultivated for its fiber and organic compounds (Cisse *et al*. 2009).

Furthermore, Roselle is one of the most famous folk medicinal plants (Ibrahim and Hussein 2006). The plant is used for treating hypertension (Faraji and Tarkhani 1999), pyrexia and liver disorders (Chen *et al*. 2003), microorganism growth limitation (Oboh and Elusiyam 2004), a diuretic, cardiotonic, laxative, cough remedy, wound dressing, a digestive and sedative (Akindahunsi and Olaleye 2003). The plant has an antioxidant activity in patients with atherosclerosis (Tsai *et al*. 2002).

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ON the other hand, success of any program to improving any plant crop depends on the genetic variability, genetic advance and characters association with the plant yield (Ibrahim and Hussein 2006). The genetic diversity is important for parent selection to recover transgressive segregates (Kiran Patro and Ravisankar 2004). Beside the genetic diversity, the environmental influences on the plant growth and formation of active constituents which play an important role in the plant morphology (El-Mergawi and Sidkey 1998, Ottai *et al*. 2004, Falusi *et al*. 2012 and 2014). Finally, the plant yield is affecting by the genotype-environmental interaction (Ottai *et al*. 2006).

However, the molecular markers have been applied and developed since 1990, including random amplified polymorphic DNA (RAPD) (Williams *et al*. 1990 and Kumar and Gurusubramanian 2011). These molecular markers have been used in several fields for the assessment of genome mapping programs, genetic diversity, genotype fingerprinting, and molecular breeding (Ismail *et. al*., 2012 and Yang *et. al*. 2013). RAPD-PCR results are believed to be useful tool in the future breeding programs in *Hibiscus* (Barik *et. al*., 2006, Abou El-Nasr *et al*., 2013 and Al-Kordy *et al*., 2013).

The aim of this study is to evaluate the genetic diversity among 15 selected Roselle genotypes through studying their yield characters, genetic parameters and molecular RAPD-PCR fingerprinting to confirm the efficacy of these genotypes as new germplasms which can be enhance the Roselle income in Egypt.

Materials and Methods

Based on the yield components, individual Roselle plants were selected from the local plantations of Qena and Aswan in Upper Egypt during the summer season 2007 and harvested separately to be parent plants of this study. The seeds of these parents were re-cultivated in the next season (2008) for propagation and selection continuity at the Experimental Station of El-Baraka Company in Sohage Governorat. At the end of the 2008 season, fifteen individual plants were selected and harvested to form new germplasms of Roselle believing that they will improve the Roselle crop if inserted in an ambitious breeding program. For two successive seasons (2009 and 2010), seeds of these selected genotypes were cultivated at the above mentioned Experimental Station. Randomized complete blocks was designed with three replications, each consisted of five lines 3.5m long and 0.6m in between, thus the plot was 10.5m^2 (1/400 faddan). Hills were 50cm 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. A representative random sample of 20 individual plants from each plot was used for recording the next Roselle yield attributes

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).

Statistical Analysis:

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 1952. Phenotypic correlation coefficient was estimated according to Steel & Torrie 1980.

DNA extraction and PCR conditions:

Three selected genotypes (1, 8 and 15 presented high, moderate and low dry sepals yield/plant DSPY, respectively at the second season) were subjected for total genomic DNA extraction using the CTAB method (Doyle and Doyle, 1987 and Cullings, 1992). DNA quality and quantity were determined by electrophoresis in 0.8% agarose gel using RAPD-PCR method. Eleven used primers (Table 1) were obtained from Pharmacia Biotech (Amersham Pharmacia Biotech UK Limited, Ebgland HP79 NA).

Table 1: Eleven primer sequences used in identification of three Roselle lines.

Primer code	Sequence	Primer code	Sequence	Primer code	Sequence
OPA-01	CAG GCC CTT C	OPA-09	GGG TAA CGC C	OPA-19	CAA ACG TCG G
OPA-02	TGC CGA GCT G	OPA-11	CAA TCG CCG T	OPA-20	GAC CAA TGC C
OPA-04	AAT CGG GCT G	OPA-16	AGC CAG CGA A	OPB-15	GGAGGGTGTT
OPA-05	AGG GGT CTT G	OPA-18	AGG TGA CCG T		

PCR reactions were performed with GoTaq® Flexi DNA Polymerase kit (Promega), in a total volume of 25 µl volume reaction mixture according to Bakr *et. al*. (2013). PCR reaction was tested on 1.8% agarose (Genetics) gel and 100 bp DNA Ladder H3 RTU (Genetics) was used as a standard marker.

