Variations In Productive Characteristics And Genetic Diversity Assessment Among Garlic Cultivars And Lines Using Dna Markers

S. Al-Otayk[1], M. Z. El-Shinawy [2] and M. I. Motawei [1]

[1] Department of Plant Production and Protection, College of Agriculture and Veterinary Medicine, Al-Qassim University, Saudi Arabia

[2] Horticulture Dept., Faculty of Agric., Ain Shams Univ., Shobra El-Khima, Cairo, Egypt.

ABSTRACT

Garlic cultivars; namely, Egyptian, Chinese and Elephant, and six Chinese lines were evaluated for its productivity in two field experiments during 2004/2005 and 2005/2006 winter seasons. The results indicated that the maximum plant height was 70.5 and 70.8 cm for Egyptian cultivar in both seasons, respectively. The maximum values of leaf area per plant were observed with Elephant cultivar and a Chinese line (L2). Elephant cultivar was superior than the other cultivars and lines in plant fresh weight. While, the Chinese line, L4, had the maximum plant dry weight and the highest chlorophyll content. Bulb fresh and dry weights of the Egyptian cultivar, Balady, was the lowest among the cultivars and lines tested. On the other hand, the Egyptian cultivar, Balady, produced more cloves number, compared with the other cultivars and Chinese lines. Elephant and Chinese cultivars gave the highest mean values of bulb diameter, compared with the Egyptian cultivar. Also, the Chinese lines, L2 and L5, gave higher mean values of bulb diameter, compared with the other Chinese lines. The same trend was observed for clove weight. Total soluble solids (TSS) content of cloves was the highest in the Egyptian cultivar and the Chinese line (L5). The marketable yield (g/m^2) showed that Elephant cultivar and the Chinese line (L5) produced the highest yield. While, Egyptian cultivar (Balady) and Chinese line 6 (L6) produced the lowest yield.

Two types of molecular markers, random amplified polymorphic DNA (RAPD) and inter-simple sequence repeat (ISSR), were assayed to determine the genetic diversity of six garlic lines and three garlic cultivars. A high level of polymorphism, among garlic cultivars and lines was found with both RAPD and ISSR markers. While, ISSR revealed higher polymorphism among Chinese lines than RAPD. The UPGMA dendrogram, generated from RAPD data, clearly indicated four main clusters. The dendrogram, generated from ISSR data, clearly indicated five clusters. Chinese line (L6) was separated from the other Chinese lines and this line gave the lowest yield and total soluble solid content, compared to the other lines and the Chinese cultivar. Polymorphic ISSRs, were abundant in garlic and demonstrated genetic diversity among related lines. Accordingly, ISSR was an additional tool for fingerprinting and detailed assessment of genetic relationships in garlic.

Keywords: Garlic cultivars and lines, productive characters, fingerprint, genetic diversity, RAPD markers, ISSR markers.

INTRODUCTION

Garlic (*Allium sativum* L.) is a perennial plant for which bulbs are economically important as a food additive. Most garlic plants are sterile and vegetatively propagated by cloves. The sterility of garlic could be due to the structural heterozygosity of chromosomes, though its definite cause is uncertain (Etoh, 1985). Garlic has a large and complex genome with two pairs of satellite chromosomes in the basic karyotype (Kim and Seo 1991; Lee *et al*., 2003). It is an unusual crop in that, despite being exclusively propagated asexually over centuries, it maintains a diverse phenotype amongst different clones. This makes garlic an ideal species for investigating heritage and diversity. Using molecular techniques, scientists are able to evaluate diversity among strains. Through the development of a phylogenetic tree, diversity is established among clones, based on their relative position within the tree. Mutations, such as single base substitutions, inversions or deletions allow for phylogenetic differentiation. A tree is then useful for breeders to select diverse parents in making crosses for the development of superior crops. Also, germplasm, from a country of origin, may help to aid in the selection of appropriate growing climates. The premise behind this is that a selection may have developed in adaptation to a particular climate, making it better suited to similar climates (Fernandez *et al*., 2003). Prior studies have used total genomic DNA to screen for molecular markers by employing such method, as RAPD's (random amplified polymorphic DNA) (Maas and Klaas,1995; Nabulsi *et al*., 2001; Ipeck *et al*., 2003). Moreover, several PCR-based DNA fingerprinting techniques, including simple sequence repeat (SSR), and amplified fragment length polymorphism (AFLP) are available for detecting genetic differences within and among cultivars (Volk *et al*., 2004). Among these, simple sequence repeat (SSR) markers are efficient, cost-effective and can detect a significantly higher degree of polymorphism in onion (Kuhl *et al*., 2003). They are ideal for genetic diversity studies and intensive genetic mapping. An alternative method to SSR, called inter-SSR (ISSR)-PCR (Nagaoka and Ogihara, 1997), has also been used to fingerprint the different plant species and cultivars (Nagaoka and Ogihara, 1997; Levi and Rowland, 1997; Wolfe *et al*., 1998; Nagaraju *et al*., 2002; Al-Humaid *et al*., 2004).

The objectives of the present study were to: (1) Evaluate garlic cultivars and lines selected from a Chinese cultivar for productivity and (2) Investigate the assessment of genetic diversity in garlic cultivars and lines, using RAPD and ISSR markers.

MATERIALS AND METHODS

Plant materials and field experiments:

Two field experiments were conducted at the Experimental Farm of the College of Agriculture and Veterinary Medicine, AL-Qassim University, Sudi Arabia. Three garlic cultivars; namely, Egyptian (Balady), Elephant, Chinease and six lines of Chinese garlic were planted in 13th and 8th October in 2004/2005 and 2005/2006 in the first and second winter seasons, respectively. Cloves of garlic cultivars and lines were planted at a spacing 7 cm of both sides of ridges, spaced 60 cm apart. The plants were fertilized with the rate of 300 kg of ammonium sulphate (20.5% N), 300 kg of calcium superphosphate (15.5% P_2O_5) and 200 kg potassium sulphate (48% K_2O) per fedddan. Drip irrigation was used.

