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Genetic Fingerprint of Five Peanut Genotypes and Study the Effect of Irrigation Intervals on Yield and Its Components.

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ABSTRACT

At Ismailia Research Station, ARC, a field experiment was carried out during two successive seasons; 2013and 2014 to study the effect of irrigation intervals (every 4, 7 and 10 days) on yield and yield components of five peanut genotypes (*Arachis hypogaea* L.) namely; Giza6, Line623, Sohag110, Hybrid11 and Line21 under newly reclaimed saline soil conditions. The Results should that irrigation every 7 days gave the highest values for all the studied characteristics except number of pods/plant. Significant differences were detected among the five peanut genotypes in all characteristics except for 100-seed weight and shelling percentage during both seasons. Line 21 was superior and gave the highest value of all the studied characteristics. In the meantime, line 21 proved to be more drought tolerant. On the other hand, five RAPD primers were used to identify these genotypes. The RAPD matrix showed low correlation (r = 0.083) between line623 and both of hybrid11and Giza6, while it showed high correlation between Giza6 and Line21 (r = 0.833). The dendrogram divided the genotypes into two clusters. Line 21 was in one cluster, while the other cluster included the other four genotypes.

Keywords: Peanut, yield, drought, phylogenetic tree, RAPD- PCR markers.

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INTRODUCTION

Peanut (*Arachis hypogaea* L.) seeds are considered the world´s fourth important source of edible oil, the third important source of vegetable protein and one of the richest sources of vitamin B1 in plants. The seed contain 44–56% oil and 22–30% protein on the dry seeds basis (Florkowski, 1994; Singh, 1995). About 80% of the world peanut production comes from seasonally rainfed areas in the semi-tropics, where climate is characterized by a low and erratic rainfall (Wright and NagesawaraRao, 1994).

Soil water is the most crucial factor in arid and semi-arid regions where yield potential is directly a function of water availability for plant growth. So, drought has been the major environmental constraint to peanut survival and to crop productivity in this area (Boyer, 1983). Drought stress has adverse influence on water relations (Babu and Rao, 1983), photosynthesis (Bhagsari *et al*., 1976), mineral nutrition, metabolism, growth and yield of groundnut (Suther and Patel, 1992). On the other hand, the crop has a good ability for growing in light soil, and thrives in improving the characteristics of the newly reclaimed sandy soils, which commonly suffer from some constraints such as poor physical properties and nutrient deficiency. Therefore, water shortage and low water quality are becoming an international issue and unfortunately it seems that rapid growth of population and water resources reduction are less in harmony with future demands (Genhua and Denise, 2006).

The DNA amplification fingerprinting (DAF) approach is a modification of the RAPD technique (Williams et al., 1990), but it is relatively more informative because the use of altered reaction conditions, shorter primers, and silver staining (Caetano-Anoll´es *et al*., 1991).

The genetic relationships and diversity among elite breeding lines and available genotypes are important for the optimal design of peanut breeding programs (Bainchi-Hall *et al*., 1993; Lu and Pickersgill 1993). They showed a survey of biochemical marker analyses in cultivated peanut which had documented a low level of genetic diversity.

Halward *et al*. (1991 and 1992) found a little or no variation using RFLP and RAPD markers in more than 25 unadapted germplasm that represented four of the six botanical varieties of peanut.

Subramanian *et al*. (2000) and Zietkiewicz, *et al*. (1994) identified RAPD polymorphism among cultivated peanuts and a molecular marker technique using (SSR) has been available and widely used for phylogenetic, diversity and mapping studies.

The main objectives of the present study were: 1) to evaluate five genotypes of peanut under different irrigation intervals and 2) to estimate the genetic diversity of the genotypes using ISSR markers.

MATERIALS AND METHODS

At Ismailia Research Station, ARC, a field experiment was carried out during two successive seasons; 2013 and 2014. The experimental plots were 4 x 4 m. The four borders of each plot were raised up to about 60 cm above soil surface. The layout of the experiment was split plot design with three replicates. The main plots were assigned for water intervals and the subplot for the genotypes. The combination of three irrigation intervals (every 4, 7 and 10 days) and five peanut genotypes (Giza 6, Line 623, Sohag110, Hybrid11and Line 21) are shown in Table2.

Recorded Data:

Ten plants were randomly taken from the inner rows of each sub sub plot to determine the following characteristics: number of pods plant^{-1}, pod weight plant^{-1}, 100-pod weight, seed weight/plant, 100-seed weight, shelling percentage, pod yield (ard fed) $^{-1}$ and oil percentage. The data was statistically analyzed according to Steel and Torrie (1980). Significance of differences among the various means of different characteristics under study was compared using Duncan's multiple range test (Duncan, 1955).