Data analysis:

Data of RAPD markers were scored as "1" for the present band and "0" for the absent band. These data were used in counting the number of total amplified markers in the three genotypes. Moreover, pairwise comparisons of the studied genotypes (based on the presence or absence of unique and shared polymorphic products) were used to determine their similarities according to Jaccard (1908) using PAST: Paleontological Statistics Software V 2.17 (Hammer *et. al*. 2001). The similarity coefficients were used to construct UPGMA: Unweighted Pair Group Method with Arithmetic averages using the mentioned software (Hammer *et. al*. 2001).

Results and Discussion

1- Analysis of variance:

Data of seven studied characters for fifteen Roselle genotypes were analyzed for two seasons (S1 and S2), separately and combined and illustrated in Tables 2 and 3, respectively. The genotypes appeared significant differences in all traits for both seasons separately and combined. Also, highly significant variations were computed between seasons for all studied characters, except fresh capsules yield/plant FCY and dry seed yield/plant DSEY were insignificant. While the interactions between genotypes and seasons were highly significant for all traits.

Table 2: Analysis of variance (mean squares) of seven quantitative characters for fifteen new Roselle genotypes grown in two seasons (2009 and 2010) separately.

Characters / Season	First Season			Second Season		
ANOVA	genotypes	Replicates	Error	genotypes	Replicates	Error
d.f.	14	2	28	14	2	28
Plant height (PH)	1369.92**	13.27	32.20	500.50**	10.07	12.07
No. of total branches/plant (NTB)	39.50**	2.47	4.11	15.12**	1.87	4.15
No. of capsules/plant (NC)	2566.55**	13.45	13.11	1221.81**	5.45	9.97
Fresh sepals yield/plant (FSPY)	37353.76**	627.81	676.66	27690.95**	406.67	181.31
Dry sepals yield/plant (DSPY)	510.26**	7.22	10.27	590.17**	4.94	9.69
Fresh capsules yield/plant (FCY)	43082.86**	901.43	904.64	19824.29**	285.0	156.07
Dry seed yield/plant (DSEY)	376.03**	0.20	16.49	57.71**	1.58	1.01

Table 3: Combined analysis of variance (mean squares) of seven quantitative characters for fifteen new Roselle genotypes grown in two seasons (2009 and 2010) and the genotypes- seasons interaction.

SOVA	Df	Plant height (PH)	No. of total branches /plant (NTB)	No. of capsules /plant (NC)	Fresh sepals yield/plant (FSPY)	Dry sepals yield/plant (DSPY)	Fresh capsules yield/plant (FCY)	Dry seed yield/plant (DSEY)
Genotypes (G)	14	568.64**	16.51**	1169.51**	21186.59**	337.38**	18915.95**	160.04**
Seasons (S)	1	302.50**	45.51**	3894.04**	5522.50**	2592.10**	90.0	0.10
G x S	14	54.83**	1.70**	93.28**	493.33**	29.43**	2053.10**	23.01**
Replicates	2	4.17	0.12	1.23	98.11	1.91	77.44	3.72
Errors	58	6.14	0.33	4.69	206.67	3.34	181.39	2.86

These results indicated that the genotypes have different genetic bases and high genetic variability of the plant in Egypt. Another say is that the genotypes carried alleles with different additive and additive x effects and these effects were constant from season to another. However, the traits of FCY and DSEY had insignificant season differences but significant differences for the genotype-season interaction indicated the genotype enhanced effects on the interaction variation. Ibrahim and Hussein 2006 found significant differences among 16 Roselle genotypes in Egypt while Falusi *et al*. 2014 found the same differences among six Roselle accessions in Nigeria.