The soil type of this farm is classified as a sandy soil (sand 96.3%, silt 1.8% and clay 1.9%) with bulk density (1.501 g/cm3). Water holding capacity, field capacity, and wilting point were 17.2, 9.6 and 4.4 %, respectively. The layout of the experiments was randomize complete block design with four replicates. The plot area was 7 X 6 m and contained 9 rows 75 cm apart.

Measurements:

Four plants were randomly chosen for recording the vegetative growth parameters, which included plant height (cm), number of leaves per plant, leaf area /plant (cm^2) and plant fresh and dry weights. Also, chlorophyll content was measured of the fifth upper leaf, using Minolta chlorophyll Meter SPAD –501.

At the harvesting stage, four plants were randomly chosen for recording yield parameters, which included bulb fresh and dry weights (g), clove fresh and dry weights (g), bulb diameter (cm), number of cloves and total soluble solids (T.S.S.) (%). Also, marketable yields per square meter were recorded.

DNA extraction:

Bulk leaf samples from garlic lines and cultivars were used. The bulk sample of leaves was first ground into fine powder with liquid nitrogen. DNA was extracted in 10 ml of CTAB buffer,consisting of: 50 mM NaCl, 10 mM Tris-HCl pH 7.5, 5 mM EDTA, and 1% CTAB. The homogenate was incubated for two hours at 65 ºC with occasional mixing. Following incubation, 5 ml of chloroform/isoamylalcohol (24:1) was added to the tubes, mixed and centrifuged at 260 g for 10 min. The aqueous phase was removed to a fresh tube and an equal volume of ice-cold isopropanol was added,

followed by centrifugation as above to precipitate the DNA. The pellet was dissolved in TE buffer (10 mM Tris-HCl, pH 8.0, 0.1 mM EDTA). The DNA concentration was assessed ,spectrophotometrically, at 260 nm, and quality was assessed by the 260/280 ratio (Sambrook *et al*., 1989). The DNA was suspended to a final concentration of 5 ng/L in 0.5X TE and stored at 4˚C.

RAPD analysis

A total of twenty 10-mer oligonucleotides, with arbitrary sequence from Operon (Table 2) were used in RAPD analysis. The PCR reaction mixture consisted of 50 ng genomic DNA, 1×PCR buffer, 2.0 mmol/L MgCl2, 100 µmol/L of each dNTP, 0.1 µmol/L primer and 1U Taq polymerase in a 25µL volume. The amplification protocol was 94 ºC for 4 min. to pre-denature, followed by 45 cycles of 94 ºC for 1 min, 36 ºC and 72 ºC for 1 min, with a final extension at 72 ºC for 10 min. Amplification products were fractionated on 1.5% agarose gel.

ISSR assay:

The ISSR-PCR method was carried out, according to Negaoka and Ogihara (1997). Amplification were carried out in 25 µl reaction volumes, containing 1X Taq polymerase buffer (50 mM KCl, 10mM Tris, pH 7.5, 1.5 mM MgCl2) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 50 ρmol of ISSR primers (Table 3), and 50 ng of total genomic DNA. Amplification was performed in a thermal cycler (Thermolyne Amplitron) programmed for 1 cycle of 2 min at 94˚C; and 35 cycles of 30 secs at 94˚C, 45 secs at 44˚C, and 1.3 min at 72˚C; followed by 20 min at 72˚C.

After completion of PCR, samples were cooled immediately to 10ºC and stored at 4ºC until gel separation. A gel-loading solution (5 µl) was addedand 10 µl of the total product volume was resolved in 1.5% agarose in 1X TAE buffer for 2 h .aside with a 100- bp ladder (Pharmacia, Germany) as the size standard. Gels were stained in ethedium bromide and images were recorded.

Data analysis:

Data were statistically analyzed by using a randomized complete block design, with three replicates, according to Snedecor and Cochran (1980). The two growing seasons were separately analyzed. The least significant differences (LSD) test was used to compare among means at the 5% level. Only significant differences (at P≤0.05) were considered in the text.

Data of RAPD and ISSR analysis were scored for computer analysis on the basis of the presence or absence of the amplified products for each ISSR primer. If a product was present in a cultivar, it was designated as “1”, if absent it was designated as “0”. Pair-wise comparisons of cultivars, based on the presence or absence of unique and shared polymorphic products, were used to generate similarity coefficients, based on SIMQUAL module. The similarity coefficients were ,then, used to construct a dendogram by UPGMA (Unweighted Pair-Group Method with Arithmetical Averages), using NTSYS-PC software version 2.0 (Exeter Software, New York) (Rohlf, 2000).

RESULTS AND DISCUSSION

Growth and yield characters:

There were differences in plant height, number of leaves and leaves area per plant among garlic cultivars and lines (Table 1). The maximum plant height was 70.5 and 70.8 cm for Egyptian cultivar in both seasons, respectively. Hussein et al. (1995) found that the maxiumum plant height for Egyptian cultivar was 74.0 cm. Whereas, Omer and Abou Hadid (1992) reported approved approximately 105.5 cm for Egyptian cultivar. Concerning the leaves number, it can be noted that Chinese line (L5) (9.3 and 8.5) had more leaves number compared with Egyptian cultivar (Balady) (7.3 and 7.5) in both seasons, respectively. The maximum value of leaves area per plant were observed with Elephant cultivar and Chinese line (L2). Elephant cultivar was superior than the other cultivars and lines in plant fresh weight (186.8 and 183.9 g) in both seasons, respectively. While, the Chinese line (L4) had the maximum plant dry weight (56.0 and 55.5 g) in both seasons, respectively (Table 2). Also, the last line and Egyptian cultivar had the highest chlorophyll content of the tested cultivars and lines in both seasons (Table 2). Ovesna *et al.* (2007) concluded that studied accessions of garlic *Allium sativum* L. representing a substantial portion of genetic resources preserved in the genebank RICP Prague, Workplace Olomouc gathered genetic diversity as revealed by morphological descriptors.

