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RESPONSE TO PLANTING DATE, STRESS TOLERANCE AND GENETIC DIVERSITY ANALYSIS AMONG OKRA (*Abelmoschus esculentus*) VARIETIES

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ABSTRACT

Six okra varieties were studied for the growth and yield at two different planting dates in 2016 and 2017 in the Research Farm of Department of vegetable crops, Faculty of Agriculture, Assiut University, Egypt. The experiments were laid out in a split plot design in randomized complete blocks arrangement. The results revealed that the measured growth, yield attributes and yield parameters of the studied varieties were significantly affected by planting dates. The early planting date '15th of March' enhanced growth and yield parameters of the okra variety 'Pusa Sawani'. The varieties 'Iraqi' and 'Hala' were less adapted to Upper Egypt weather. The okra variety 'Pusa Sawani' showed the highest performance under Assiut weather and was suggested to cultivate at summer season in Upper Egypt. The six okra varieties were grouped into four clusters based on agro-morphological traits. Among these varieties, 'Pusa Sawani' variety was separated only in a cluster which had the highest mean values for the most of agro-morphological traits. The six varieties were screened for drought, salinity and heat stress at early growth seedling. Significant variation was observed among the varieties. These varieties were grouped into two main clusters based on tolerance indices. Three molecular marker techniques, ISSR, SRAP and SSR were used to study the genetic diversity among the six varieties and to detect markers related to agro-morphological traits and drought, salinity and heat stress tolerance. These results revealed at least one marker related to agro-morphological traits and/or drought, salinity and heat tolerance.

Key words: ***Okra, Planting dates, Heat, Salinity, Drought, Genetic diversity.***

INTRODUCTION

Okra (*Abelmoschus esculentus*) is one of the most consumed vegetables in Egypt and worldwide. The total area cultivated with okra in Egypt was 11393 feddan and the total production 55166 tons of immature pods (Mousa *et al* 2012 and FAOSTAT, 2014). Fruits of okra are very rich in calcium, rich in protein, carbohydrates and fats and a good source of vitamins A, Band C (Adiroubane and Letachoumanane 1992). There is a limited number of vegetables able to be grown under Upper Egypt extreme dominant weather during summer including high temperature and light intensity. Therefore, okra was considered one of the most important sources of daily cash for numerous poor families in summer in Upper Egypt (Mousa *et al* 2012). The Upper Egypt farmers normally start the growing season of summer okra at late February until middle of March, which allows early yield and high prices. However, low germination level, delay of flowering and fruit setting, weak plants and low number of harvested fruits are the major challenges facing the early planting of okra (Mohammed *et al* 2007

and Mousa *et al* 2012). Therefore, optimum planting dates of okra plants has a great impact on yield and quality of okra (Mousa *et al* 2012).

High-temperature, drought and salinity are the most important environmental stresses limiting plant growth at early seedling stage (Taherkhani *et al* 2013), these problems are widespread in several areas around the world (Soltani *et al* 2006). Salinity and drought stress act by decreasing the rate of seeds germination and seedling growth leading to reductions in plant growth and final crop yield (Macar 2009)**.** Drought stress caused in, decreases of water availability, changing the percentage and velocity of seed germination and growth of seedlings adversely. The damaging effects of salinity on seed germination and seedling establishment are attributed to a decrease in osmotic potential of the growing medium, specific ion toxicity and nutrition deficiency (Greenway and Munns 1980). High-temperature effect on plant growth through the indirect and direct injuries, the indirect damage includes inactivation of enzymes, inhibition of protein synthesis while the direct injuries due to increased fluidity of membrane lipids, and cause protein denaturation (Howarth 2005).

A broad base of genetic variations among okra genotypes is very useful for plant breeders to produce new varieties that meet the changing needs regarding adaptation to growing conditions, resistance to environmental stresses product yield or specific quality requirements (Prakash *et al* 2011). Information on the level of variation for important agro-morphological and morpho-physiological traits of okra is limited. Knowledge of genetic diversity among the okra genotypes may play an important role in breeding programs to improve agronomic traits and resistance to environmental stresses. Physiological and agro-morphological diversity have been studied by several studies (Salameh and Kasrawi 2011, Bello *et al* 2006, Rahman *et al* 2012, Umrao *et al* 2014, Amoatey *et al* 2015, Kaur *et al* 2013, Kyriakopoulou *et al* 2014, Cong-Ying *et al* 2015**,** Younis *et al* 2015, Singh *et al* 2018 and El-Sherbeny *et al* 2018). Morpho-physiological and agro-morphological traits are not adequate for the development of gene pools since either these traits are influenced by environmental conditions and stage of plant growth or they reveal only limited variation (Terzopoulos and Bebeli 2008). Molecular markers are powerful tools for the assessment of genetic diversity among different genotypes (Cong-Ying *et al* 2015). They have the advantage of providing thorough genome assessments that are not influenced by environmental factors. Different types of molecular marker techniques were used to study the genetic diversity and relationships among okra genotypes. In addition, they can be used to identify unique genotypes and markers that are associated with or linked to important agro-morphological traits and tolerance to heat, drought and salinity stresses. Among the several DNA

based techniques, random amplified polymorphic DNA (RAPD, Kaur *et al* 2013, Ikram *et al* 2013 and Patel *et al* 2018), inter-simple sequence repeat (ISSR, Cong-Ying *et al* 2014 and 2015 and Younis *et al* 2015 and El-Sherbeny *et al* 2018), amplified fragment length polymorphism (AFLP, Kyriakopoulou *et al* 2014 and Younis *et al* 2015), sequence related amplified polymorphism (SRAP; Gulsen *et al* 2007).Simple sequence repeats (SSRs, Kumar *et al* 2017, Patel *et al* 2018, Ouedraogo *et al* 2018 and Ravishankar *et al* 2018).

The present study aimed to a) assessment of the performance of the growth and yield of six okra varieties under two planting dates at Assiut Governorate, b) studying the genetic diversity among okra varieties using agro-morphological, morpho-physiological and molecular markers, c) screening of okra varieties, on the basis of early growth parameters, for drought, salinity and heat tolerance by exposing them to different stress regimes, and d) identifying molecular markers associated with agro-morphological traits, drought, salinity and heat tolerance of okra varieties.

MATERIALS AND METHODS

Experimental site and statistical design

Field experiments were conducted in 2016 and 2017 cropping seasons at the Research Station of Vegetables Department, Faculty of Agriculture, Assiut University (lat 27° 03' N, long 31° 01' and alt 70 m asl.), to study the response of 6 okra varieties to planting dates. The soil was clay, having pH 7.8 and field capacity 42%. The soil contents of NPK and some micronutrient elements were 1.78% (N^+), 0.624% (P^+), 3.3% (K^+), 0.6 ppm (Fe^+), 4 ppm (Zn^+) and 11 ppm (Mn^+). The experiments were laid out using a split plot design in randomized complete blocks arrangement 3 replicates. The planting dates were distributed in the main plots, while okra varieties were devoted to the sub-plots.

Plant materials

Seeds of the six okra varieties (*Abelmoschus esculentus*) var 'Balady Assiut', 'Pusa Sawani', 'Iraqi', 'Hala', Emerald and 'Balady Qina' were obtained from Vegetable Research Station, Giza, Egypt.

Planting dates and experimental site preparation

The performance of six okra varieties was tested at two planting dates (15^{th} March and 15^{th} April) in the two cropping seasons 2016 and 2017. The experimental site was prepared as recommended for okra planting by Mousa *et al* (2012) with modifications. The experimental site was supplied with 10 ton/fed animal manure and a basal dose of 100 $kgfed^{-1}$ calcium super phosphate (36%, P_2O_5), 100 kg fed^{-1} ammonium nitrate (NH_4NO_3, 33.5% N) and 100 $kgfed^{-1}$ ammonium sulphate (38% $(NH4)_2SO_4$). The remaining amount of ammonium nitrate (200 kg fed^{-1}) and 100 kg fed^{-1} potassium sulphate (48%, K_2O) were provided to okra at vegetative,

flowering and fruit setting stages. The planting space was 30 cm (between two adjacent okra plants in the same row) and the row spacing was 70 cm (between each two rows). Cultural practices (irrigation, fertilization and pests and diseases control) were applied as recommended for okra production (Mousa *et al* 2012).

Measurements

The following parameters were measured for okra plants: days to flowering, plant height (cm) and no. of branches/plant at end of growing season (cm), no. of fresh pods/plant, weight of fresh pods/plant (g), average length of fresh pods (cm) and total yield of fresh pods mt/fed^{-1}.

Correlation between measured parameters

The simple linear correlation coefficients between the growth, yield components and yield parameters during the cropping seasons 2016 and 2017 were calculated. The correlation significances were tested at probability level of 5% (Gomez and Gomez 1984).

Data Analysis

Analysis of variance of split plot design and RCBD experiments as described by (Gomez and Gomez 1984) was performed. The treatment means were compared by Duncan test at 5% probability level.

Genetic distance based on agro-morphological traits

Euclidean distance (ED) was computed from all data collected for okra varieties after standardization (subtracting the mean value and dividing it by the standard deviation). The distance matrix from phenotype traits was used to construct dendrogram based on the Unweighted Pair-group Method with Arithmetic Means (UPGMA).

Screening for salt, drought and heat stress at seedling stage

In order to study the effects of water stress, using polyethylene glycol (PEG), salinity stress using sodium chloride (NaCL) and heat stress on early seedling growth parameters in okra, an experiment was conducted at the biotechnology laboratory, Department of Genetics, Faculty of Agriculture, Assiut University, Assiut, Egypt. The experiment of design used in this study was completely randomized design (CRD) with three replications (each replicate containing five pots containing a mixture of sand, peat and soil in equal sizes, each pots containing 10 grains per each genotype). Grains of the six okra cultivars were subjected to four stress levels of PEG-6000 (0.0%, 5%, 10%, and 15%), according to methods by Michael and Kaufmann (1973), four stress levels of NaCL (0.0%, 50 mM, 100 mM and 150 mM) and two temperature regimes [optimum temperature (28/22 °C day/night) and high temperature (40/30 °C day/night] according to methods by Singh *et al* (2012). After four weeks the data were recorded on the following parameters: Shoot length (Sh L), Root length (R L), Shoot fresh weight (Sh FW), Shoot dry weight (Sh DW), Root fresh weight (R

FW) and Root dry weight (R DW). For all investigated parameters, analysis of variance was performed using the MSTAT-C software package. Significant differences among the mean values were compared by LSD test ($P < 0.05$).

Molecular analysis

DNA isolation

Genomic DNA was extracted from young leaf tissues (2-weeks-old seedlings) of six okra varieties following the CTAB method described by Doyle and Doyle (1990). The quality of DNA was checked on 0.8 % agarose gel and the concentration was measured using UV spectrophotometer at 260 nm.