2- Genotypes performance in the growth characters:

Performance of genotypes characters in S_1 and S_2 was tabulated as mean values in Table, 4. Generally, the genotype mean values were increased in S_2 for most of the studied traits. The genotype 9 presented the highest mean value of plant height (PH) 177.3 ± 1.5 and 176.7 ± 0.9 cm and fresh sepals yield/plant (FSPY) 556.7 ± 12.2 and 546.7 ± 3.3 in both S_1 and S_2, respectively. While the genotype 13 had the maximum mean value of FCY 550.0 ± 28.9 and 490.0 ± 5.8 g and DSEY 53.0 ± 1.7 and 45.0 ± 0.6 g in the generations, respectively.

Whilst 20.0 ± 0.0 and 18.3 ± 0.3 were recorded for the maximum number of total branches/plant (NTB) in both seasons and corresponded to the genotype 2. Whereas genotype 10 presented the highest number of capsules/plant (NC) 127.3 ± 1.5 and genotype 3 had the highest dry sepals yield/plant (DSPY) 103.0 ± 1.0 g in S_1. But, in S_2 the maximum NC was 131.0 ± 0.6 recorded for genotype 2 and the value of 122.3 ± 1.5 was the highest DSPY related to genotype one in S_2. On the other hand, the lowest mean value for NC, FSPY and DSPY corresponded to genotype 15 for both seasons. But the lowest FCY and DSEY corresponded to genotype 4, the smallest PH related by genotype 2 and the lowest NTB recorded for genotype 13 (Table, 4). The maximum value of PH is less than 206 cm observed by Ibrahim and Hussein 2006, similar with 169 cm, approximately observed by Atta *et al*. 2011, but higher than 141cm observed by Falusi *et al*. 2014. While, Amir *et al*. 2008 and Mahadevan *et al*. 2009 said that Roselle plant is about 3.5 m tall. The highest NTB was inagreement with 24.5 branches/plant recorded by Falusi *et al*. 2014 and nearest to 36.73 branches/plant recorded by Atta *et al*. 2011. Meanwhile, the maximum NC was less than 156.07 capsules/plant recorded with Atta *et al*. 2011, but higher than 27 capsules/plant resulted by Ibrahim and Hussein 2006 and 58.16 capsules/plant recorded by Falusi *et al*. 2014. The result of FSPY was higher than those of Ottai and Abd El-Khair 2004 who found FSPY of Sudani and Masri cultivars were 115.6 and 173.2 g/plant for the cultivation by direct seeds and 237 and 172.4 g/plant for the cultivation by transplantation. Hence, the highest DSPY was enhancing than 17.8 and 19.8 g/plant for the cultivation by direct seeds of Sudani and Masri cultivars, respectively and 26.7 and 23.1 g/plant for the cultivation by transplantation resulted by Ottai and Abd El-Khair 2004, enhancing than 13.96 g/plant resulted with Ibrahim and Hussein 2006 and 29.88 g/plant resulted with Ibrahim *et al*. 2013a. The result of FCY was less than 1 kg/plant found by Osman *et al.* 2011. The highest DSEY was higher than 15.23 g/plant found by Ibrahim and Hussein 2006, 23.3 g/plant found by Ibrahim *et al*. 2013a and 26.04 g/plant found by Falusi *et al*. 2014. Generally, these results confirm the genetic diversity among the studied genotypes and can be exploited and used in the improvement of Roselle in Egypt. Also, the traits different values among genotypes can be attributed to genetic causes as well as their interactions with the environment (Ibrahim *et al*. 2013a).

Table 4: Mean values of seven characters of fifteen Roselle genotypes grown in two seasons.