For bulb fresh weight, it is apparent in Table 3 that Elephant cultivar and Chinese line (L5) produced the highest bulb fresh weight (117.6 and 118.6 g) and (68.7 and 67.6 g) in both seasons, respectively. Also, Chinese lines (L2 and L5) produced the highest bulb dry weight. Bulb fresh and dry

weight of Egyptian cultivar (Balady) was the lowest among the cultivars and lines tested. On the other hand, Egyptian cultivar (Balady) produced more cloves number (32.0 and 29.5) compared with the other cultivars and Chinese lines in both seasons, respectively. These results are in agreement with Omer and Abou Hadid (1992) who reported that Chinese cultivars produced larger bulbs in comparison with Egyptian cultivars.

The character of bulb diameter is among the major harvestable yield components that contribute the ultimate development of different cloves order, number and diameter of arranged cloves attached the disc steam structure. In this respect the data in Table 4 cleared that Elephant and Chinese cultivars gave the highest mean values of bulb diameter compared with Egyptian cultivar. Also, Chinese lines (L2 and L5) gave higher mean values of bulb diameter compared with the other Chinese lines. The same trend was observed for clove weight, where Elephant cultivar and Chinese line (L2) gave the highest mean values of cloves fresh and dry weight compared with the other cultivars and lines. Total soluble solid (TSS) content of cloves was highest in Egyptian cultivar (Balady) and Chinese line 5 (L5) (37.0 and 38.8 %) and (38.6 and 38.4 %) in both seasons respectively (Table 5). On the other hand, Elephant cultivar and Chinese line 6 (L6) gave the lowest total soluble solid content. Sing and Chand (2003) found that total soluble solid content of cloves was significantly differed among garlic cultivars and clones. Marketable yield (g/m2) showed that Elephant cultivar and Chinese line 5 (L5) resulted in the highest yield (2352.0 and 2372.4 g/m2) and (1373.2 and 1352.2 g/m2) in both seasons, respectively (Table 5). While, Egyptian cultivar (Balady) and Chinese line 6 (L6) produced the lowest yield (729.8 and 723.1 g/m2) and (743.3 and 714.9 g/m2) in both seasons, respectively. Similar results were reported by Hussein et al. (1995) who found that Balady cultivar produced the lowest garlic yield.

Table 1.Means of plant height, number of leaves and leaf area of garlic cultivars and lines during 2004/05 and 2005/06 seasons.

Cultivars & Lines	Plant height (cm)		No. of leaves/plant		Leaf area /plant (cm^2)	
	2004/05	2005/06	2004/05	2005/06	2004/05	2005/06
Line 1 (L1)	61.0 c*	62.5 b	8.8 ab	7.8 ab	256.6 bc	262.9 f
Chinese garlic (Ch)	54.5 d	57.2 d	8.5 ab	8.0 ab	226.8 bc	235.3 g
Line 2 (L2)	60.5 c	60.5 c	9.3 a	8.5 a	353.2 b	378.1 b
Line 3 (L3)	60.8 c	58.0 d	7.8 bc	7.0 b	198.3 c	212.2 h
Line 4 (L4)	64.3 b	63.0 b	8.3 abc	8.0 ab	350.8 b	350.6 c
Line 5 (L5)	59.3 c	60.0 c	9.3 a	8.5 a	346.4 b	340.9 d
Line 6 (L6)	69.3 a	63.3 b	8.3 abc	7.5 ab	281.9 bc	284.5 e
Egyptian garlic (B)	70.5 a	70.8 a	7.3 c	7.5 ab	244.9 bc	146.6 i
Elephant garlic (El)	53.8 d	51.5 e	8.8 ab	8.5 a	517.9 a	535.4 a

*, Means followed by the same letter are not significantly different according to LSD at 0.05 level of probability.

Table 2. Means of plant fresh and dry weight and chlorophyll content of garlic cultivars and lines during 2004/05 and 2005/06 seasons.

Cultivars & Lines	Plant fresh weight (g)		Plant dry weight (g)		Chlorophyll (Value)	
	2004/05	2005/06	2004/05	2005/06	2004/05	2005/06
Line 1 (L1)	134.5f *	130.4 f	43.5 b	40.0 e	61.7 f	62.5 f
Chinese garlic	142.3 e	143.8 e	44.3 b	45.8 c	66.1 d	66.0 d
Line 2 (L2)	167.9 c	165.4 c	55.0 a	52.5 b	64.5 e	64.5 e
Line 3 (L3)	160.5 d	160.3 d	52.1 a	51.1 b	69.8 c	71.0 c
Line 4 (L4)	171.9 c	170.3 b	56.0 a	55.5 a	71.9 b	75.6 b
Line 5 (L5)	147.2 e	145.4 e	45.1 b	44.2 cd	70.8 bc	70.6 c
Line 6 (L6)	164.9 cd	163.0 cd	52.9 a	53.5 ab	69.5 c	72.1 c
Egyptian garlic	178.2 b	171.2 b	53.5 a	53.8 ab	75.0 a	76.6 a
Elephant garlic	186.8 a	183.9 a	40.1 c	41.9 de	69.9 c	70.6 c

*, Means followed by the same letter are not significantly different according to LSD at 0.05 level of probability.

Table 3. Means of bulb fresh and dry weight and number of cloves of garlic cultivars and lines during 2004/05 and 2005/06 seasons.