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Materials: The Five peanut genotypes along with the origin are shown in Table 1.

Table (1): Name and origin of five peanut genotypes.

Genotype	Name	Origin
1	Giza6	Egypt
2	Line623	USA
3	Sohag110	Egypt
4	Hybrid11	Egypt
5	Line21	Egypt

Molecular studies: Phylogenetic tree and relationships among the five genotypes of *A. hypogaea* were studied.

DNA Extraction:

Fresh leaf samples of *A. hypogaea* were taken for DNA extraction according to Bio basic kits protocol.

PCR- amplification of RAPD: Amplification reaction was carried out in 25µl reaction mixture contained 2µl of genomic DNA, 3µl of the primer, 2.5µl of 10X Taq DNA polymerase reaction buffer, 1.5 units of Taq DNA polymerase and 200 µm of each dNTPs. The following PCR program was used in a DNA Thermocycler (PTC-100 PCR version 9.0-USA); initial denaturation at 94°C for 5 min , followed by 35 cycles of 94°C for 30 Sec., 42°C for 90 Sec., 72°C for 90 Sec. Then, final extension at 72°C for 2 min.

Amplification products of RAPD-PCR were separated on 1.5% agarose gel in 1X TAE buffer and detected by staining with ethidium bromide according to Sambrook *et al.* (1989). DNA ladder 100bp was used.

PCR products were visualized by UV-transilluminator and photographed by gel documentation system, Biometra - Bio Doc. The amplified bands were scored as (1) for presence and (0) for the absence of all studied peanut cultivars according to gel analyzer protocol.

RESULTS AND DISCUSSION

Irrigation intervals effect

Results presented in Table (2) show that irrigation of peanut plants at medium intervals (every 7 days) led to significant increase and gave the highest values of all studied characteristics in both seasons and their combined analysis than those irrigated at short or long intervals *i.e.* every 4 and 10 days, respectively. The previous results are in full agreement with those reported by, Reddy and Reddy (1995), Vorasoot *et al.* (2003), Shinde and Laware (2010) and Abdzad Gohari and Babaei Bazkiyaei (2012).

Genotype differences

Results presented in Table (2) show that all studied characteristics except for shelling percentage of the five genotypes in both seasons and their combined analysis were significantly differed. It was evident that line 21 surpassed on the other four genotypes (Giza6, line623, Sohag110 and hybrid 11) in all studied characteristics. In the meanwhile, line 623 and Giza 6 surpassed on the other two genotypes in most characteristics. Significant varietal differences regarding those traits were reported by Waliyar *et al.* (2003) and Hamid Ziaeidoustan *et al.* (2013).

Interaction effect

As shown in the combined analysis**,** the interaction effects between irrigation intervals and peanut genotypes in Tables (2) were significant. The data indicated that line 21 under medium irrigation intervals (7days) gave the highest values of 100-pod weight, number of seeds plant^{-1}, seed weight plant^{-1}, 100-seed weight, shelling percentage, oil percentage and pod weight (ard fed) $^{-1}$, while the lowest values were obtained by hybrid 11 under long irrigation intervals (10 days).

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Table (2): Effect of irrigation intervals on the studied traits of peanut in two seasons