Season	Code No.	Plant height (PH)	No. of total branches /plant(NTB)	No. of capsules /plant (NC)	Fresh sepals yield/plant (FSPY)	Dry sepals yield /plant (DSPY)	Fresh capsules yield /plant (FCY)	Dry Seed yield/plant (DSEY)
First	1	168.3±1.7	16.7±0.3	116.7±1.7	433.3±8.8	101.7±1.7	403.3±3.3	32.3±1.5
	2	134.0±2.1	20.0±0.0	126.0±1.2	435.0±2.9	102.7±1.5	390.0±5.8	32.7±1.5
	3	149.0±1.0	13.0±0.6	117.3±1.5	513.3±6.7	103.0±1.0	453.3±3.3	37.7±1.2
	4	170.0±2.9	14.7±0.3	111.7±1.7	330.0±5.8	102.3±1.5	283.3±12.0	27.7±1.2
	5	161.7±1.7	12.3±0.3	101.7±1.7	466.7±16.7	101.0±1.0	393.3±6.7	35.7±0.7
	6	176.0±2.1	13.7±0.3	84.3±1.2	473.3±14.5	102.7±0.5	473.3±14.5	37.7±1.5
	7	177.0±1.5	13.3±0.3	82.0±1.2	510.0±5.8	102.3±1.5	493.3±6.7	44.3±0.3
	8	152.7±1.5	13.7±0.3	98.7±1.3	490.0±5.8	100.7±0.7	406.7±6.7	37.3±1.5
	9	177.3±1.5	12.3±0.3	116.7±1.7	556.7±12.2	101.7±0.9	486.7±8.8	40.7±0.7
	10	162.7±1.5	13.7±0.3	127.3±1.5	480.0±11.6	100.7±0.7	400.0±0.0	34.0±0.6
	11	172.7±1.5	12.7±0.3	82.7±1.5	433.3±8.8	95.7±0.7	303.3±3.3	28.0±1.2
	12	171.0±1.0	12.3±0.3	96.7±1.2	416.7±10.1	86.3±0.9	400.0±0.0	40.7±0.7
	13	172.3±1.5	12.0±0.0	88.0±1.2	473.3±14.5	85.7±07	550.0±28.9	53.0±1.7
	14	155.0±2.9	13.7±0.3	92.3±1.5	433.3±16.7	86.0±0.6	390.0±5.8	35.7±0.7
	15	171.7±0.9	12.3±0.3	77.3±1.5	313.3±8.8	82.7±1.5	393.3±6.7	31.7±1.7
	G.	164.7±1.8	13.8±0.3	101.3±2.5	450.6±9.7	96.9±1.1	414.7±10.3	36.6±1.0
Second	1	175.7±0.7	17.3±0.3	127.3±1.5	450.0±5.8	122.3±1.5	440.0±5.8	39.0±1.0
	2	152.0±1.2	18.3±0.3	131.0±0.6	445.0±2.9	117.3±1.5	435.0±2.9	36.0±1.0
	3	158.3±0.9	15.7±0.3	122.0±1.2	500.0±5.8	116.0±1.0	446.7±3.3	39.3±0.7
	4	174.0±0.6	15.0±0.3	122.7±1.5	360.0±5.8	112.3±1.5	306.7±6.7	25.3±0.6
	5	166.3±0.9	14.3±0.3	113.3±1.7	500.0±5.8	111.7±1.5	410.0±5.8	36.0±0.6
	6	175.0±1.2	15.3±0.3	107.3±1.5	493.3±3.3	111.0±1.0	453.3±3.3	39.3±0.7
	7	170.0±1.2	15.3±0.3	112.3±1.5	506.7±6.7	108.7±0.7	403.3±3.3	38.3±0.9
	8	159.3±0.7	15.7±0.3	120.0±0.7	520.0±5.8	109.0±0.6	426.7±3.3	37.7±0.9
	9	176.7±0.9	14.0±0.6	124.3±0.7	546.7±3.3	106.7±0.9	445.0±2.9	39.0±0.6
	10	168.0±1.2	15.7±0.3	129.3±0.7	520.0±5.8	105.7±0.7	410.0±5.8	36.0±0.6
	11	168.3±0.9	14.3±0.3	100.0±0.0	443.3±3.3	103.3±0.9	325.0±2.9	30.7±0.7
	12	172.0±1.2	14.7±0.3	103.3±0.9	445.0±2.9	100.7±0.7	396.7±3.3	35.7±0.7
	13	176.0±0.6	14.0±0.0	100.7±0.7	465.0±2.9	101.0±0.6	490.0±5.8	45.0±0.6
	14	162.3±1.2	14.7±0.3	106.0±0.6	445.0±2.9	96.0±0.6	400.0±2.9	36.0±0.6
	15	172.3±1.5	15.2±0.3	96.3±0.9	353.3±3.3	92.3±1.5	401.7±1.7	34.7±0.9
	G.	168.4±1.1	15.2±0.2	114.4±1.7	466.2±8.1	107.6±1.2	412.7±6.9	36.5±0.7

3- Genotypes performance under the genetic parameters:

Estimates of four genetic parameters; genotypic and phenotypic coefficient of variations (GCV and PCV, respectively), broad sense heritability (h^2b) and expected genetic advance % (GA) for all studied characters of the selected Roselle genotypes grown in S_1 and S_2 seasons are given in table (5). In general, the values of all

genetic parameters were higher in s_1 for all characters comparing with those in S_2 except h^2b values for FSPY, DSPY, FCY and DSEY as well as GA value for DSPY were higher in S_2. Lowest values of GCV and PCV, 12.82 and 13.27, respectively in S_1 and 7.58 and 7.85, respectively in S_2 were exhibited for PH trait. The highest GCV and PCV values 29.92 and 31.90, respectively in S_1 were computed for the trait of DSEY and 20.54 and 20.74, respectively for FSPY in S_2. Ibrahim *et al*. 2013a found that the highest GCV was for DSEY in two seasons. Meanwhile Ibrahim and Hussein 2006 exhibited the maximum GCV for DSPY and DSEY in two seasons. However, NTB presented the lowest h^2b values (0.742 and 0.468) and lowest GA values (6.09 and 2.70) for S_1 and S_2, respectively. Opposite to NC which presented the highest GA values (59.64 and 40.90) in S_1 and S_2, respectively. The maximum h^2b (0.985 and 0.981) were similar in both seasons approximately and belonging to NC in M_1 but to FSPY in S_2 (Table, 5). Generally, most of the heritability estimates found to be high values unlike with Ibrahim *et al*. 2013a who found low heritability estimates among 13 yield components of Roselle genotypes. However, Ibrahim and Hussein 2006 found the high h^2b coupled with high GA for PH, DSPY and DSEY. Ottai *et al*. 2006 reported that the characters which appear high estimates of heritability and genetic gain such as DSPY and FSPY would to be useful criteria as a base selection on the phenotypic performance. A large proportion of the phenotypic variance for such traits was due to genetic effects, so selection for these traits will be effective.

Table 5: Four genetic parameters for seven characters of Roselle genotypes grown in two seasons.

Items	Season	Plant height (PH)	No. of total branches /plant (NTB)	No. of capsules /plant (NC)	Fresh sepals yield /plant (FSpY)	Dry sepals yield/plant (DSpY)	Fresh capsules yield /plant (FCY)	Dry seed yield/plant (DSeY)
GCV	S1	12.82	24.96	28.80	24.54	13.33	28.59	29.91
	S2	7.58	12.60	17.56	20.54	12.93	19.62	10.42
PCV	S1	13.27	28.99	29.02	25.21	13.73	29.50	31.90
	S2	7.85	18.41	17.78	20.74	13.25	19.85	10.70
h^2b	S1	0.933	0.742	0.985	0.948	0.942	0.940	0.879
	S2	0.931	0.468	0.976	0.981	0.952	0.977	0.949
GA	S1	42.01	6.09	59.64	-	25.81	-	21.14
	S2	25.32	2.70	40.90	-	27.96	-	8.73

GCV=Genotypic coefficient of variation, PCV Phenotypic coefficient of variation, h2b=heritability broad sense and GA = Genetic advance

4- Phenotypic correlation coefficients:

Estimates of correlation coefficients for seven studied characters of Roselle genotypes grown in two successive seasons were tabulated in Table 6. Highly significant and negative correlation was detected between PH and NTB for both seasons.

Table 6: Correlation coefficients for seven characters of Roselle genotypes grown in two successive seasons.

Season	Trait	(PH)	(NTB)	(NC)	(FSW)	(DSW)	(FCW)
First	Plant height (PH)	1.00					
	No. of total branches/plant (NTB)	-0.574**	1.00				
	No. of capsules/plant(NC)	-0.502**	0.537**	1.00			
	Fresh sepals weight/plant (FSW	-0.028	-0.135	0.222	1.00		
	Dry sepals weight/plant (DSW)	-0.168	0.403**	0.536**	0.413**	1.00	
	Fresh capsules weight/plant (FCW)	0.191	-0.239	-0.138	0.631**	-0.073	1.00
	Dry seed yield/plant (DSY)	0.219	-0.363*	-0.227	0.520**	-0.231	0.875**
Second	Plant height (PH)	1.00					
	No. of total branches/plant (NTB)	-0.433**	1.00				
	No. of capsules/plant(NC)	-0.315*	0.691**	1.00			
	Fresh sepals weight/plant (FSW	-0.128	0.132	0.355*	1.00		
	Dry sepals weight/plant (DSW)	-0.195	0.736**	0.753**	0.274	1.00	
	Fresh capsules weight/plant (FCW)	-0.023	0.183	0.118	0.523**	0.153	1.00
	Dry seed yield/plant (DSY)	0.128	-0.010	0.114	-0.235	0.095	0.264