Cultivars & Lines	Bulb fresh weight (g)		Bulb dry weight (g)		No. of cloves	
	2004/05	2005/06	2004/05	2005/06	2004/05	2005/06
Line 1 (L1)	49.9 f*	52.7 ef	19.1 e	20.0 d	13.5 b	14.0 b
Chinese garlic	54.7 e	55.2 e	24.9 c	22.4 c	8.5 ef	9.0 ef
Line 2 (L2)	62.7 c	63.3 c	34.9 a	32.6 a	7.8 f	8.0 f
Line 3 (L3)	51.6 f	50.9 f	20.7 d	20.8 d	10.0 d	9.5 d
Line 4 (L4)	57.03d	58.4 d	21.5 d	21.1 d	9.3 de	9.3 de
Line 5 (L5)	68.7 b	67.6 b	26.9 b	24.4 b	11.3 c	11.8 c
Line 6 (L6)	37.2 g	36.9 g	13.7 f	13.1 e	11. 0 c	10.5 c
Egyptian garlic	36.5 g	36.2 g	11.7 g	21.1 e	32.0 a	29.5 a
Elephant garlic	117.6 a	118.6 a	23.8 c	24.6 b	5.3 g	4.8 g

*, Means followed by the same letter are not significantly different according to LSD at 0.05 level of probability.

Table 4. Means of bulb diameter, and cloves fresh and dry weight of garlic cultivars and lines during 2004/05 and 2005/06 seasons.

Cultivars & Lines	Bulb diameter (cm)		Cloves fresh weight (g)		Cloves dry weight (g)	
	2004/05	2005/06	2004/05	2005/06	2004/05	2005/06
Line 1 (L1)	5.4 c*	5.7 c	3.3 d	3.3 d	1.4 f	1.4 d
Chinese garlic	6.2 b	5.9 b	6.2 c	5.4 cd	2.7 e	2.9 b
Line 2 (L2)	6.7 b	5.9 b	7.7 b	7.8 b	4.0 b	4.5 a
Line 3 (L3)	5.5 c	5.3 d	4.9 c	5.0 d	2.2 d	2.0 c
Line 4 (L4)	5.1 d	5.9 b	5.9 c	5.9 c	2.2 d	2.3 c
Line 5 (L5)	6.0 b	6.0 b	5.5 c	5.3 cd	1.9 e	2.3 c
Line 6 (L6)	5.1 d	5.1 e	2.9 d	3.4 d	1.2 f	1.2 d
Egyptian garlic	5.1 d	5.1 e	1.0 e	1.1 e	0.4 g	0.4 e
Elephant garlic	7.4 a	7.2 a	19.3 a	24.0 a	5.2 e	4.5 a

*, Means followed by the same letter are not significantly different according to LSD at 0.05 level of probability.

Table 5.Means of T.S.S and marketable yield of garlic cultivars and lines during 2004/05 and 2005/06 seasons.

	Cloves T.S.S %		Marketable yield g /m2	
Cultivars & Lines	2004/05	2005/06	2004/05	2005/06
Line 1 (L1)	37.3 b*	38.3 a	999.1 f	1055.2 b
Chinese garlic	35.6 bc	34.9 c	1095.9 e	1104.8 e
Line 2 (L2)	36.1 bc	36.8 ab	1254.7 c	1265.2 c
Line 3 (L3)	34.5 cd	35.1 c	1032.4 f	1018.2 f
Line 4 (L4)	36.0 bc	35.5 bc	1140.6 d	1167.6 e
Line 5 (L5)	38.8 a	38.4 a	1373.2 b	1352.2 b
Line 6 (L6)	33.2 d	32.7 d	743.3 g	714.9 g
Egyptian garlic	37.0 b	38.6 a	729.8 g	723 1 g
Elephant garlic	26.8 e	27.8 c	2352. 0 a	2373.4 a

*, Means followed by the same letter are not significantly different according to LSD at 0.05 level of probability.

Genetic diversity

For RAPD analysis, random primers (Operon Technologies Alameda, Calif.) reported to be polymorphic in previous studies for garlic (Maas and Klaas, 1995; Ipek *et al.*, 2003) were tested. Eight primers of arbitrary nucleotide sequence were used to amplify DNA segments from garlic cultivars and lines. The number of amplification bands per primer varied between 5 and 11. Analysis of the 8 primers among garlic cultivars and lines included in this study generated 62 bands, 52 of which were polymorphic among garlic cultivars and lines (Table 6). There were 6.5 polymorphic bands per primers in average. While, analysis of RAPD primers among Chinese garlic lines generated only 4 polymorphic bands. Ipek *et al*. (2003) found that all garlic clones shared 100% of RAPD bands within each group. Examples of polymorphism are shown in figure 1. The UPGMA dendrogram generated from RAPD data clearly indicated four main clusters (Fig 2). The first cluster contained Chinese lines L1, L2, and L3. The second cluster contained Chinese cultivar and lines L4, L5 and L6. The Chinese line 4 was genetically closed to Chinese cultivar. The third cluster contained Egyptian cultivar (Balady) (*Allium sativum*). The fourth

cluster contained Elephant garlic (*Allium ampeloprasum*). Etoh *et al. (*2003) found that the genetic similarity among the Iberian garlic clones using RAPD marker was high, and poor genetic diversity was estimated among the clones from Spain and Portugal, while the genetic

similarity among the Central Asian clones was comparatively low, and greater genetic diversity was estimated among those Central Asian clones.

Operon primers	Amplified products	Polymorphic Fragments among garlic cultivars and lines	Polymorphic fragments among Chinese lines
OPA-01	11	10	1
OPA-13	6	4	0
OPF-03	6	4	0
OPF-04	6	6	0
OPF-05	12	10	1
OPF-06	5	4	1
OPF-07	7	5	0
OPF-08	9	9	1

Table 6. RAPD primers with the number of amplified products, polymorphic fragments among garlic cultivars and lines, and polymorphic fragments among Chinese lines.