ISSR, SRAP and SSR detection and analyses

A total of 10ISSR primers, 10SRAP primer combinations and 15SSR primer pairs (Table 1), obtained from Metabion International AG Company (Germany) were used. The reaction conditions were optimized and mixtures (25 µL total volume) were composed of 11.7 µL dH_2O, 3.0 µL 10X reaction buffer, 3.0 µL dNTP's mix (2.5 mM each dNTP; Promega), 2.0 µL primer (2.5 µM) for (ISSR), 1.0 µL forward primer, 1.0 µL reverse primer for (SRAP and SSR), 4.0 µl $MgCl_2$ (25 mM), 0.3 µL *Taq* DNA polymerase (5 U per µL; Promega) and 1 µL Template DNA (50 ng per µL). PCR procedures were carried out in a Lab Cycler (Model SensoQuest, GmbH, Germany).

The PCR amplification conditions were; For ISSR; was as follows: initial denaturation for 5 min at 94ºC, 45 cycles of 1 min denaturation at 92ºC, 1 min annealing at 38ºC - 44ºC and 2 min extension at 72ºC, 10 min final extension at 72ºC, then followed by a final hold at 4ºC.

For SRAP; was as follows initial denaturation for 4 min at 94ºC, 10 cycles of 1min denaturation at 92ºC, 1 min annealing at 35ºC and 2 min extension at 72ºC, 35 cycles of 1 min denaturation at 92ºC, 1 min annealing at 50-55ºC and 2 min extension at 72ºC, 10 min final extension at 72ºC, then followed by a final hold at 4ºC.

For SSR was as follows: initial denaturation at 94ºC for 4 min, followed by 35 cycles of denaturation, annealing and polymerization steps (94ºC for 1 min, 50-60ºC for 1 min and 72ºC for 2 min, respectively. A final extension of polymerization was done at 72ºC for 7 min, then followed by a final hold at 4ºC.

Amplification products were separated on agarose 1.5%, 2% and 2.5% for ISSR, SRAP, and SSR, respectively. Gels were stained with ethidium bromide (EB) (0.5 µg/ml) and DNA fragments were visualized using GelDoc-It®^{2} Imager

Table 1. List of ISSR, SRAP and SSR primer combinations used in the study.

Primers		Sequences	Primers		Sequences
HB10		GAG AGA GAG AGA CC	HB14		GTG GTG GTG GC
ISSR-814		CTCTCTCTCTCTCTCTTG	HB08		GAG AGA GAG AGA GG
HB15		GTGGTGGTGGC	HB09		GTG TGT GTG TGT GG
HB06		GACAGACAGACAGACA	UBC820		GTG TGT GTG TGT GTG TC
HB12		CACCACCACGC	HB07		ACACACACACACACACT
SSR-1	F R	CTCAACTTGGATGGATGAGT CCTCTCGAACTGAGAAAGAA	SRAP-1	F R	TGA GTC CAA ACC GGA TA GAC TGC GTA CGA ATT AAT
SSR-2	F R	CGTCGAAGGATTTCACTATC CAATTGCACATCAGATTCAC	SRAP-2	F R	TGA GTC CAA ACC GGA TA GAC TGC GTA CGA ATT GAC
SSR-3	F R	TGTTTTACCCCTAACCGTCCC TACCCGTACCCGTCCGTA	SRAP-3	F R	TGA GTC CAA ACC GGA TA GAC TGC GTA CGA ATT CAA
SSR-4	F R	TAGCAAAAGCGATGATTGTCTG CCCCTAAACCCTAATCCTGACT	SRAP-4	F R	TGA GTC CAA ACC GGA GC GAC TGC GTA CGA ATT TGA
SSR-5	F R	TCAAGACAAGGATAAAGTCGCAC TAATGCAGGGTGATTTAGATGG	SRAP-5	F R	TGA GTC CAA ACC GGA GC GAC TGC GTA CGA ATT CAA
SSR-6	F R	TAGAGGGATAAATTGGGAGATGC CGTTGGGACTACTGTGACTCTG	SRAP-6	F R	TGA GTC CAA ACC GGA AT GAC TGC GTA CGA ATT GAC
SSR-7	F R	GTGATTATGGTTGCCTGAAT CCCACTGACAGCTTATTGAA	SRAP-7	F R	TGA GTC CAA ACC GGA CC GAC TGC GTA CGA ATT GAC
SSR-8	F R	TAACGCAAACTGAACTCTCGTTA ACCTAACCCTAACCCTAAACCG	SRAP-8	F R	TGA GTC CAA ACC GGA CC GAC TGC GTA CGA ATT CTG
SSR-9	F R	CATTTTAAGGAGCGAGTGTC CTCTTCCTCAACAAAACCAG	SRAP-9	F R	TGA GTC CAA ACC GGA CA GAC TGC GTA CGA ATT AAT
SSR-10	F R	CGTCGAAGGATTTCACTATC CAATTGCACATCAGATTCAC	SRAP-10	F R	TGA GTC CAA ACC GGA GC GAC TGC GTA CGA ATT AAT

For each primer, the presence (1) or absence (0) of DNA bands in each genotype was visually scored and entered into a binary matrix. The pairwise comparisons between the tested genotypes were used to calculate the coefficient of genetic similarity matrix (Gs) according to Dice (1945). A dendrogram was constructed based on similarity estimates using NTSYS-pc version 2.11T (Rolhf 2002). The correlation between the different molecular marker systems as well as between molecular markers and phenotypic traits were calculated using Mantel test (Mantel 1967). The three parameters viz., Polymorphic information content (PIC), Marker index (MI) and Resolving power (Rp), was calculated as follows: "PIC"= 1- $[(p)^2 + (q)^2]$ (Ghislain *et al* 1999), "MI"= PIC x ηβ (Powell *et al* 1996) and "Rp"=∑Ib, (Prevost and Wilkinson 1999).

RESULTS

Growth parameters

There were observed significant variances due to days to flowering due to the effects of studied okra varieties, planting dates and their interaction in both seasons (Table 2). The results showed that the early planting significantly enhanced early flowering of okra variety 'Pusa Sawani' as compared with other studied varieties. The results revealed that plants of 'Pusa Sawani' produced flowers after 41.60 and 42.60 days for planting dates 15th March and 15th April, respectively in 2016.

Table 2. Growth parameters, yield and yield attributes of okra varieties as affected by planting date.

Traits	Okra varieties	2016			2017		
		15thMarch	15thApril	Mean	15thMarch	15thApril	Mean
Days to flowering	Balady Assiut	6.13E+02	54.30c	57.80E	59.60e	51.60b	55.60C
	Balady Qina	57.30d	53.60b	55.45C	55.30d	50.60b	52.95B
	Emerald	59.60d	54.30c	56.95C	56.30d	52.60c	54.45C
	Pusa Sawani	41.60a	42.60a	42.10A	40.30a	41.30a	40.80A
	Iraqi	76.30f	54.60c	65.45D	73.60f	52.30c	62.95D
	Hala	54.60c	52.30b	53.45B	53.30c	51.60b	52.45B
	Mean	58.45B	51.45A		56.40B	49.33A	
Plant height (cm)	Balady Assiut	130.6d	175.5b	153.1B	122.5c	165.3a	143.9B
	Balady Qina	95.3f	100.1f	97.7E	87.9f	97.6e	92.8E
	Emerald	120.1e	130.4d	125.3D	113.6d	118.5c	116.1D
	Pusa Sawani	180.9a	175.7b	178.3A	165.7a	171.6a	168.7A
	Iraqi	130.7d	140.6c	135.7C	121.7c	133.7b	127.7C
	Hala	100.3f	80.5g	90.4F	95.7e	76.2g	86.0F
	Mean	126.3B	133.8A		117.9B	127.2A	
No. branches/plant	Balady Assiut	5.012d	3.321f	4.167D	4.985d	3.167f	4.076D
	Balady Qina	7.362b	4.633d	5.998B	7.262b	4.531d	5.897B
	Emerald	6.013c	4.366e	5.190C	5.956c	4.289e	5.123C
	Pusa Sawani	2.321g	3.355f	2.838F	2.217g	3.167f	2.692E
	Iraqi	8.641a	5.013d	6.827A	8.421a	8.311a	8.366A
	Hala	4.621d	3.031f	3.826E	4.423d	4.126e	4.275D
	Mean	5.662A	3.953B		5.544A	4.599B	
No. fresh pods/plant	Balady Assiut	33.610g	49.510c	41.560C	31.118g	47.974c	39.546C
	Balady Qina	37.350f	45.320e	41.335C	35.318f	43.268e	39.293C
	Emerald	48.110d	50.160c	49.135B	46.402d	48.704c	47.553B
	Pusa Sawani	63.150a	62.110b	62.630A	61.291a	59.124b	60.208A
	Iraqi	31.620h	32.130g	31.875E	29.883h	31.456g	30.670D
	Hala	34.120g	37.330f	35.725D	31.691g	26.296i	28.994E
	Mean	41.327B	46.093A		39.284B	42.804A	
Fresh pod length (cm)	Balady Assiut	3.71E+01	3.621e	3.667C	3.544d	3.440d	3.492C
	Balady Qina	4.632c	4.841b	4.737B	4.576c	4.810c	4.693B
	Emerald	4.251d	4.061d	4.156C	4.148d	3.935d	4.041C
	Pusa Sawani	5.371b	6.187a	5.779A	5.406b	6.322a	5.864A
	Iraqi	4.492c	4.592c	4.542B	4.419c	4.531c	4.475B
	Hala	5.132b	4.813b	4.973B	5.137b	4.779c	4.958B
	Mean	4.599B	4.686A		4.538B	4.636A	
Fresh pods weight /plant (g)	Balady Assiut	306.1h	335.1d	320.6C	284.7h	310.6d	297.7C
	Balady Qina	321.7f	380.6c	351.2B	299.2e	354.0c	326.6B
	Emerald	318.6g	327.9e	323.3C	294.3f	304.9e	299.6C
	Pusa Sawani	410.6a	396.1b	403.4A	380.9a	368.4b	374.6A
	Iraqi	218.5k	231.6j	225.1E	201.2k	215.4j	208.3E
	Hala	238.1i	242.6i	240.4D	221.4i	225.6i	223.5D
	Mean	302.3B	319.0A		280.3B	296.5A	
Total yield of fresh pod (ton/fed.)	Balady Assiut	5.653g	6.351e	6.002C	5.157g	5.906f	5.532D
	Balady Qina	6.707d	7.162c	6.935B	6.138d	6.651c	6.395B
	Emerald	6.013f	6.233e	6.123C	5.592e	5.787e	5.690C
	Pusa Sawani	8.160a	7.536b	7.848A	7.589a	7.118b	7.353A
	Iraqi	4.321j	4.861i	4.591E	4.019i	4.521h	4.270F
	Hala	5.116h	5.261h	5.189D	4.758h	4.893g	4.825E
	Mean	5.995B	6.234A		5.542B	5.813A	

[1] **Means in the same Colum followed by the same small letter(s) do not significantly different at 0.05 level of probability.**

In addition, 'Pusa Sawani' plants produced the earliest flowers at planting date15th March (40.30 days) and 15th April (41.30 days) in 2017 (Table 2). On the contrary, plants of the variety 'Iragi' were the latest to produce flowers with 76.30 and 73.60 days in 15th March 2016 and 2017, respectively. Regarding height of okra plants, the results revealed a significant interaction between the studied varieties and planting dates. Tallest plants were recorded for 'Pusa Sawani' with 180.90cm at planting dates 15th March in 2016 and 171.60cm at planting date 15th April in 2107. The shortest plants were observed for 'Hala' which recorded 80.50cm and 76.20cm at planting date 15th April in 2016 and 2017, respectively. Plants of the okra variety 'Iraqi' produced the highest number of branches at planting date 15th March with 8.641 in 2016 and 8.421 in 2017. Moreover, 'Iraqi' plants produced the highest number of branches/plant at planting date 15th April in 2017 (8.311). As presented in Table (2), the least number of branches were observed for plants of 'Pusa Sawani' at planting date 15th March in 2016 (2.321) and 2017 (2.217).