Also, highly negative significant correlation and negative significant correlation were estimated between PH and NC in S_1 and SA_2, respectively. NTB exhibited positive high significant association with both NC and DSPY in both S_1 and S_2. However, NC revealed positive and high significant correlation with DSPY in both seasons. Also, high positive significantly correlation was detected between FSPY with FCY in both seasons. DSEY showed negative significant and highly significant positively association with NTB and FSPY, respectively in the S_1 only. Ultimately, positive significant correlation was computed between NC and FSPY only in S_2 (Table 6). These results harmonize with the results of Ottai *et al*. 2004 and Ibrahim and Hussein 2006. While, Ibrahim *et al*. 2013b mentioned that the genotypic correlation coefficient exceeded the phenotypic correlation coefficient for the most characters of Roselle lines. All the correlated characters are important for the breeders because the improvement of one character may cause improvement or deterioration in associated

character/s. So knowledge of associations between these plant attributes is very essential to determine the most efficient breeding procedure.

5- RAPD-PCR Fingerprinting:

Three Roselle genotypes; high, moderate and low of DSPY during S_2 were subjected for molecular RAPD-PCR identification using eleven primers (Fig. 1 and Table, 7). PCR products revealed of 94 amplified DNA fragments with different sizes ranged from 150-2200bp. Whereas 77 from them were monomorphic bands and the other 17 fragments were polymorphic bands with 18.1 % polymorphism.

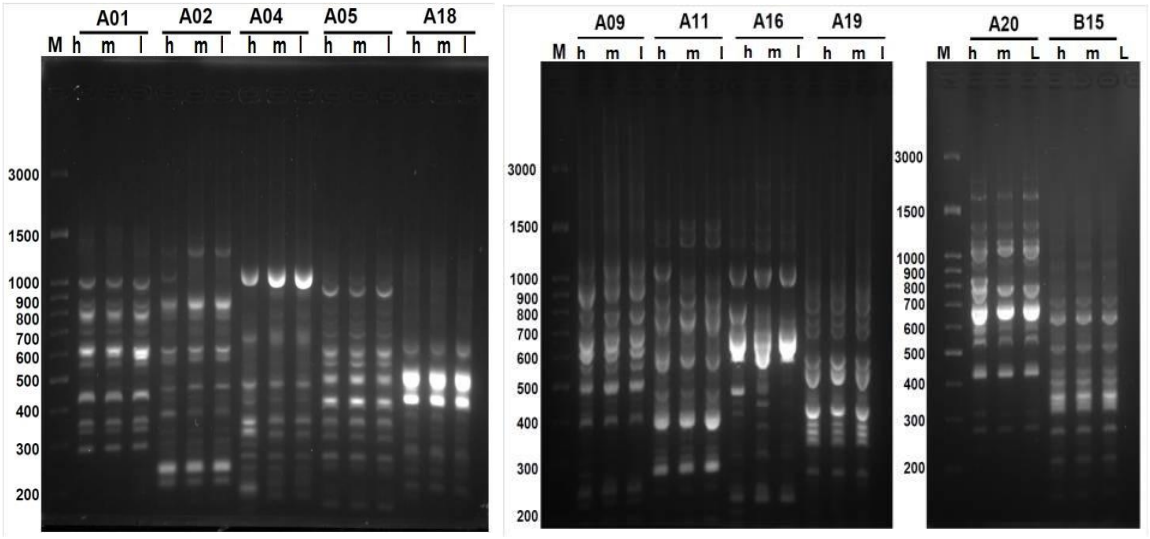

Fig. 1: DNA amplified fragments using eleven random primers for three Roselle genotypes M: DNA ladder markers, h: high, m: moderate and l: low DSPY genotypes.