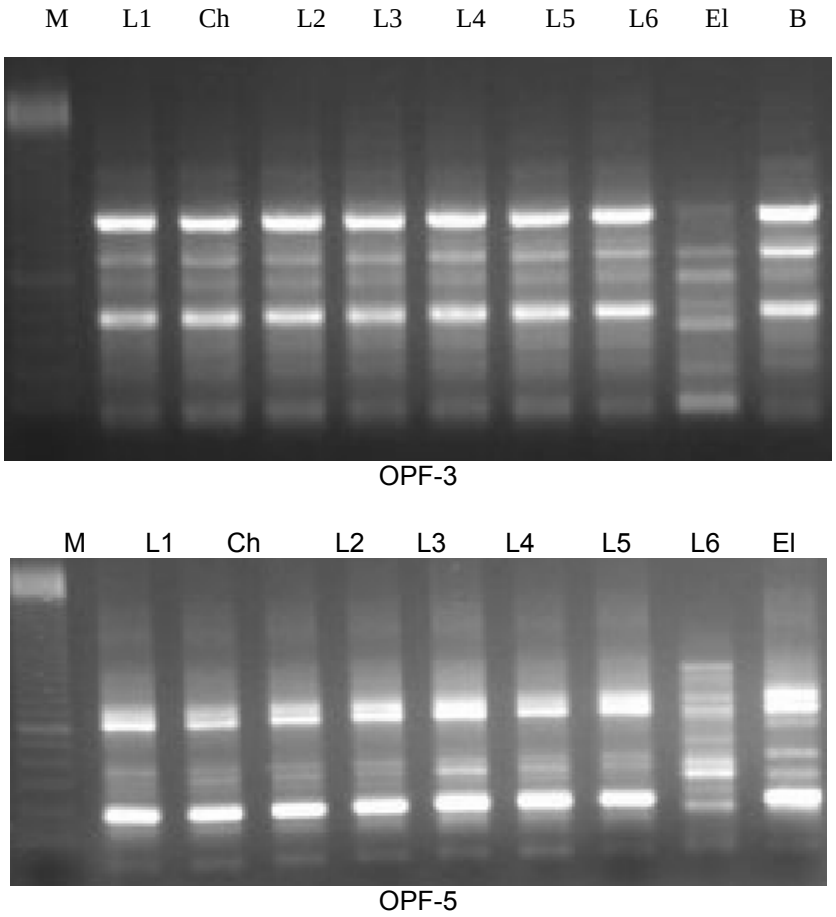

Fig. 1: Polymorphism revealed using primer OPF-3 and OPF-5 to amplify genomic DNA purified from the tested garlic cultivars and lines. M lane is 1 kbp ladder DNA marker.

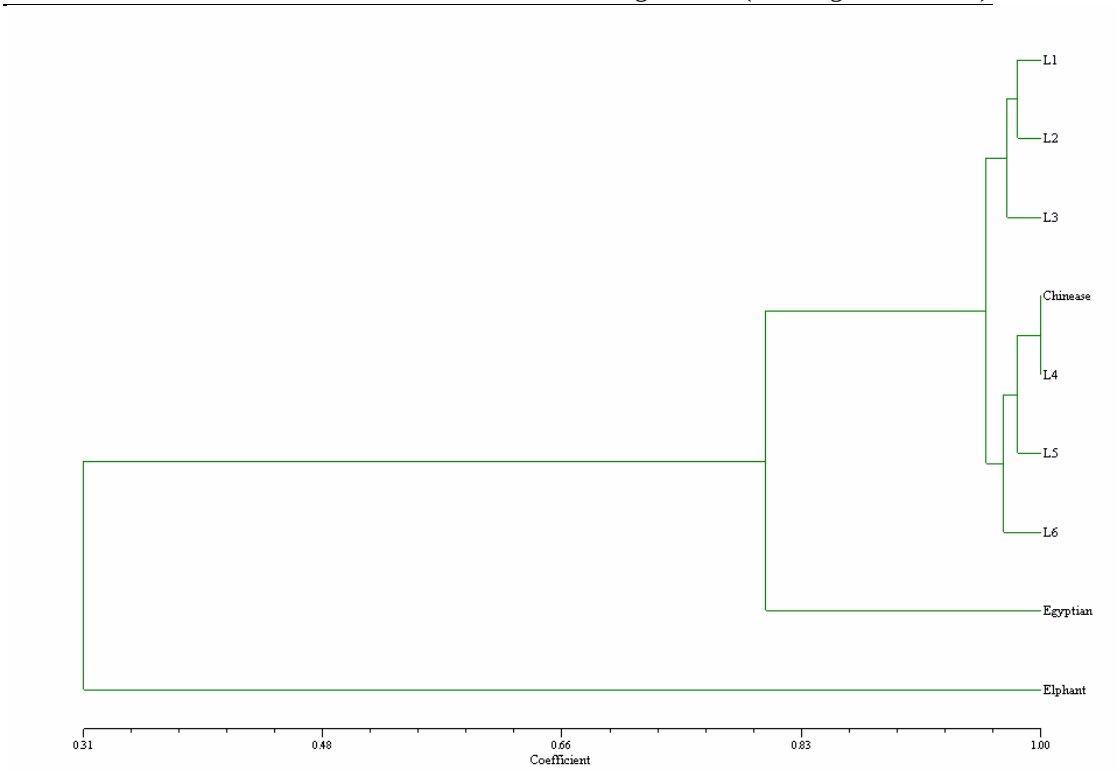

Fig. (2): Dendrogram constructed from similarity coefficients and showing the clustering of the tested garlic cultivars and lines using RAPD markers.