Genotype	Number of seeds pl.$^{-1}$		Seed weight pl.$^{-1}$		100-seed weight	
	2013	2014	2013	2014	2013	2014
Giza 6	23.3	27.3	40.4	50.6	188.2	193.2
Line 623	23.2	23.2	43.5	56.2	195.1	194.6
Sohag 110	21.2	31.1	38.3	58.0	179.6	192.5
Hybrid 11	22.8	29.8	40.2	54.9	192.7	192.2
Line 21	39.2	40.3	80.3	85.9	230.0	235.6
L.S.D (G) 0.05	0.938	1.889	-	3.298	4.02	-
Irrigation after 4days	24.8	27.0	47.0	53.3	201.4	197.3
Irrigation after 7days	25.9	29.02	48.0	76.5	200.7	205.1
Irrigation after 10 days	21.1	28.6	35.2	50.9	196.3	185.7
L.S.D (I) 0.05	1.11	1.278	4.41	2.21	4.45	2.89
Giza 6 (after4days)	49.7	35.4	36.5	49.7	193.	207.7
Giza 6 (after7days)	30.4	34.0	48.6	66.1	205.9	207.3
Giza 6 (after10days)	14.5	25.3	33.0	41.7	187.7	201.9
Line 623 (after4days)	25.9	35.8	38.6	57.3	192.7	197.2
Line 623 (after7days)	25.6	37.9	52.8	77.1	217.7	207.4
Line 623 (after10days)	21.1	31.1	36.7	48.9	193.4	197.6
Sohag 110(after4days)	22.0	32.4	49.0	58.0	161.8	197.2
Sohag 110(after7days)	24.6	41.7	36.3	76.9	196.8	205.5
Sohag 110(after10days)	25.3	38.1	41.1	57.5	195.3	190.8
Hybrid 11 (after4days)	21.9	27.5	37.8	49.2	197.0	193.6
Hybrid 11 (after7days)	26.9	42.0	52.7	85.3	203.6	214.2
Hybrid 11 (after10days)	21.5	25.9	39.9	45.7	189.9	175.6
Line 21 (after4days)	35.2	33.6	30.3	69.3	209.1	211.3
Line 21 (after7days)	36.3	30.2	31.3	75.3	223.6	225.9
Line 21 (after10days)	23.2	23.9	19.6	60.3	199.0	201.2
L.S.D(GxI)	2.213	2.558	8.83	4.29	8.19	5.77
Giza 6	39.6	45.4	30.8	39.6	85.1	87.4
Line 623	40.4	52.6	33.2	45.4	98.5	95.1
Sohag 110	34.7	53.6	27.5	46.9	86.1	81.1
Hybrid 11	38.7	38.9	28.8	26.3	89.3	88.2
Line 21	79.5	76.4	80.3	79.6	101.2	103.4
L.S.D (G) 0.05	1.73	4.32	2.82	3.21	-	-
Irrigation after 4days	35.2	43.1	28.3	36.4	86.3	84.5
Irrigation after 7days	44.6	61.2	36.9	51.8	90.6	93.6
Irrigation after 10 days	40.4	48.1	30.9	40.6	89.4	82.0
L.S.D (I) 0.05	2.19	3.12	2.43	2.09	2.31	3.57
Giza 6 (after4days)	43.6	49.4	39.6	35.2	85.3	92.9
Giza 6 (after7days)	49.4	54.8	40.9	52.6	95.9	105.0
Giza 6 (after10days)	42.2	45.2	25.4	37.0	76.3	70.6
Line 623 (after4days)	38.8	46.9	37.3	45.1	87.5	88.9
Line 623 (after7days)	46.1	69.9	37.6	58.6	94.6	97.6
Line 623 (after10days)	32.9	542.5	35.6	48.4	79.5	72.7
Sohag 110(after4days)	38.3	49.6	34.8	51.1	83.5	75.3
Sohag 110(after7days)	35.3	75.1	30.3	63.2	72.5	77.2
Sohag 110(after10days)	37.9	50.6	25.6	45.5	80.8	61.8
Hybrid 11 (after4days)	47.6	48.6	39.3	33.0	98.1	95.8
Hybrid 11 (after7days)	38.3	44.0	28.1	31.6	90.3	82.9
Hybrid 11 (after10days)	36.3	38.8	32.5	28.9	79.8	70.9
Line 21 (after4days)	69.3	563	70.3	60.2	102.3	106.4
Line 21 (after7days)	71.8	60.0	79.3	77.4	109.8	111.2
Line 21 (after10days)	45.2	44.3	42.1	42.6	99.8	101.2
L.S.D(GxI) 0.05	4.35	6.25	4.86	4.19	4.63	7.15
Giza 6	69.9	71.7	25.5	26.3	47.7	48.7
Line 623	71.4	72.3	26.5	26.7	48.3	50.1
Sohag 110	69.7	69.2	23.9	25.0	45.8	47.6
Hybrid 11	66.6	72.6	24.5	26.1	45.5	45.7
Line 21	75.3	75.0	30.6	29.8	49.3	50.2
L.S.D (G) 0.05	-	-	0.98	0.69	0.44	1.05
Irrigation after 4days	72.3	72.01	24.5	25.3	49.0	49.5
Irrigation after 7days	66.9	71.9	27.5	28.6	47.0	48.8
Irrigation after 10 days	69.4	71.8	27.4	28.7	47.3	48.8
L.S.D (I) 0.05	-	-	0.97	0.93	0.80	0.65

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Table (2): Cont.