Yield and its attributes

The results of yield and its attributes of six okra varieties grown at two planting dates are presented in Table (2). The results showed that planting okra variety 'Pusa Sawani' at 15th March significantly increased number of fresh pods/plant in 2016 (63.15 pods/plant) and 2017 (61.29 pods/plant). The varieties 'Balady Assiut' and 'Haa' produced the least no. of fresh pods/plant when planted in 15th March 2016 and 2017. The results were not significantly differed from that observed for 'Iraqi' in 2016 (32.13 pods/plant) and 2017 (31.46 pods/plant). Regarding length of fresh pods (cm), the okra variety 'Pusa Sawani' produced the longest pods at planting date of 15th April with an average pods length of 6.19 and 6.32cm in 2016 and 2017, respectively. The 'Balady Assiut' registered the shortest pods at 15th March and 15th April planting date in 2016. In addition, 'Balady Assiut' and 'Emerald' produced the shortest pods, while the differences between both varieties were not significant (Table 2). The weight of fresh pods was significantly affected by studied okra varieties, planting dates and their interaction. The greatest weight of pods/plant was recorded for plants of the variety 'Pusa Sawani' when planted at 15th March with an average weight of 410.60g and 380.90g in 2016 and 2017, respectively. Planting the okra variety 'Hala' at middle of March and April 2017 significantly reduced the weight of pods/plant (221.40g and 225.60g for middle of March at April, respectively). Moreover, 'Iraqi' variety produced the least weight of pods/plant when planted in the middle of April 2016 and the results were not significant different from that were observed for 'Hala'. The total yield of fresh pods (ton/fed) was significantly differed due to studied okra varieties, planting dates and their interaction. Planting okra variety 'Pusa

Sawani' at 15th March significantly increased total yield of fresh pods in 2016 (8.16 ton/fed) and 2017 (7.59 ton/fed) as compared to other studied varieties. The least yield of fresh pods was produced by 'Iraqi' when planted at 15th March 2016 (4.32 ton/fed) and 2017 (4.02 ton/fed). The prevailed weather conditions at Upper Egypt particularly Assiut region during March and April significantly enhanced yield and yield attributes of the okra variety 'Pusa Sawani' as compared with other studied varieties. On the contrary, the varieties 'Hala' and 'Iraqi' was not adapted to the weather of Assiut region during March and April, therefore both varieties showed the lowest performance with regard growth, yield and its attributes.

Correlation between measured parameters

The data of the correlation between growth, yield and yield attributes are presented in Table (3).

Table 3. Correlation coefficients among growth, yield and yield attributes of okra varieties as affected by planting dates.

Traits	Days to Flowering	Plant height (cm)	No. branches	Pod length (cm)	No. of pods/plant	Pod weight/ plant (g)	Total yield (ton/fed)
15th March							
Days to Flowering	**1**	**-0.368**	**0.855****	**-0.567**	**-0.726****	**-0.655***	**-0.750****
Plant height		**1**	**-0.382**	**0.133**	**0.653***	**0.5**	**0.44**
No. branches			**1**	**-0.277**	**-0.604***	**-0.473**	**-0.515**
Pod length				**1**	**0.427**	**0.299**	**0.414**
No. pods/plant					**1**	**0.871****	**0.863****
Pod weight/plant						**1**	**0.973****
Total yield							**1**
15th April							
Days to Flowering	**1**	**-0.406**	**0.732****	**-0.549**	**-0.684***	**-0.691***	**-0.785****
Plant height		**1**	**-0.385**	**0.174**	**0.721****	**0.5**	**0.46**
No. branches			**1**	**-0.261**	**-0.641***	**-0.661***	**-0.677***
Pod length				**1**	**0.364**	**0.301**	**0.441**
No. pods/plant					**1**	**0.875****	**0.867****
Pod weight/plant						**1**	**0.973****
Total yield							**1**

***and ** indicate significant at 0.05 and 0.01 probability levels, respectively.**

The results revealed significant and positive correlations between many measured traits for okra varieties planted in 15th March. The positive correlations were observed between days to flowering and no. of branches/plant (r = 0.855**), plant height and no. of pods/plant (r=0.653*), no. of pods/plant and weight of pods/plants (r=0.875**) and total yield of fresh pods (r=0.867**). Moreover, significant positive correlation was

observed between weight of pods/plant and total yield of fresh pods (r=0.973**). On the contrary, significant negative correlations were observed between days to flowering and no. of pods/plant (r= -0.726**), pods weight/plant (r= -0.655**) and total yield of fresh pods (r= -750**). In addition, significant and negative correlation was observed between no. of branches/plant and no. of pods/plant (r= -0.604*). The correlations between growth, yield and yield attribute parameters of okra varieties planted at 15th April are presented in Table (3). Significant and positive correlations were observed between days to flowering and no. of branches (r= 0.732**) and between plant height (cm) and no. of pods/plant (r= 0.721**). In addition, significant positive correlations were observed between no. of pods/plant and weight of pods/plant (r= 0. 875**) and total yield of fresh pods (r= 0.867**). The correlation between weight of pods/plant and total yield of fresh pods was positive and highly significant (r= 0.973**). Highly significant and negative correlations were observed between days to flowering and no. of pods/plant (r= -0.684**), pods weight/plant (r= -0.691**) and total yield of fresh pods (r= -0.785**), and between no. of branches and no. of pods/plant (r= -0.641*), weight of pods/plant (r= -0.661*) and total yield of fresh pods (r= -0.677*).

Agro-morphological traits analysis

The genetic distance of six okra varieties was measured using Euclidean distance based on 7 agro-morphological traits. The genetic distances for all possible pairs of six okra varieties ranged from 1.45 to 6.66 with a mean of 4.06. The most distant varieties were Pusa Sawani and Iraqi (6.66) followed by Pusa Sawani and Hala (5.38). The lowest genetic distance was exhibited between Balady Assiut and Emerald (1.45) followed by Balady Qina and Emerald (1.63).

Cluster analysis of the six okra genotypes based on the standardized value of agro-morphological traits was performed by UPGMA method and a dendrogram was constructed as depicted in Fig. 1. Okra varieties were divided into four main clusters. The cluster I comprised of Pausa Sawani which had the highest mean values for pod length, number of pods/plant, pods weight/plants, yield/plant and plant height and lowest values for number of branches/plant and day to 50% flowering. Cluster II contained Iraqi which had the highest mean value for number of branches/plants and days to 50% flowering. Cluster III included the Hala which had the lowest mean value for plant height compared to the other varieties. Cluster IV consisted of three genotypes Balady Assiut, Balady Qina and Emerald which had moderate values for the most agro-morphological traits. This cluster represented 50% of the total number of varieties. In general, the varieties clustered together in one cluster are less divergent than those placed in different clusters.

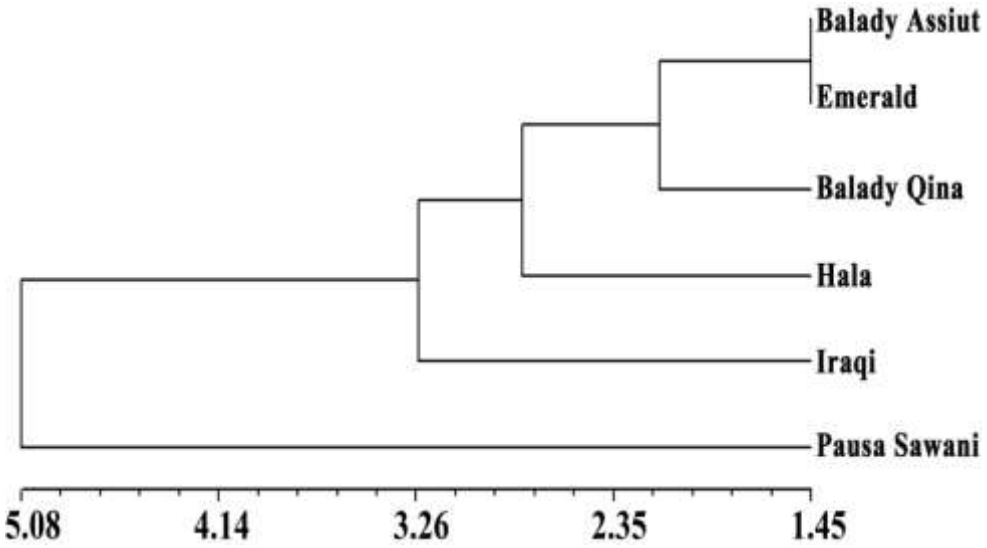

Fig. 1. Dendrogram of the genetic dissimilarities among six varieties of okra, achieved by the UPGMA method based on the Euclidian coefficient from seven agro-morphological traits.

Screening for salt, drought and heat tolerance at seedling stage

Effect of salt stress

Salt stress significantly reduced the shoot length, root length, shoot and root fresh weight and shoot and root dry weight in all studied okra varieties but the highest reduction in all traits was noted in plants exposed to 150 mM NaCl stress followed by 100 and 50 NaCl. Plants grown under non-saline conditions exhibited the highest values compared to those subjected to elevated salinity levels. The comparison of means reflected that maximum values for all traits under salt stress were noted with 'Hala' variety followed by 'Pusa Sawani', while the lowest values were recorded with 'Emerald' variety followed by 'Balady Qina'. Significant differences were found among genotypes as well as between concentrations of NaCl (Table 4). The ranking of the varieties was done on the basis of their salt tolerance index for each trait and under each NaCl concentration, separately. The varieties were ranked as 1–6 with '1' as the most tolerant and '6' as susceptible based upon their abilities to withstand saline conditions. The ranks under each NaCl concentration were pooled to calculate an overall ranking of the varieties on the basis of the traits in this study. Ranks depict the performance of varieties under all salt concentrations. The ranking of varieties (Table 5) depicted that, varieties like 'Balady Assiut', 'Pausa Sawani', 'Iraqi' and'Hala' can be categorized as the highly salt tolerant while 'Balady Qina' and 'Emerald' as sensitive ones.

Effect of drought stress

Table 4. Analysis of variance for effect of varieties and stress (salinity, drought and heat) levels on seedling trait indices of six okra varieties.