In ISSR analysis, the number of amplification bands per primer varied between 0 and 10. Examples of polymorphism are shown in figure 3. The trinucleotide repeats (CTC)n primer had more bands than (CAC)n (CTC)n and (GTG)n primers, and dinucleotide repeats (CA)n primer (Table 7). Also, among seven ISSR primers, poly (CTC) based primers accounted 37.5% of total polymorphic bands among garlic cultivars and lines. Analysis of ISSR primers among Chinese garlic lines generated six polymorphic bands. Therefore, ISSR revealed higher polymorphism among Chinese lines than RAPD. In barley genome, the percentage of ISSR polymorphic bands was 98.1% (Hou et al., 2005).

Table 7. ISSR primers with the number of amplified products and polymorphic

Primers	Sequence 5` to 3`	Amplified products	Polymorphic Fragments among garlic cultivars and lines	Polymorphic fragments among Chinese lines
P02	$(ATCG)_4$	0	0	0
D12	$(GA)_6CG$	0	0	0
D14	$(CAC)_3GC$	4	2	1
D24	$(CA)_6CG$	4	3	1
HB 13	$(GAG)_3GC$	6	3	0
HB 14	$(CTC)_3GC$	10	6	2
HB 15	$(GTG)_3GC$	4	2	2

fragments among garlic cultivars and lines, and polymorphic fragments among Chinese lines.

The dendrogram generated from ISSR data clearly indicated five clusters (Fig 4). The first cluster included Chinese lines 1, 2, 4, and 3. The second cluster included Chinese cultivar and line (L5). The third cluster contained Chinese
line (L6). The fourth cluster contained Egyptian cultivar (Balady) (*Allium sativum*). The fifth cluster contained Elephant garlic (*Allium ampeloprasum*). It should be noted that Chinese line (L6) was separated from the other Chinese lines and this line gave the lowest yield and total soluble solid content compared to the other lines and Chinese cultivar. Bradley *et al.* (1996) reported that bolting and intermediate/nonbolting garlic forms could be separated from each other based on cluster analysis of RAPD markers. In conclusion, ISSR technology is a useful tool for analysis of genetic diversity of garlic along with productive characters and RAPD markers. ISSR markers can provide a better approximation to true variation among garlic lines.

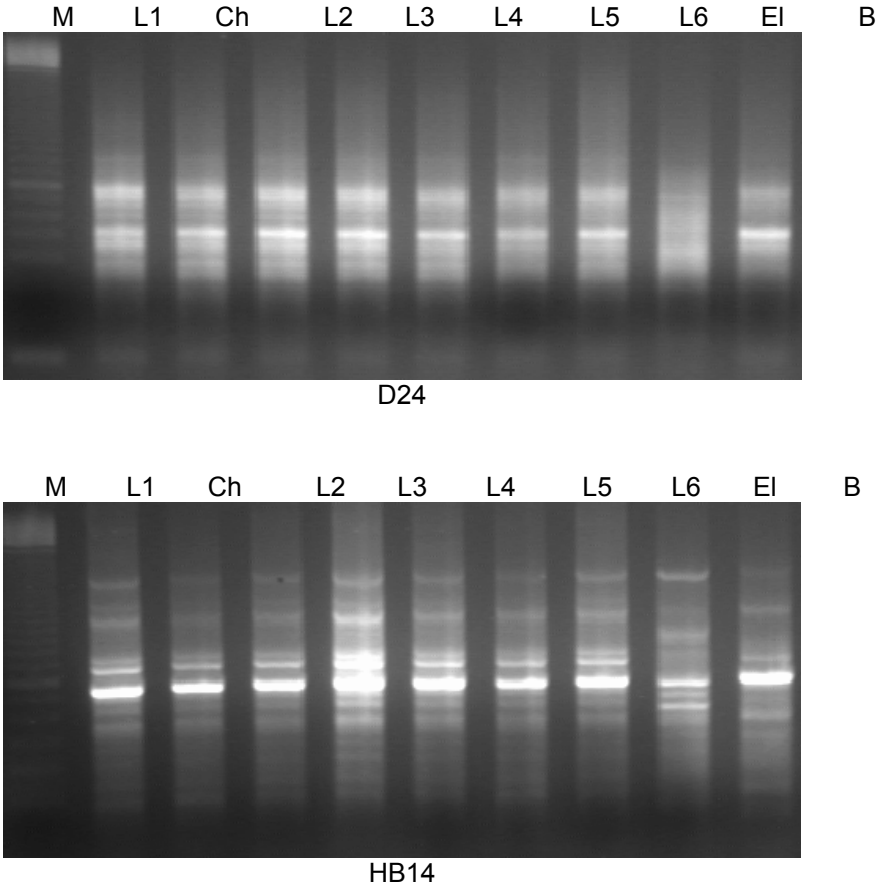

Fig. 3: Polymorphism revealed using primer D24 and HB14 to amplify genomic DNA purified from the tested garlic cultivars and lines. M lane is 1 kbp ladder DNA marker.