Genotype	Shelling (%)		Pod yield (ard Fed^{-1})		Oil (%)	
	2013	2014	2013	2014	2013	2014
Giza 6 (after4days)	75.7	71.6	25.1	35.6	51.1	51.0
Giza 6 (after7days)	72.6	76.2	26.8	27.2	47.9	48.8
Giza 6 (after10days)	64.2	66.9	31.3	30.8	46.3	48.6
Line 623 (after4days)	70.8	72.8	25.06	29.6	46.2	47.4
Line 623 (after7days)	68.6	74.4	25.6	30.9	51.3	50.9
Line 623 (after10days)	68.0	67.9	24.1	28.3	41.6	50.8
Sohag 110(after4days)	78.0	74.1	30.6	32.0	47.4	51.1
Sohag 110(after7days)	77.5	67.0	30.6	29.5	46.6	47.8
Sohag 110(after10days)	59.1	64.9	28.8	31.9	44.5	38.0
Hybrid 11 (after4days)	72.7	79.0	20.6	30.6	49.2	50.8
Hybrid 11 (after7days)	74.4	74.9	23.8	23.3	47.1	47.9
Hybrid 11 (after10days)	67.9	68.9	21.4	26.9	42.1	43.6
Line 21 (after4days)	73.2	70.2	25.4	26.0	48.2	45.0
Line 21 (after7days)	75.0	74.8	31.2	32.0	50.2	46.8
Line 21 (after10days)	71.0	70.2	21.0	22.0	46.8	45.2
L.S.D(GxI) 0.05	5.96	5.25	1.94	1.85	1.61	1.29

Molecular analysis: Five random primers revealed 36 different alleles. 23 out of them were polymorphic with polymorphism 63.9%. The average of alleles was 7.2/primer (Fig.1& Table3).

Cluster analysis in Table (4) grouped the 5 genotypes into two main clusters with Jaccard's similarity coefficient ranging from 0.083 to 0.83 (Fig. 2). The highest similarity was found between Giza6 and Line 21, while the lowest similarity was among Giza6, Line623 and Hybrid11. The first cluster included Line21 only, which was high tolerant to drought and gave high yield. However, the second cluster contained the rest of the genotypes.

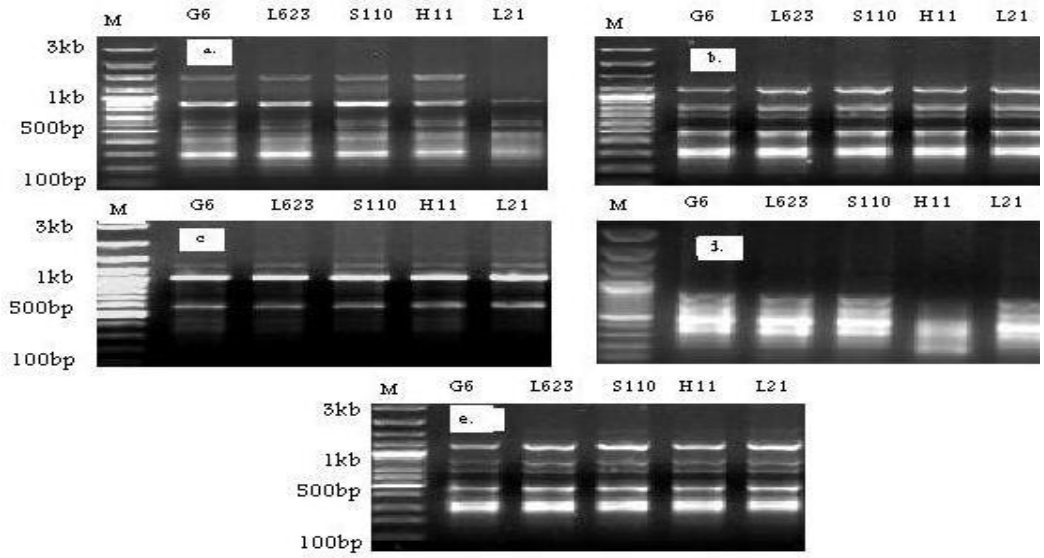

Fig. 1: RAPD- PCR banding patterns of the five peanut genotypes using five *10-mer* random primers; (a) OP-A02, (b) OP-A04, (c) OP-B10, (d) OP- O14 and (e) OP-O19; MS = 100-bp ladder.

Level of polymorphism varied from one primer to another. Primer OPB-10 showed the highest level of polymorphism (83.3%), while primers OPA-02 and OPO-14 showed the lowest one (57.1 %). The average of polymorphic bands/primer was 4.6 (Table 3).