Stress	SOV	df	MS					
			ShL	R L	Sh FW	R FW	Sh DW	R DW
NaCL	Rep	2	3.576	0.671	0.0858	0.0065	0.0033	0.007
	Geno	5	53.35**	18.84**	0.5834**	0.122**	0.0133**	0.015**
	Cont	3	252.741**	179.317**	1.674**	0.909**	0.129**	0.098**
	G*C	15	10.472**	6.831**	0.031	0.056**	0.0036	0.0041
	Error	46	1.587	0.489	0.0352	0.009	0.0033	0.0037
	SOV	df	MS					
PEG	Rep	2	2.906	0.165	0.564	0.1669	0.0188	0.0244
	Geno	5	62.212**	26.806**	1.013**	0.286*	0.0115	0.0041
	Cont	3	66.924**	32.362**	0.6104	0.422**	0.045**	0.0109
	G*C	15	12.923**	6.142**	0.241	0.223**	0.0093	0.0196
	Error	46	0.394	0.671	0.255	0.0857	0.0065	0.0105
	SOV	df	MS					
HEAT	Rep	2	0.311	0.424	0.0017	0.0002	0.0006	0.0007
	Geno	5	51.992**	15.764**	0.0693**	0.0794**	0.010**	0.0077**
	Cont	1	273.627**	152.564**	1.579**	0.3383**	0.157**	0.0205**
	G*C	5	6.855**	8.154**	0.026**	0.0536**	0.0047**	0.0006
	Error	22	0.612	1.154	0.00541	0.0015	0.0009	0.00087

***and ** indicate significant at 0.05 and 0.01 probability levels, respectively.**

Table 5. Salinity, drought and heat tolerance trait indices (STTIs) of six traits studied on six okra at each stress level.

Stress	Genotype	ShL	RotL	ShFW	RotFW	ShDW	RotDW	Mean	RANK
NaCL	G1	1.21	0.84	1.54	1.30	0.98	1.20	1.18	4.00
	G2	1.18	0.89	1.07	0.77	0.77	0.60	0.88	5.00
	G3	0.76	0.89	0.80	1.07	0.51	0.61	0.77	6.00
	G4	1.25	1.26	2.01	1.54	0.90	1.41	1.39	2.00
	G5	0.65	0.90	0.85	2.98	0.75	1.00	1.19	3.00
	G6	1.13	0.92	1.73	2.43	1.51	1.39	1.52	1.00
		ShL	RotL	ShFW	RotFW	ShDW	RotDW	Mean	RANK
PEG	G1	1.18	0.85	0.87	0.57	0.64	0.55	0.78	5.00
	G2	1.18	0.89	0.78	1.35	0.49	0.45	0.86	4.00
	G3	0.76	0.97	0.57	0.54	0.62	0.56	0.67	6.00
	G4	1.24	1.30	3.41	4.06	1.10	1.61	2.12	1.00
	G5	0.63	0.77	0.94	2.37	0.78	1.18	1.11	3.00
	G6	1.13	0.92	1.38	2.19	0.85	1.20	1.28	2.00
		ShL	RotL	ShFW	RotFW	ShDW	RotDW	Mean	RANK
HEAT	G1	0.79	0.81	0.81	0.59	1.24	0.71	0.83	4.00
	G2	1.23	0.97	0.82	1.37	1.31	0.95	1.11	2.00
	G3	0.50	0.81	0.47	1.76	0.52	1.16	0.87	3.00
	G4	1.42	1.11	0.84	1.25	1.71	0.89	1.20	1.00
	G5	0.74	0.56	0.50	0.35	0.58	0.67	0.57	6.00
	G6	0.46	0.56	0.54	0.64	0.90	0.54	0.61	5.00

Balady Assiut (G1), Balady Qina (G2), Emerald (G3), Pusa Sawani (G4), Iraqi (G5) and Hala (G6).

Drought stress had an inhibiting effect on all traits at the seedling stage, therefore a significant reduction in all traits was noted in investigated okra varieties. The highest reduction in all traits was noted in plants exposed to 15% PEG stress followed by 10 and 5% PEG. The comparison of means reflected that maximum values for all traits under drought stress were noted with 'Pusa Sawani' variety while the lowest value was recorded for 'Emerald variety'. Significant differences were found among varieties for all traits, except shoot and root dry weight traits, also Significant differences were found between concentrations of PEG for all traits except root dry weight trait (Table 4). Based on ranking regarding the shoot and root length, shoot and root fresh and dry weight traits, the varieties 'Pusa Sawani', 'Iraqi' and 'Hala' can be categorized as the highly drought tolerant while 'Balady Assiut', 'Balady Qina' and 'Emerald' as sensitive ones(Table 5).

Effect of heat stress

Heat stress significantly reduced the shoot length, root length, shoot and root fresh weight and shoot and root dry weight in all studied okra varieties compared to the control treatment. The comparison of means reflected that maximum values for all traits under heat stress were noted with 'Pusa Sawani' variety followed by 'Balady Qina' variety while the lowest value was recorded with 'Iraqi' variety followed by 'Hala' variety. Significant differences were found among varieties as well between

temperature treatments (Table 4). Ranks depict the performance of genotypes under heat stress conditions (Table 5) revealed that, varieties 'Balady Qina' and 'Pusa Sawani' can be categorized as the highly tolerant for heat while the remaining varieties as sensitive ones.

Cluster analysis of the six okra varieties based on the standardized value of stress tolerance indices was performed by UPGMA method and a dendrogram was constructed as depicted in Fig. 2. The dendrogram distinguished the six okra varieties into two main clusters. Cluster I comprised of 'Pusa Sawani' which separated in a single branch from the other varieties, this variety had the highest tolerance indices for NaCl, PEG and heat stress. Cluster II contained the five remaining varieties and divided into two sub-clusters. Sub-cluster I comprised of 'Iraqi' and 'Hala' which had the lowest tolerance index for heat stress at seedling stage (highly susceptible for heat stress). Sub-cluster II included three varieties, 'Balady Assiut', 'Balady Qina' and 'Emerald' which had the lowest tolerance index for drought stress (highly susceptible for drought stress).

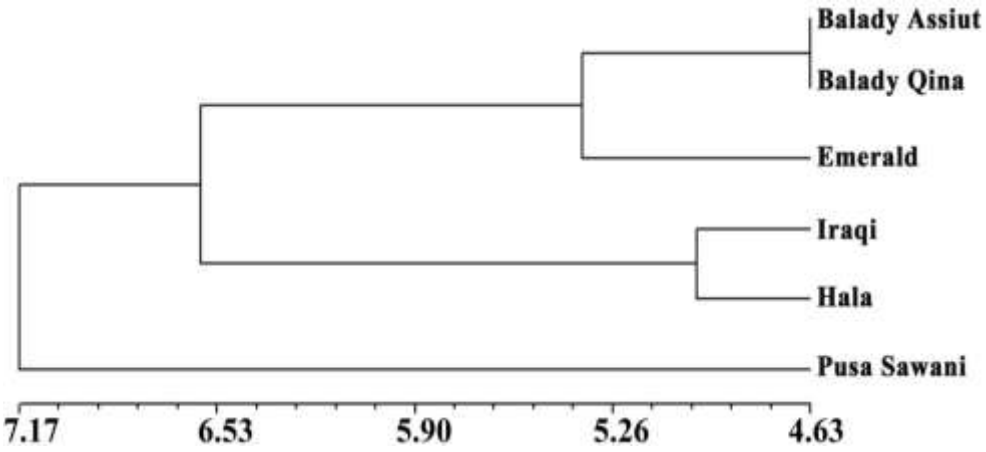

Fig. 2. Dendrogram of the genetic dissimilarities among six varieties of okra, achieved by the UPGMA method based on the Euclidian coefficient from tolerant indexes for salinity, drought and heat stress at seedling stage.

Molecular analysis

In total, 10ISSR, 10SRAP, and 10SSR primers or primer pairs gave reproducible results that were further considered for data analysis.

For ISSR, a total of 88 reproducible bands were generated by 10 ISSR primers with an average of 8.8 bands per primer and ranged in sizes from 130 bp (HB10) to 1119 bp (ISSR 814) (Fig. 3). Of the 88 bands, 49 were polymorphic, with an average of 4.9 polymorphic bands per primer. The percentage of polymorphism ranged from 40.0 % (ISSR 814) to a maximum of75.0 % (HB14), with an average of 56.08 % (Table 6).

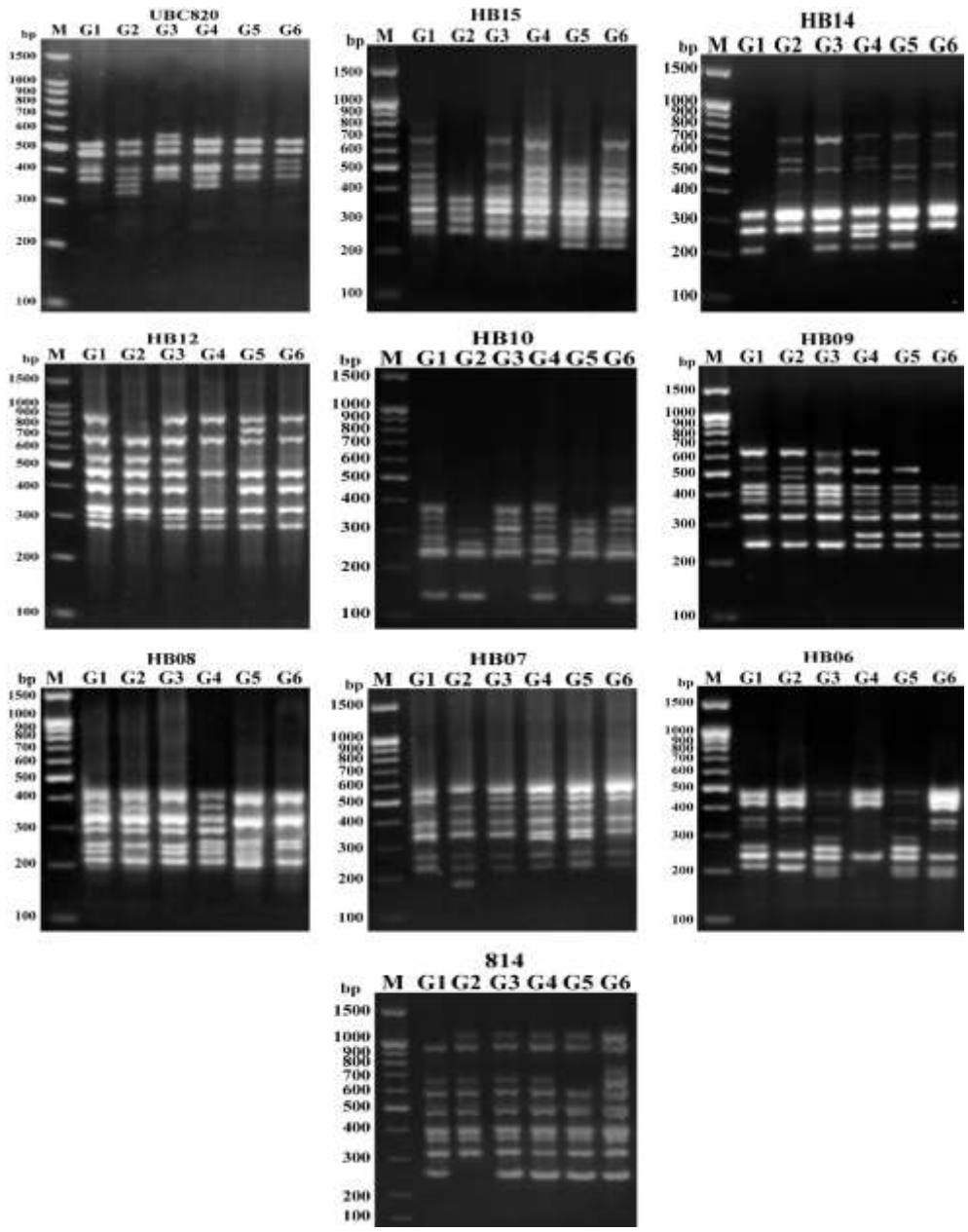

Fig. 3. ISSR profiles of six okra genotypes, Balady Assiut (G1), Balady Qina (G2), Emerald (G3), Pusa Sawani (G4), Iraqi (G5) and Hala (G6).