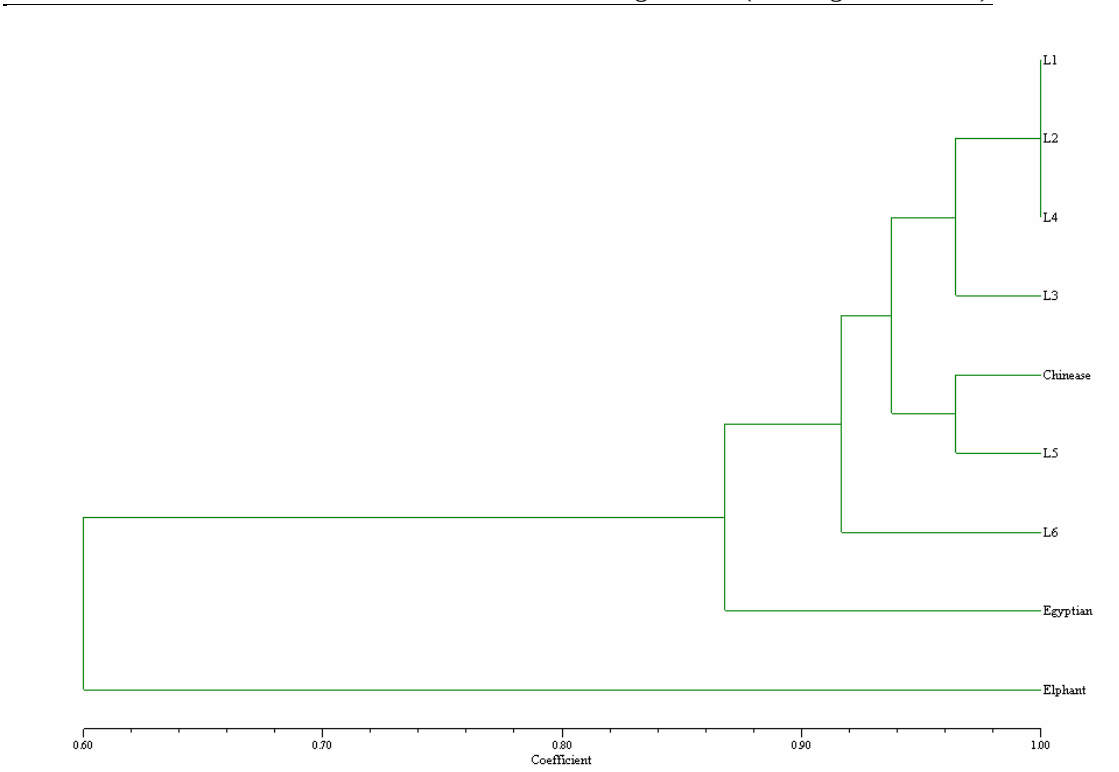

Fig. (4): Dendrogram constructed from similarity coefficients and showing the clustering of the tested garlic cultivars and lines using ISSR markers.

REFERENCES

Al-Humaid, A., M.I. Motawei, M.Y. Abdalla, and F. Mana. 2004. Detection of genetic variation and fusarium resistance in turfgrass genotypes using PCR-based markers (ISSR and SCAR). Food, Agriculture & Environment 2(3&4):225-229.

Bradley, K. F., M. A. Reiger and G.G. Collins. 1996. Classification of Australian garlic cultivars by DNA fingerprinting. Australian Journal of Experimental Agriculture. 36: 613-618.

Etoh, T. 1985. Studies on the sterility in garlic, *Allium sativum* L. Mem. Fac. Agr. Karoshima Univ. 21: 77–132.

Etoh, T., W. Hideki, and I. Sumio. 2003. RAPD Variation of Garlic Clones in the Center of Origin and the Westernmost Area of Distribution. Memoirs of the Faculty of Agriculture, Kagoshima University 37: 21-27.

Fernandez, M., M. Ipek, A. Ipek, and P. Simon. 2003. Development of a phylogenetic tree in garlic (*Allium sativum* L.) using targeted mtDNA-PCR and RAPD analysis. Biology 37: 51-54.

Hou Y.C., Z.H. Yan, Y.M., Wei, and Y.L. Zheng. 2005. Genetic diversity in barley from west China. Barley Genetics Newsletter 35: 9-22

Hussein, N.S., H.M. El-Saeid, and E.A. Omer. 1995. Development of growth and Yield of some lines of Chinese garlic . Egypt. J. Hort. 22(1) 19-23.

Ipek, M., A. Ipek and P.W. Simon. 2003. Comparison of AFLPs, RAPD markers, and isozymes for diversity assessment of garlic and detection of putative duplicates in germplasm collections. J. Amer. Soc. Hort. Sci. 128:246-252.

Kim, Y.K., and Seo, B.B. 1991. Karyotyping variation in callus and regenerated plant of *Allium sativum* L. Kor. J. Genet. 13: 147–155.

Kuhl, J.C., F. Cheung, Q. Yuan, W. Martin, Y. Zewdie, J. McCallum, A. Catanach, P. Rutherford, K.C. Sink, M. Jenderek, J. P. Prince, C.D. Town, and M.J. Havey. A Unique Set of 11,008 Onion Expressed Sequence Tags Reveals Expressed Sequence and Genomic Differences between the Monocot Orders Asparagales and Poales. The Plant Cell (16): 114-125.

Lee, H., E. Eom, Y. Lim, J. Bang, and D. Lee. 2003. Construction of a garlic BAC library and chromosomal assignment of BAC clones using the FISH technique. Genome 46: 514-520.

Levi, A. and L.J. Rowland. 1997. Identifying blueberry cultivars and evaluating their genetic relationships using randomly amplified

polymorphic DNA (RAPD) and simple sequence repeat (SSR) anchored primers. J. Amer. Soc. Horti. Sci. 122: 74-78.

Maas, H. I. and M. Klaas. 1995. Infraspecific differentiation of garlic (*Allium sativum* L.) by isozyme and RAPD markers. Theoretical Applications in Genetics 91: 89-97.

Nabulsi, I., B. Al-Safadi, N.M. Ali, and M.I.E. Arabi. 2001. Evaluation of some garlic (*Allium sativum* L.) mutants resistant to white rot disease by RAPD analysis Annals of Applied Biology 138 (2): 197–202.