Primer OPA-02 showed 7 bands. Three of them were monomorphic and four were polymorphic. Primer OPA-04 revealed 8 bands; three monomorphic and five polymorphic.

On the other hand, primer OPB-10 revealed six bands; one of them was monomorphic and five polymorphic.

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Finally, primers OPO-14 and OPO-19 revealed three monomorphic bands. Primer OPO-14 showed four polymorphic bands, while primer OPO-19 revealed five polymorphic bands.

Results revealed moderate of polymorphism(63.9%) that agree with (Gepts, 1993), whereas the low DNA polymorphism in peanut in contrast to the high diversity for agronomic traits may be due to the selective neutrality of molecular markers, while morphological traits have been subjected to intense selection.
Development of a genetic map of the cultivated peanut may also enrich the existing map of *Arachis* and thus facilitate an accelerated improvement of this crop.

Table (3): Total number, monomorphic, polymorphic bands and average polymorphism of the five peanut genotypes using five RAPD-PCR primers.

No.	Primer code	Sequence (5`→3`)	Monomorphic bands	Polymorphic bands	Total bands	Polymorphism%
1	OPA-02	CAGGCCCTTC	3	4	7	57.1
2	OPA-04	AATCGGGCTG	3	5	8	62.5
3	OPB-10	CTGCTGGGAC	1	5	6	83.3
4	OPO-14	AGCATGGCTC	3	4	7	57.1
5	OPO-19	CAATCGCCGT	3	5	8	62.5
Average	-	-	2.6	4.6	7.2	63. 9%
-	-	Total	13	23	36	

Table 4): Proximity Matrix of five peanut genotypes.

Case	Matrix File Input				
	Giza6	Line623	Sohag110	Hybrid11	Line21
Giza6	1.000				
Line623	.083	1.000			
Sohag110	.167	.167	1.000		
Hybrid11	.083	.417	1.000	1.000	
Line21	.833	1.000	.417	.500	1.000

Our results revealed that the chosen RAPD markers are distributed in the peanut genome and may be useful to investigate the genetic diversity among the studied peanut genotypes.

El-Adawy *et al.* (2002) revealed that RAPD was more useful than SSR in classifying maize inbred lines and generating a dendrogram more fitted to their pedigree, while He *et al*. (2003) reported that AFLP markers were better than RAPD and ISSR markers in terms of the number of polymorphic bands detected and the experimental stability. Raina *et al.* (2001) reported that it was possible to identify accessions, particularly those of divergent origins, by RAPD and/or ISSR fingerprints and marker-based genetic improvement in peanut.

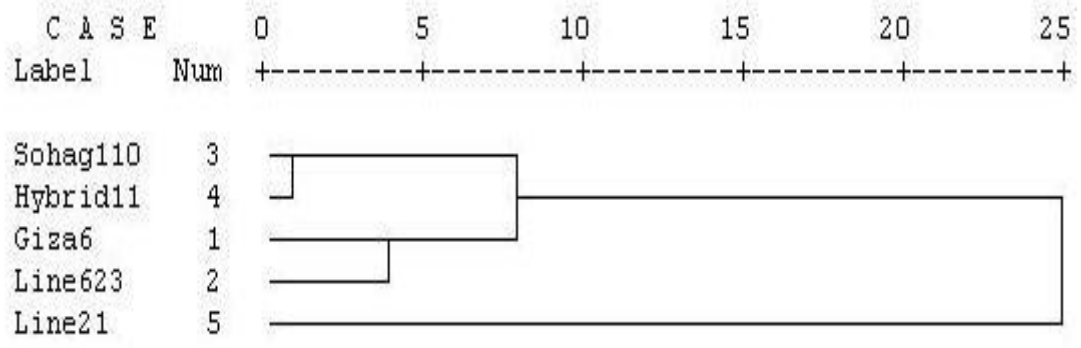

Fig.2): Dendrogram using average linkage among five peanut genotypes.

CONCLUSIONS

From our result, it was found that Line 21 genotype was highly tolerant for drought. This is likely to produce the largest number of unique and potentially agronomic useful alleles. The success of our study in identifying polymorphic loci might be due to these three factors, (I) the genotypes analyzed in this study were properly characterized and differed in tolerance for drought, (II) use of five RAPD primers for prescreening, and (III) use of phylogenetic tree for genotypes which was indicator for breeding with selection. RAPD markers succeeded in detecting relationships among these genotypes and showed the correlations between yield traits and molecular traits. The present study will help identifying linked molecular loci against drought in cultivated peanut. In addition to, the distinct genotypes can be used to produce mapping population for detection of quantitative trait loci.

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