The ISSR 814 primer exhibited the lowest value for PIC, MI and RP (0.11, 0.44 and 1.33, respectively) while the HB06 primer recorded the highest values for the three parameters (0.27, 1.63 and 4.0, respectively) (Table 6). ISSR primers (HB08 and UBC820) possessed the highest Rp values (3.33 and 4.0, respectively) and each was able to distinguish all six okra varieties.

Table 6. Summary of RAPD, ISSR and SRAP primer combination characteristics

No	Markers	TB	PB	PPB	PIC	MI	RP
1	ISSR-1	8	5	62.5	0.22	1.08	2.33
2	ISSR-2	8	6	75	0.25	1.5	2.67
3	ISSR-3	10	4	40	0.11	0.44	1.33
4	ISSR-4	8	4	50	0.24	0.94	3.33
5	ISSR-5	9	5	55.56	0.19	0.96	2.33
6	ISSR-6	10	5	50	0.18	0.89	2.67
7	ISSR-7	9	6	66.67	0.27	1.63	4
8	ISSR-8	8	4	50	0.16	0.64	1.67
9	ISSR-9	9	6	66.67	0.23	1.37	3
10	ISSR-10	9	4	44.44	0.16	0.64	2
TOTAL		88	49	---	---	---	---
AVER		8.8	4.9	56.08	0.2	1.01	2.53
No	Markers	TB	PB	PPB	PIC	MI	RP
1	SRAP-1	15	7	46.67	0.16	1.14	3.33
2	SRAP-2	13	8	61.54	0.25	1.98	5.33
3	SRAP-3	12	6	50	0.15	0.92	2.33
4	SRAP-4	14	6	42.86	0.16	0.95	3.33
5	SRAP-5	16	9	56.25	0.18	1.63	4
6	SRAP-6	11	9	81.82	0.27	2.45	4
7	SRAP-7	11	7	63.64	0.19	1.34	2.67
8	SRAP-8	13	9	69.23	0.26	2.38	5.33
9	SRAP-9	11	3	27.27	0.09	0.27	1.33
10	SRAP-10	10	5	50	0.19	0.97	3
TOTAL		126	69	---	---	---	---
AVER		12.6	6.9	54.93	0.19	1.4	3.47
No	Markers	TB	PB	PPB	PIC	MI	RP
1	SSR-1	5	4	80	0.27	1.07	2
2	SSR-2	8	5	62.5	0.24	1.22	3
3	SSR-3	4	2	50	0.18	0.36	1
4	SSR-4	8	3	37.5	0.19	0.56	3
5	SSR-5	8	6	75	0.23	1.38	2.33
6	SSR-6	8	4	50	0.16	0.64	1.67
7	SSR-7	7	3	42.86	0.17	0.5	1.67
8	SSR-8	7	3	42.86	0.14	0.43	1.33
9	SSR-9	4	4	100	0.32	1.28	1.67
10	SSR-10	8	7	87.5	0.31	2.14	3.33
TOTAL		67	41	---	---	---	---
AVER		6.7	4.1	62.82	0.22	0.96	2.1

TNB total number of bands, NPB number of polymorphic bands, PPB percentage of polymorphic bands, PIC polymorphic information content, MI marker index, RP resolving power.

For SRAP analysis, the 10 SRAP primer combinations generated a total of 126 bands, with an average of 12.6 bands per primer combinations and ranged in sizes from 75 bp (SRAP-4) to 1350 bp (SRAP-5) (Fig. 4).

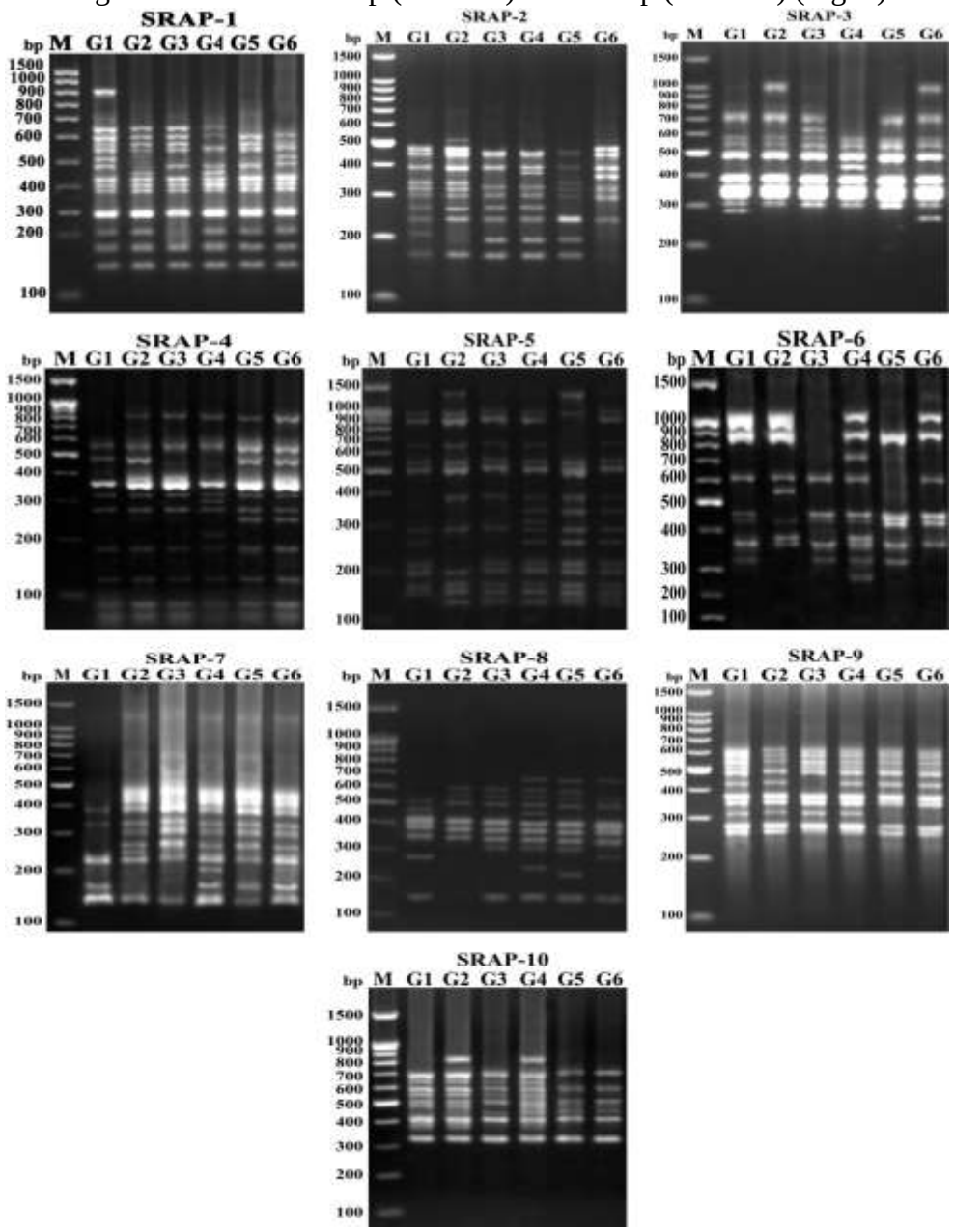

Fig. 4. SRAP profiles of six okra genotypes, Balady Assiut (G1), Balady Qina (G2), Emerald (G3), Pusa Sawani (G4), Iraqi (G5) and Hala (G6).

The maximum number of bands was recorded for SRAP-5 primer combination, while the minimum number of bands was recorded for SRAP-10. Out of 126 bands generated, 69 were polymorphic, generating an average of 6.9 per primer pairs. Maximum percentage of polymorphism was observed using primer SRAP-6 (81.82 %), while minimum percentage was observed using primer SRAP-9 (27.27 %), with an average of 54.93 % (Table 5). The SRAP-6 primer combination possessed the highest values for PIC and MI (0.27 and 2.45, respectively) while the lowest values for the two parameters (0.09 and 0.27, respectively) was recorded for SRAP-9 (Table 6). The resolving power (RP) ranged from 1.33 for primer combinations SRAP-9to 5.33 for primer combinations SRAP-2and SRAP-8with a mean of 3.47 (Table 6). The two SRAP primer combinations (SRAP-2 and SRAP-8) possess the highest Rp values (5.33) and each was able to distinguish all six okra varieties.

For SSR data, a total of 15 SSR primer pairs were screened for the PCR amplification, out of which five primers did not produce amplified polymorphic products, thus SSR analysis of six okra varieties was done using 10 SSR primer pairs. The approximate size of the amplified products ranged from 161 bp (SSR-1) to 981bp (SSR-5) (Fig. 5). In total, 41 polymorphic bands out of 67amplified fragments were obtained, with an average of 4.1per primer pair. An average percentage of polymorphism was 62.82 %, ranging from 37.5 % (SSR-4) to a maximum of 100 % (SSR-9) (Table 6). The PIC value for SSR primers found between 0.14 (SSR-8) and 0.32(SSR-9) with a mean of 0.22 (Table 6). The resolving power (RP) ranged from 1.0 (SSR-3) to 3.33 (SSR-10) with a mean of 2.1 (Table 6). The three SSR primer pairs (SSR-2, SSR-4 and SSR-10) possessed the highest Rp values (3.0, 3.0 and 3.33, respectively) and each was able to distinguish all six okra varieties.

Combined analysis of molecular data

In order to obtain more accurate genetic estimates, combined analysis was carried out using three molecular marker systems data. The three molecular marker systems (ISSR, SRAP and SSR), amplified a total of 281 band classes, with an average of 9.37 bands/primer, and the average of their polymorphism was 56.58(Table 5). The variety ‘Pusa Sawani’ displayed the highest number of DNA fragments (228 bands) followed by ‘Hala’ (208 bands), while the variety ‘Balady Qina’ revealed the least number of bands (199 bands). The three molecular marker systems were successful in characterizing all okra varieties by unique positive and/or negative markers (Table 7).

Genetic similarity (GS) coefficients were obtained with UPGMA algorithm using Dice coefficient (Dice, 1945) based on combined data of ISSR, SRAP and SSR markers.