Nagaoka T and Y Ogihara. 1997. Applicability of inter-simple sequence repeat polymorphism in wheat for use as DNA markers in comparison to RFLP and RAPD markers. Theor Appl Genet., 94: 597-602

Nagaraju, J., M. Kathirvel, R. Ramesh Kumar, E. A. Siddiq, and S. E. Hasnain. 2002. Genetic analysis of traditional and evolved Basmati and non-Basmati rice varieties by using fluorescence-based ISSR-PCR and SSR markers. PNAS. 99 (9): 58-36.

Omer, E. A. and A.F.Abou-Hadid. 1992. Evaluation of some lines of Chinese garlic comparing with balady cultivar Egypt. J. Hort. 19(2): 17-20.

Ovesna, J., L. Kucera, J. Kralova, L. Leisova, H. Stavelikova, and J. Velisek. 2007. Genetic diversity among garlic clones as revealed by AFLP, phenotypic descriptors and S-amino acids level. Vegetable Crops Research Bulletin 66: 105-116.

Rohlf, F. J. 2000. NTSYS-PC numerical taxonomy and multivariate system, version 2.1 Applied Biostatistics Inc., New York.

Sambrook, J., E.F. Fritsch, and T. Maniatis. 1989. Molecular cloning: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NJ.

Singh, Y. and R. Chand. 2003. Performance studies of some garlic (*Allium sativum* L.) clones. Himachal Journal of Agricultural Research 29 (1/2): 35-42.

Snedecor, G. W. and W. G. Cochran, (1980). Statistical methods. Sixth Edition, Iowa state university press, Ames., Iowa, U.S.A.

Wolf, K. and Q.Y. Xiang, and S.R. Kephart. 1998. Assessing hybridization in natural population of *Penstemon* (*Scrophulariaceae*) using hypervariable inter-simple sequence repeat (ISSR)bands. Molecular Ecology 7(9): 1107-1125.

Volk G.M., A.D. Henk, and C.M. Richards. 2004. Genetic Diversity among U.S. Garlic clones as detected using AFLP methods. J. Amer. Hort. Sci.129: 559-569.

الملخص العربي

الاختلافات في الصفات الإنتاجية وتقدير الاختلافات الوراثية بين التراكيب الوراثية للثوم باستخدام دلائل الحامض النووي الـ (DNA)

سليمان محمد العتيق * و محمد زكى الشناوي و محمد إبراهيم مطاوع***

*قسم الإنتاج النباتي ووقايته – كلية الزراعة والطب البيطري – جامعة القصيم – المملكة العربية السعودية.

** قسم البساتين – كلية الزراعة – جامعة عين شمس – مصر.

المستخلص. تم تقيم الإنتاجية صنف الثوم المصري والصيني وصنف الثوم الفيل وستة سلالات من الثوم الصيني في تجربتين حقليتين في الموسم الشتوي لعامي 2004- 2005 م و 2005 – 2006 م وذلك في مزرعة محطة البحوث والتجارب الزراعية بكلية الزراعة والطب البيطري - جامعة القصيم – المملكة العربية السعودية . أظهرت النتائج أن صنف الثوم المصري كان اعلي في طول النبات حيث كان طول النبات 70.5 و 70.8 سم في الموسمين على الترتيب وسجل صنف ثوم الفيل أعلي قيمة لمساحة الأوراق للنبات تبعه صنف الثوم الصيني السلالة 2 .وكان أيضا صنف ثوم الفيل متفوقا في الوزن الطازج للنبات عند مقارنته بباقي أصناف الثوم وكذلك السلالات بينما سجلت السلالة رقم 4 اعلي وزن جاف للنبات وكذلك في محتوي للكلوروفيل بأوراقه. وكان الصنف البلدي المصري اقلهم في وزن الرأس طازج أو جاف بمقارنته بالأصناف تحت الدراسة ومن ناحية أخري كان الصنف البلدي المصري اعلي الأصناف في عدد الفصوص بمقارنته بباقي الأصناف أو السلالات .وقد أعطى كلا الصنفين الفيل والصيني أعلى قيم متوسطات لقطر البصلة مقارنة بالصنف المصري. أيضا السلالة (2 ،5) التابعة للصنف الصيني أعطت أعلى قيم متوسطات لقطر البصلة مقارنة بالسلالات الأخرى. وقد لوحظ نفس الاتجاه في وزن الفص. وقد سجل الصنف المصري والصنف الصيني السلالة رقم 5 أعلى محتوي من المواد الصلبة الذائبة في الفص.وأعطي صنف الفيل والسلالة رقم 5 اعلي محصول قابل للتسويق (جرام/ م2). بينما الصنف المصري (البلدي) والصنف الصيني السلالة رقم 6 سجل أقل إنتاجية.

و استخدم نوعين من الدلائل الجزيئية هما RAPD and ISSR لتقدير الاختلافات الوراثية بين ستة سلالات ثوم و ثلاثة أصناف ثوم.و وجد نسبة كبيرة من الاختلافات بين أصناف و سلالات الثوم باستخدام كلا من RAPD و ISSR بينما أظهرت الدلائل الجزيئية ISSR اختلافات بين سلالات الثوم الصيني بنسبة اكبر من الدلائل الجزيئية RAPD. و أوضح التحليل التجميعي لنتائج RAPD انه تم تقسيم أصناف و سلالات الثوم إلي أربع مجموعات بينما قسمت إلي خمس مجموعات بتحليل نتائج ISSR .

و كانت سلالة الثوم الصيني رقم 6 منفصلة عن باقي السلالات كما أنها اقل السلالات في المحصول و نسبة المواد الصلبة الذائبة. وكانت الاختلافات الناتجة من الدلائل الجزيئية ISSR متوفرة في الثوم و أظهرت الاختلافات الوراثية بين سلالات و أصناف الثوم، و لذلك تعتبر هذه الطريقة إضافية لعمل البصمة الوراثية و تقدير العلاقات الوراثية في الثوم.