Table 7: Genetic polymorphism between three Roselle genotypes.

Primers	Total bands	Monomorphic bands	Polymorphic bands	Polymorphism %
OP-A01	16	15	1	6.25%
OP-A02	15	14	1	6.67%
OP-A04	16	10	6	37.5%
OP-A09	14	12	2	14.29%
OP-A16	19	13	6	31.58%
OP-A20	14	13	1	7.14%
All primers	94	77	17	18.10%

The amplification fragments varied from primer to another, where the primer A16 showed the highest fragments (19 fragments) while the lowest 14 fragments were produced by primers A09 and A20. Furthermore, the primer A04 revealed the highest polymorphism percentage (37.5), however the lowest polymorphism percentage (6.25) was produced by the primer A01. The amplification banding patterns of five primers A05, A18, A19, A11 and B15 were similar in all three tested genotypes, therefore, they were neglected. However, the other six primers A01, A02, A04, A09, A16 and A20 showed many specific markers or polymorphic bands. The polymorphic bands 570bp using primer A01, while 700bp, 520, 220bp and 200bp using primer A04, as well as 530bp using primer A16 were detected in moderate and low genotypes. Moreover, A02 and A20 primers revealed a unique specific marker only in high genotype at molecular weight 1020bp and 820bp, respectively. Also, only in high genotype, the primers A04 and A09 showed four specific markers, two of them at 300bp and 210bp were detected by A04 and the other markers were amplified by A09 at molecular weight 300bp and 280bp. The primer A16 showed many polymorphic bands, 530bp appeared only in moderate genotype, 490bp and 420bp detected in high and moderate genotypes, and 380bp presented in high and low genotypes. The similarity index was calculated for the three tested genotypes and illustrated in Fig. (2) and Table, 8. The highest similarity (98%) was found between the moderate and low genotypes. However, the similarity percentage 91% was found between the high genotype with both moderate and low genotypes. The genetic diversity between the tested genotypes was in the range of 91-98% similarity. Same results were reported by Hanboonsong *et al.* 2000 and Omalsaad *et al.* 2014.

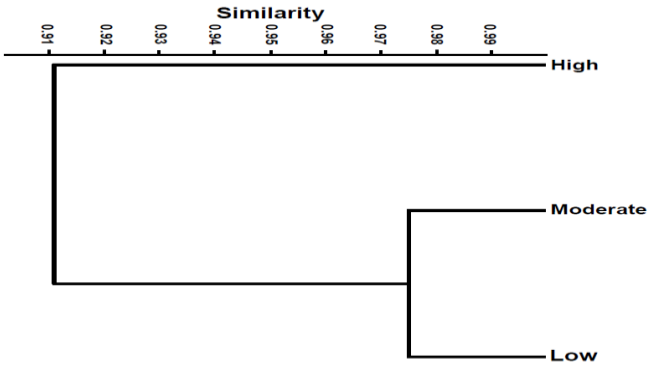

Fig. 2: Dendrogram represented the genetic relationships among the three Roselle genotypes, using UPGMA cluster analysis of Jaccard genetic similarity coefficients generated from eleven RAPD markers. (H: High, M: Moderate and L: Low DSPY genotypes).

Table 8: Similarity indices between three Roselle genotypes (High, Moderate and Low DSPY) with RAPD markers based on Jaccard's coefficients.

	High	Moderate	Low
High	1		
Moderate	0,91	1,00	
Low	0,91	0,98	1

Moreover, the dendrogram constructed by the UPGMA method revealed the genetic relationship between the studied genotypes and divided them into two groups; the first contained the moderate and the low genotypes, and the second cluster included only the high genotype. RAPD-PCR is a good technique for identification of studied Roselle genotypes and the same results detected by Khafaga 2013. These results could refer to that the artificial selection procedure is necessary for plant breeding for development new genotypes which could be produced by performing in their genome that might be occurred by mutations or in a segregation of plant population. Our results are in agreement with Cleveland *et al.* 2000 who reported that genetic diversity is the basis for genetic improvement through plant selection within segregating populations, which changes the genetic makeup of the population. Moreover, Cooner 2003 exhibited that the artificial selection can be used to increase the variation. In general, the genetic variation is required for starting plant breeding program.

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