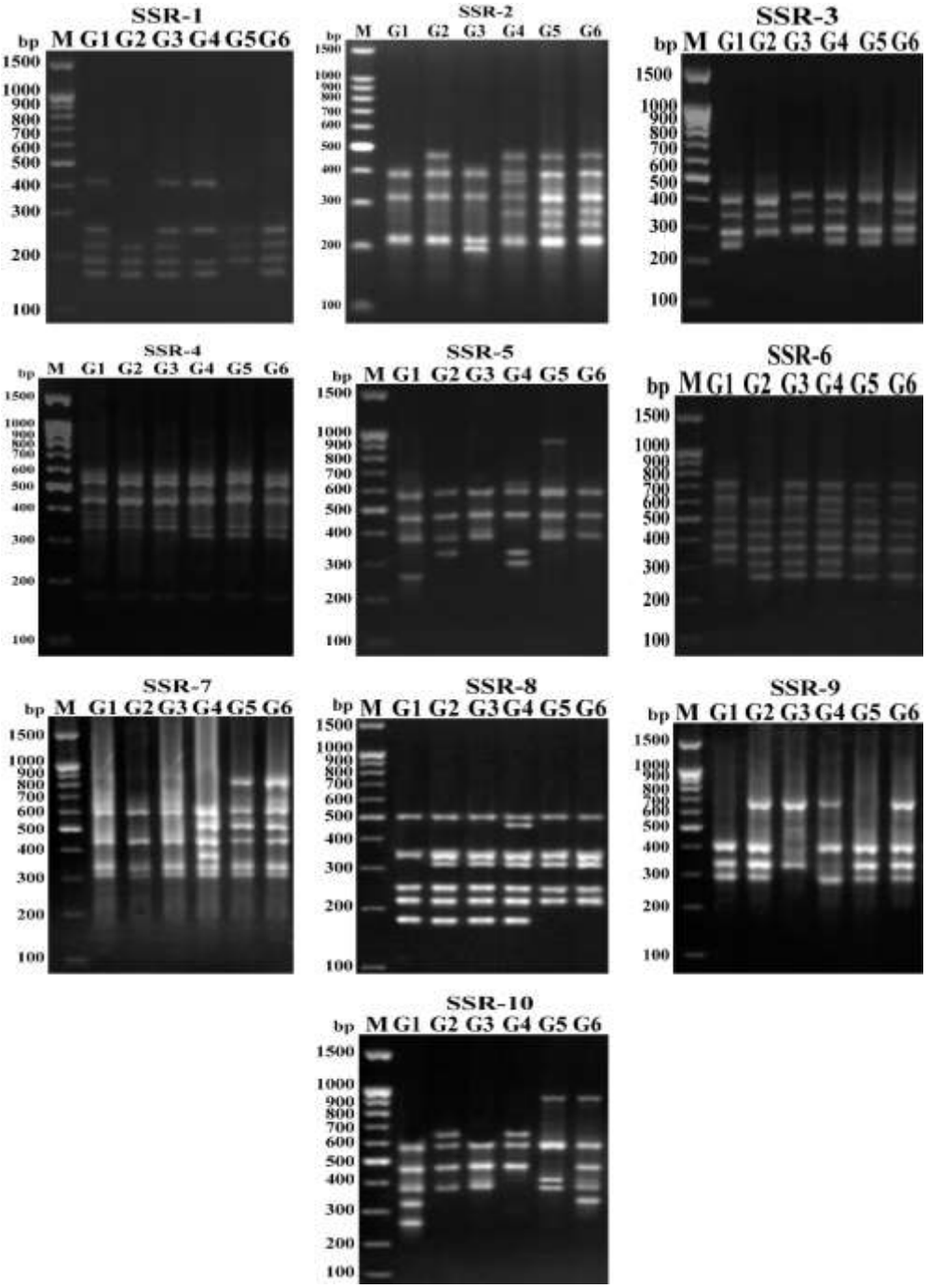

Fig. 5. SSR profiles of six okra genotypes, Balady Assiut (G1), Balady Qina (G2), Emerald (G3), Pusa Sawani (G4), Iraqi (G5) and Hala (G6).

Table 7. Unique DNA bands generated by RAPD, ISSR and SRAP markers.

Genotype	Positive	Negative
G1	812 bp (SRAP-1), 205 bp (SRAP-2), 284 bp (SRAP-3), 261 bp (SSR-5), 263 bp (SSR-10)	687 bp (ISSR-2), 501 bp (ISSR-2), 824 bp (SRAP-4), 393 bp, 142 bp (SRAP-5), 271 bp (SSR-6), 1119 bp (ISSR-3), 310 bp (SSR-8), 463 bp, 425 bp, 307 bp (SRAP-7), 529 bp (SRAP-8) 449 bp (SRAP-10)
G2	513 bp (SRAP-2), 544 bp (SRAP-6), 248 bp (SRAP-7), 480 bp (ISSR-6), 324 bp (ISSR-8), 186 bp (ISSR-10)	333 bp, 272 bp (ISSR-1), 259 bp (SSR-1), 485 bp (SRAP-1), 220 bp (SRAP-5), 709 bp (SSR-6), 250 bp (ISSR-3), 448 bp (SRAP-6), 490 bp, 394 bp (ISSR-5), 138 bp (SRAP-8), 383 bp, 265 bp(ISSR-9), 502 bp (SRAP-9), 526 bp (PSSR-10)
G3	195 bp (SRAP-2), 661 bp (SRAP-3), 554 bp (ISSR-8)	205 bp (SRAP-1), 864 bp (SRAP-6), 438 bp (SRAP-9), 405 bp, 290 bp (SSR-9), 485 bp (SRAP-10)
G4	210 bp (ISSR-1), 238 bp (ISSR-2), 382 bp (SSR-2), 452 bp (SRAP-3), 299 bp, 208 bp (SRAP-4), 315 bp (SRAP-5), 645 bp, 304 bp (SSR-5), 545 bp (SSR-6), 382 bp (SSR-7), 457 bp (SSR-8), 721 bp, 264 bp (SRAP-6), 204 bp (SRAP-7), 230 bp (SRAP-8), 342 bp (ISSR-6)	222 bp (SSR-1), 580 bp (SRAP-1), 708 bp (SRAP-3), 394 bp (SSR-5), 356 bp, 209 bp (ISSR-7), 385 bp (ISSR-9), 330 bp (SSR-9), 390 bp (SSR-10)
G5	447 bp (ISSR-2), 981 bp (SSR-5), 211 bp (SRAP-8), 730 bp (ISSR-9), 420 bp (SSR-10)	161 bp (SSR-1), 340 bp (SSR-3), 912 bp (SRAP-5), 663 bp (ISSR-3), 606 bp (SRAP-6), 474 bp (SSR-10)
G6	276 bp (SRAP-3), 731 bp (ISSR-3), 438 bp (ISSR-8)	159 bp (SRAP-2), 523 bp (ISSR-6)

Balady Assiut (G1), Balady Qina (G2), Emerald (G3), Pusa Sawani (G4), Iraqi (G5) and Hala (G6).

The genetic similarity ranged from 0.797 (‘Balady Qina’ and ‘Iraqi’) to 0.893 (‘Iraqi and ‘Hala’). The dendrogram grouped the six varieties into four main clusters (Fig. 6). Cluster I comprised of the two genotypes ‘Iraqi’and ‘Hala’which closely related with genetic similarity 0.893. Cluster II contained one genotype Pusa Sawani’. Cluster III included only ‘Balady Qina’. Cluster IV contained ‘Balady Assiut’ and ‘Emerald’ with genetic similarity 0.863.

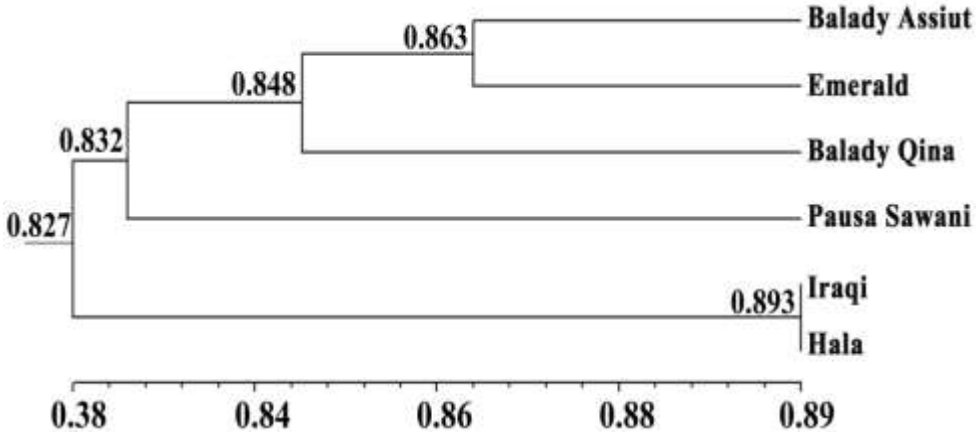

Fig. 6. Dendrogram showing clustering of six okra varieties constructed using UPGMA based on Dice coefficient obtained from ISSR, SRAP and SSR combines analysis.

The matrices for ISSR, SRAP and SSR markers were compared using Mantel's test (Mantel 1967) for matrix correspondence. The correlation between the matrices of cophenetic correlation coefficient values for the dendrogram based on ISSR + SRAP data was (r=0.232), ISSR + SSR (r= 0.032), SRAP + SSR (r= 0.358), ISSR + (SRAP + SSR) (r= 0.146), SRAP + (ISSR + SSR) (r= 0.412) and SSR + (ISSR + SRAP) (r= 0.214), which indicates a good fit among ISSR, SRAP and SSR marker systems.

DNA fragments at molecular sizes 557 bp (HB14), 345 bp (UBC820), 466 bp (SRAP-1), 378 bp (SRAP-6), 856 bp (SRAP-9), 336 bp (SSR-5) and 669 bp(SSR-9) appeared only in the two varieties ('Balady Qina' and 'Pusa Sawani') which had the highest tolerance index for heat stress (highly tolerant) but did not appear in the other varieties. Fragments at molecular size 260 bp (HB08), 269 bp (HB09), 348 bp (SRAP-5), 642 bp (SRAP-8), 278 bp (SSR-2) and 333 bp (SSR-4) appeared in the three varieties namely, 'Pusa Sawani', 'Iraqi' and 'Hala'which were the highly tolerant for drought stress, but were not observed in the other varieties. Fragments at molecular size 438 bp (HB15), 267 bp (SRAP-5), 164 bp (SRAP-7) and 249 bp (SSR-3) appeared in the four varieties, 'Balady Assiut', 'Pusa Sawani', 'Iraqi and 'Hala'which had the highest tolerance index for salinity stress. Three DNA fragments at 212 bp (HB114), 228 bp (HB08) and 317 bp (SRAP-6) were found in 'Balady Assiut', 'Emerald', 'Pusa Sawani' and Iraqi' varieties which showed the highest mean value for plant height but not appear in 'Balady Qina' and 'Hala' varieties which had the lowest mean for plant height. Fragment at molecular size 486 bp (SSR-2) was generated in 'Balady Qina', 'Pusa 'Sawani', Iraqi' and 'Hala' varieties which possessed the highest pod length. Fragment at molecular

size 1350 bp (SRAP-5) appeared only in the varieties ‘Balady Qina’ and ‘Iraqi’ which had the highest mean for number of branches/ plant. The ten DNA fragments at molecular sizes 291 bp (HB08), 629 bp (HB09), 288 bp (HB12), 665 bp (SRAP-1), 264 bp (SRAP-2), 318 bp (SRAP09), 646 bp and 558 bp (SRAP-10), 315 bp (SSR-6) and 174 bp (SSR-8) appeared in ‘Balady Assiut’, ‘Balady Qina’, ‘Emerald’ and ‘Pusa Sawani’ varieties which had the highest average for number of pod/plant, weight of pod and yield/plant.

Dendrogram generated from UPGMA based on combined data of morphological traits, stress tolerance indexes and molecular markers classified the six okra varieties into three main clusters (Fig. 7).

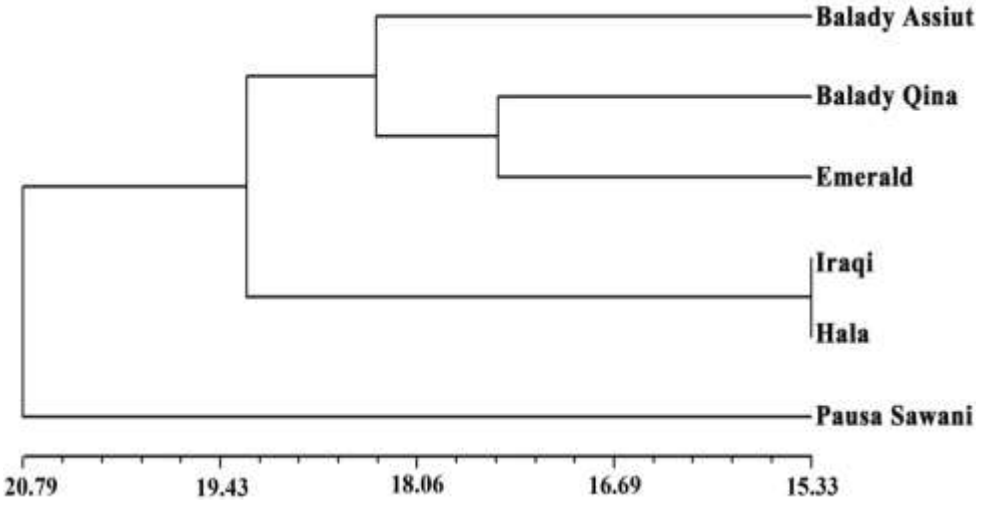

Fig. 7. Dendrogram of the genetic dissimilarities among six varieties of okra, achieved by the UPGMA method based on the combined data of agro-morphological traits, stress tolerant indexes and three molecular marker systems.

Cluster I consisted of only one variety (‘Pusa Sawani’) which separated in a single branch from the other cultivars. This variety had the highest mean values for pod length, number of pods/plant, pods weight/plants, yield/plant and plant height and lowest values for number of branches/plant and day to 50%floweringalso this variety categorized as the highly tolerant for salinity, drought and heat stress at seedling stage. Cluster II comprised of the two varieties (‘Iraqi’ and ‘Hala’) which had the lowest mean values for number of pod/plant, pod weight and yield/plant, also these varieties categorized as tolerance for salinity and drought and highly susceptible for heat stress. In this cluster, the variety (‘Iraqi’) had the highest value on day to 50% flowering and the highest mean value for number of branches/plant while the variety (‘Hala’) showed the shortest plant height compared to other varieties. Cluster III contained three varieties

('Balady Assiut', 'Balady Qina' and 'Emerald') which showed the moderate mean values for most agro-morphological traits and categorized as the highly susceptible for drought at seedling stage. This cluster further divided into two sub-clusters. Sub-cluster I comprised of 'Balady Qina' and 'Emerald'which were categorized as the highly susceptible for salinity at seedling stage. Sub-cluster 2 contained only 'Balady Assiut'which had the lowest mean value for pod length and categorized as tolerant for salinity at seedling stage

The Mantel Z test statistics showed a highly significant correlation coefficient (r=0.512, 0.371, 0.421 and 0.491) between the matrices based on molecular markers and agro-morphology, molecular markers + stress tolerance indexes, agro- morphology + stress tolerance indexes and molecular markers and (Agro-morphology + Stress tolerance), respectively.

DISCUSSIONS

Okra is a warm season crop, the optimal temperature for germination ranged from 30-35ºC, and the optimum temperature for growth and quality ranged from 25-30 ºC (Mousa *et al.* 2012). The dominant weather at Upper Egypt particularly Assiut governorate during March is optimum for growing okra including temperature, humidity, light and wind. However, the genetic background of the cultivated okra varieties particularly affects the plant performance. The dominant weather of 15th March was optimal for the genetics of the variety 'Pusa Sawani', thus recorded the highest growth parameters as compared with other studied varieties. It was reported that okra growth parameters were significantly affected by variety and planting date. Dash *et al* (2013) found that okra plant height, days to flowering and number of branches were significantly affected by studied variety and planting date. They attributed these variations to dominate proper environments during February which enhanced okra growth and vigor. Late cultivation of okra varieties (25th March) significantly affected plant height, number of branches and days to flowering (Dash *et al* 2013). Early planting (2nd February) and late planting (25th March) caused a significant delay of the flowering of okra varieties (Amjad *et al* 2001 and Dash *et al* 2013).

Okra production is greatly influenced by varieties (genetic background), agronomic practices (including plant spacing and planting dates) and environmental conditions (temperature, humidity and light). Early planting gives the longest growth cycle (Izquierdo *et al.* 2003). The results of the present study were in line with that observed by Dash *et al* (2013), who reported that yield and yield attributes including no. of pods/plant and weight of pod/plant were significantly affected by studied okra varieties and planting dates. The authors found that the okra variety 'Annie Oakley' produced the greatest no. of pods/plant, weight of pod/plant and total yield of fresh pods at planting date 2ndFebruary in both cropping

seasons. El-hag and Ahmed (2014) found that no. of pods/plant, seed yield/plant and seed yield/ha were significantly affected by planting dates and okra varieties. The okra variety 'Wad Gammer' produced the greatest no. of pods/plant, seed yield/plant and seed yield/ha at planting date 1st July as compared to other studied varieties and planting dates. In addition, they recommended early sowing (March – April) of okra for fresh pod production since seeds produced during July- August would be affected by rain and fungal diseases.

The data of the correlation between growth, yield and yield attributes in Table (2) revealed significant positive and/or negative correlations between many measured traits for okra varieties. Our results are inconformity with the findings of Chattopadhyay *et al* (2011), who observed correlations between growth, reproductive characters and seed yield. The authors found that seeds per pod and test weight were significantly and positively correlated with seed yield. Significant correlations were observed between growth, yield and yield attributes parameters (Akinyele and Osekita 2006).

The environmental stresses such as temperature, salinity and drought are serious obstacles for field crops. Early seedling growth is the most sensitive stage to abiotic stress (Patade *et al* 2011 and Shahi-Gharahlar *et al* 2010). However, the salt, drought and heat tolerance at early growth stages may not be correlated with that to the subsequent growth stages (Zeng *et al* 2002 and Wahid *et al* 2007). In this study, therefore, we focused on evaluating the potential of salt, drought and heat tolerance in okra varieties at early growth stage i.e. at seedling stage. Screening of varieties for salt, drought and heat tolerance at early stages may be important for screening salt tolerance as there is considerable saving in time. In the present investigation, six okra varieties were subjected to salinity, drought and heat stress regimes in order to screen them on the bases of their tolerance against the salinity, drought and heat stresses. On the basis of various growth attributes at seedling stage, the results of this study showed that all parameters were affected significantly by the salinity, drought and heat stresses. These results are in accordance with Baghizadeh and Mahmood (2011), who observed that drought treatment using PEG caused reduction of germination rate and seedlings growth in okra. Sixteen okra genotypes were subjected to heat stress in order to screen them on the bases of their tolerance against the high temperature stress, the result of this study showed that all parameters at seedling stage were affected significantly by the heat stress (Asghar 2016). Our results are in agreement with Abbas *et al* (2014), who reported that salt stress reduced seedling growth attributes like seedling shoot length, seedling root length, seedling fresh and dry weights in studied okra cultivars. Results showed different effects on seedling

growth parameters under these stresses, this suggesting that NaCl, heat and PEG acted through different mechanisms (Murillo *et al* 2002). The variety 'Pusa Sawani' showing less decrease in growth parameters (shoot and root length and shoot and root fresh and dry weight) was categorized as salt, drought and heat-tolerant variety while 'Emerald' variety with maximum decline in these attributes was placed in salt, drought and heat sensitive category. On the basis of this experiment, it can be concluded that reductions in seedling shoot length, seedling root length, seedling fresh and dry weights were highly associated with salt, drought and heat stress and these can serve as an effective tool for the assessment of salt, drought and heat tolerance potential of okra varieties. Literature also depicts that these attributes can be used as screening tools for salinity tolerance potential (Dasgan *et al* 2002). The screened salt, drought and heat-tolerant okra cultivars like 'Pusa Sawani' variety having excellent genetic capacity to tolerate the excessive salt, drought and heat.

The cluster analysis based on tolerant indexes of heat, drought and salinity stress showed that the six okra varieties were grouped in three clusters (Fig. 2). The results revealed that the genotype 'Pusa Sawani' which formed cluster I is more genetically diverse and had the highest mean values for drought, salinity and heat tolerance indexes. These results suggest that 'Pusa Sawani' can be used as a potential parent in hybridization programs to develop stress-tolerant varieties.

Knowledge of genetic diversity among okra genotypes can give breeders and geneticists important information on the allelic diversity present in gene bank materials and may help to identify genetically diverse pools for use in breeding programs to improve agronomic traits, quality and resistance to biotic and abiotic stresses of okra crops either by means of hybridization or direct selection of genotypes for their desirable traits. (Naser 2014). Phenotypic and agronomic traits have been widely used for estimation of genetic variations since they provide a simple way of quantifying genetic diversity (Fufa *et al* 2005). The cluster analysis classified genotypes into clusters. In general, inter- cluster distance was much more than intra-cluster distances. This suggesting that within cluster genotypes have the same genetic constitution, i.e. homogeneous are less divergent than those occurred in a different cluster. Therefore, genotypes belonging to these inter clusters may be used in hybridization programs to obtain transgressive segregants with broad spectrum of genetic variability for yield and yield component traits to isolate high yielding genotypes in okra (Umrao *et al* 2014).

In the present investigation, the range of Euclidean distance among the six okra varieties genotypes (1.45 – 6.66) is relatively high. Highest similarities belonged to varieties 'Balady Assiut' and 'Emerald' (1.45)

while the least similarity belonged to varieties ‘Pusa Sawani’ and ‘Iraqi’ (6.66). This indicated that the amount of agro-morphological variation among the varieties is relatively high. These values, which are assumed to reflect the genetic diversity of the loci controlling these traits, indicates the possibility of selecting varieties that have a diverse genetic background and the prospect of obtaining broad segregation for the characters. Therefore, the results of the genetic distance have shown that there is a room for the genetic improvement of okra varieties and the information generated can be used to plan-wide crosses and exploit genetic diversity. These results are in accordance with the findings of Salameh and Kasrawi (2011), Bello and Aminu (2017), Umrao *et al* (2014), Amoatey *et al* (2015) and Singh *et al.* (2018).

Since the diversity based on agro-morphological, agro-physiological and agronomic traits usually influenced by environmental factors and show continuous variation, in addition, the evaluation of these traits requires growing the plants to full maturity prior to identification (Vogel *et al* 1996). The molecular analysis of the genome can provide a greater advantage because DNA sequences are the same in all of the living cells of a plant, regardless of physiological or developmental state of the tissue. In the present study, we used three different marker systems (ISSR, SRAP and SSR markers). These markers differ in technical principle, type of inheritance, reproducibility, distribution in the plant genome, amount of polymorphism and in their costs (Li and Quiros2001, Zietkiewicz *et al* 1994 and Provan *et al* 1999).

A total of 281DNA fragments were generated by 10ISSR, 10 SRAP and 10 SSR primers with an average percentage of polymorphism of 56.58, which implies the presence of prominent genetic diversity among six okra varieties. The number of DNA fragments generated by the SRAP markers was obviously higher than that obtained by amplification with ISSR and SSR (Table 5). SSR markers showed higher PPB (62.82%) than the ISSR and SRAP markers (56.087% and 54.93%, respectively). Also, the polymorphism information content is more for SSR than for ISSR and SRAP markers (Table 5). The UPGMA cluster analysis based on ISSR, SRAP and SSR data was similar with slight differences.

The correlation between Dice’s similarity coefficients value resulted in high significant correlations between ISSR and SRAP (r = 0.232), SSR and SRAP (r = 0.358), high correlation for ISSR + SRAP and SSR data (r = 0.214), SRAP and ISSR + SSR (r = 0.412) and low correlation between ISSR and SSR (0.032), also between ISSR and SSR +SRAP (r = 0.146). This shows that SRAP data is slightly more close to ISSR and SSR data. The difference in resolution of three marker systems is that the three marker techniques targeted different sequences of the genome. The ISSR markers

scattered throughout the genome which revealed the diversity of the entire genome, SRAP markers only amplified target sequence of open reading frame (ORF), while SSR marker target tandem sequence repeats of short DNA motifs (1–6 nucleotides long) (Li and Quiros2001, Zietkiewicz *et al* 1994 and Provan *et al* 1999). Since each DNA maker system has its own advantages, it is important to use more than one DNA marker system, such as ISSR, SRAP and SSR, in the analysis of genetic diversity. All three marker types were able to generate unique bands in particular genotypes but not in other genotypes (Table 6). These unique bands could be used as positive and/or negative markers for this genotype. The utility of these markers for detection of genotypes differences agrees with previous reports, in other species (Gulsen *et al* 2007, Patel *et al* 2018, Ouedraogo *et al* 2018 and Kaur *et al.* 2013). These results suggested that, ISSR, SRAPand SSR approaches showed considerable potential for identifying and discriminating okra varieties.

Since, salinity, heat and drought tolerance are contributed by several regions of the genome; it requires identification which can be accomplished by the use of molecular markers as they offer a faster way to identify salinity, heat and drought tolerance related regions.

In the present investigation, ISSR, SRAP and SSR analyses were undertaken with the aim of developing a drought, salinity and heat tolerance associated DNA markers. For three marker systems analysis presented here okra varieties reported to be heat, drought and salinity tolerant/sensitive (on the basis of stress tolerance indexes performance) were used. Results in Fig. 1, 2 and 3indicating that DNA fragments at 557 bp (HB14), 345 bp (UBC820), 466 bp (SRAP-1), 378 bp (SRAP-6), 856 bp (SRAP-9), 336 bp (SSR-5) and 669 bp (SSR-9) were present only in the two varieties (‘Balady Qina’ and ‘Pusa Sawani’) which showed the highest tolerance index for heat stress (highly tolerant), indicating that these fragments may be related to heat tolerance genes and could be used as positive markers for heat tolerance using such tested primers. Fragments at 260 bp (HB08), 269 bp (HB09), 348 bp (SRAP-5), 642 bp (SRAP-8), 278 bp (SSR-2) and 333 bp (SSR-4) appeared in ‘Pusa Sawani’, ‘Iraqi’ and ‘Hala’ which were the highly tolerant for drought stress, but were not observed in the other varieties, indicating that these fragments may be linked to drought tolerance genes. Fragments at 438 bp (HB15), 267 bp (SRAP-5), 164 bp (SRAP-7) and 249 bp (SSR-3) appeared in the four varieties, ‘Balady Assiut’, ‘Pusa Sawani’, ‘Iraqi’ and ‘Hala’ which had the highest tolerance index for salinity stress indicate that these fragments may be associated with salinity tolerance genes when using these primers. These results indicated that these primers could be used as a putative marker for screening drought, heat and

salinity tolerant varieties. These results showed similarity with the results obtained by El-Sherbeny *et al* (2018) and Pakniyat and Tavakol (2007).

All three marker systems also generated DNA fragments with different molecular sizes which may be associated with some agro-morphological traits, for example, three DNA fragments at 212 bp (HB114), 228 bp (HB08) and 317 bp (SRAP-6) were amplified only in 'Balady Assiut', Emerald', 'Pusa Sawani' and 'Iraqi' varieties which showed the highest mean for plant height, Fragment at 486 bp (SSR-2) was generated in 'Balady Qina', 'Pusa Sawani', 'Iraqi'and 'Hala' varieties which possessed the highest pod length, fragment at 1350 bp (SRAP-5) appeared only in the varieties 'Balady Qina'and 'Iraqi' which had the highest mean value for number of branches/ plant and DNA fragments at 291 bp (HB08), 629 bp (HB09), 288 bp (HB12), 665 bp (SRAP-1), 264 bp (SRAP-2), 318 bp (SRAP09), 646 bp and 558 bp (SRAP-10), 315 bp (SSR-6) and 174 bp (SSR-8) appeared in 'Balady Assiut', 'Balady Qina', 'Emerald and 'Pusa Sawani' varieties which had the highest average for number of pod/plant, weight of pod and yield/plant. Such these fragments may be linked with gene (s) controlled these traits.

Dendrogram generated from UPGMA based on combined data of agro-morphological traits, stress tolerance indexes and molecular markers classified the six okra varieties into three main clusters (Fig. 7).

In this study, in order to investigate the genetic relationships among six okra varieties and to compare the extent of agreement among dendrograms derived from agro-morphological traits, stress tolerance indexes and molecular markers, a distance matrix was constructed for each assay and compared using the Mantel matrix correspondence test. The Mantel Z test statistics showed highly significant correlation (r=0.512, 0.371, 0.421 and 0.491) between the matrices based on molecular markers and agro-morphology, molecular markers + stress tolerance indexes, agro-morphology + stress tolerance indexes and molecular markers and (Agro-morphology + Stress tolerance), respectively. The significant correlation indicates that these independent sets of data likely reflect the same pattern of genetic diversity and validate the use of these data to calculate the different diversity statistics for agro-morphological traits in the okra genotypes. These results are in agreement with many investigators, who studied the genetic diversity of okra using molecular markers with morphological traits. Our results are in accordance with the findings of Cong-Ying *et al* (2014), Ravishankar *et al* (2018), Kumar *et al* (2017), Kumar *et al* (2017), Gulsen *et al* (2007), Patel *et al* (2018), Ouedraogo *et al* (2018), Kaur *et al (*2013), Kyriakopoulou *et al* (2014), Cong-Ying *et al* (2015), Younis *et al* (2015), Singh *et al* (2018) and El-Sherbeny *et al* (2018).

In Conclusion, we used different marker systems, agro-morphological, morpho-physiological and molecular parameters (ISSR, SRAP and SSR) to generate pre-breeding data that can be applied to the choice of appropriate parents to introduce more genetic diversity into okra breeding programs and to help breeders reach new strains that are capable of coping with environmental stresses. The DNA fingerprinting data generated in the study could also be used for variety description in the future. This study demonstrates that the ISSR, SRAP and SSR marker systems are powerful and easy methods for fingerprinting and distinguishing okra varieties. These results suggest that 'Pusa Sawani' can be used as a potential parent in hybridization programs to develop stress-tolerant varieties of okra.

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تحليل الاستجابة لميعاد الزراعة وتحمل الاجهاد والتنوع الوراثي لبعض أصناف الباميا

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أجريت هذه الدراسة بالمزرعة البحثية بقسم الخضر – كلية الزراعة – جامعة اسيوط ، لدراسة ستة أصناف من الباميا في موعدين مختلفين للزراعة خلال الموسم الصيفي لأعوام ٢٠١٦ و ٢٠١٧. نفذت التجارب الحقلية باستخدام تصميم القطع المنشقة مرة واحدة ووزعت المعاملات عشوائيا باستخدام تصميم القطاعات الكاملة العشوائية (RCBD). أظهرت النتائج وجود تأثيرات معنوية لمواعيد الزراعة المدروسة علي صفات النمو والمحصول ومكوناته للأصناف الستة من الباميا. وأدي موعد الزراعة المبكر ١٥ مارس إلي تفوق صنف الباميا 'Pusa Sawani' في صفات النمو وصفات المحول ومكوناته بما في ذلك صفات موعد الازهار، ارتفاع النبات (سم)، عدد الفروع، طول القرون (سم)، عدد القرون/نبات، وزن قرون النبات (جم) والمحصول الكلي من القرون الخضراء (طن/فدان)، وذلك مقارنة مع باقي الأصناف موضع الدراسة. كما اوضحت النتائج ان اصناف الباميا 'Iraqi' و 'Hala' لم تتأقلم علي الظروف الجوية في صعيد مصر حيث أزهرت متأخرة وأعطت أقل ارتفاع للنبات (سم)، عدد الفروع ، طول القرون (سم)، عدد القرون لكل نبات، وزن قرون النبات (جم) والمحصول الكلي من القرون الخضراء (طن/فدان). النتائج الجيدة للصنف 'Pusa Sawani' تحت الظروف الجوية بأسيوط تجعله مقترحاً جيدا للزراعة خلال موسم الصيف في صعيد مصر. التحليل العنقودي المعتمد على الصفات المورفولوجية قسم أصناف الباميا الستة لأربعة مجاميع حيث انعزل الصنف 'Pusa Sawani' منفرداً في مجموعة متميزاً بامتلاكه لأعلى متوسطات لقيم الصفات المورفولوجية. تم اختبار أصناف الباميا الستة تحت ظروف اجهادات الجفاف والملحية و الحرارة في مرحلة النمو المبكر للبادرات. وقد أظهرت النتائج اختلافات معنوية بين الأصناف المختبرة وانتظمت هذه الأصناف حسب دليل تحملها لهذه الاجهادات في مجموعتين رئيسيتين. كما تم استخدام ثلاث أنواع من الواسمات الوراثية هي :ISSR و SRAP و SSR لدراسة التنوع الوراثي بين الأصناف الستة واكتشاف سمات وراثية مرتبطة بالصفات المورفولوجية وتحمل الاجهادات. وأظهرت النتائج ارتباط واسم وراثي واحد على الأقل بالصفات المورفولوجية و/أو بالجفاف، الملوحة و الحرارة.

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