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Variation in Productive Characteristics and Diversity Assessment of Garlic Cultivars and Lines Using DNA Markers

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Abstract. Garlic cultivars Egyptian, Chinese, and Elephant, and sex Chinese lines were evaluated for their 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 value of leaves area per plant were observed with Elephant cultivar and Chinese line (L2). Elephant cultivar was superior on 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 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 compared with the other cultivars and Chinese lines. 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. Total soluble solid (TSS) content of cloves was highest in Egyptian cultivar (Balady) and Chinese line 5. Marketable yield (g/m^2) showed that Elephant cultivar and Chinese line 5 (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 sex 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 Chinese cultivar. Polymorphic ISSRs are abundant in garlic and demonstrated genetic diversity among related lines. Therefore, ISSR is 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 sativivum* L.) is a perennial plant whose bulb is 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 between strains. Through the development of a phylogenetic tree, diversity is established between 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 will 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 and 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; Wolf, *et al*., 1998; Nagaraju, *et al*., 2002 and Al-Humaid, *et al*., 2004).

The objectives of this study were to: (1) evaluate garlic cultivars and lines selected from Chinese cultivar for the 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

Field experiments were conducted at the Experimental Farm of the College of Agriculture and Veterinary Medicine, Al-Qassim University. Three garlic cultivars, namely Egyptian (Balady), Elephant, and Chinese, and six lines of Chinese garlic were planted in 13th and 8th October in 2004 and 2005 in the first and second seasons, respectively. Cloves of garlic cultivars and lines were planted at 7 cm spacing of both sides of ridges spaced 60 cm apart. The plants were fertilized at 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. Data in Table 1 shows specific soil characteristics. The layout of the experiments was completely randomized design with four replicates.

Table 1. The soil mechanical analysis of experimental site.

Mechanical analysis			Bulk density g /cm3	Water holding capacity (%)	Field capacity (%)	Wilting point (%)
Sand	Silt	Clay				
96.3 %	1.8 %	1.9 %	1.501	17.17	9.6	4.35

Measurements

Four plants were randomly chosen for recording the vegetative growth parameters which included, plant height (cm), number of leaves per plant, leaves area /plant (cm^2), plant fresh and dry weight. 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 weight (g), clove fresh and dry weight (g), bulb diameter (cm), number of cloves, and total soluble solid (T.S.S) (%). Also, marketable yield 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 2 hours at 65 ºC with occasional mixing. Following incubation, 5 ml of chloroform/isoamylalcohol (24:1) were 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.

Table 2. Plant height, number of leaves and leaf area of garlic cultivars and lines during 2004/05 and 2005/06 seasons.

Cultivars	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

-Data are expressed as mean

-Means within the same column and followed by the same coefficient are not significant different from each other ($p \leq 0.05$).

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 $MgCl_2$) 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 added, and 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.

Table 3. 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

-Data are expressed as mean

-Means within the same column and followed by the same coefficient are not significant different from each other ($p \leq 0.05$).

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 analyzed separately. The least significant differences (LSD) test was used to compare means at the 5% level. Only differences significant at $P \leq 0.05$ are 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 “1”, if absent it was designated “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 2). 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 maximum 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 3). Also, the last line and Egyptian cultivar had the highest chlorophyll content of the tested cultivars and lines in both seasons (Table 3).

For bulb fresh weight, it is apparent in Table 4 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.

Table 4. 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

-Data are expressed as mean

-Means within the same column and followed by the same coefficient are not significant different from each other ($p \leq 0.05$).

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 5 illustrated 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.

Table 5. 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	1.4 f	3.3 d	1.4 d
Chinese garlic	6.2 b	5.9 b	6.2 c	2.7 c	5.4 cd	2.9 b
Line 2 (L2)	6.7 b	5.9 b	7.7 b	4.0 b	7.8 b	4.5 a
Line 3 (L3)	5.5 c	5.3 d	4.9 c	2.2 d	5.0 d	2.0 c
Line 4 (L4)	5.1 d	5.9 b	5.9 c	2.2 d	5.9 c	2.3 c
Line 5 (L5)	6.0 b	6.0 b	5.5 c	1.9 e	5.3 cd	2.3 c
Line 6 (L6)	5.1 d	5.1 e	2.9 d	1.2 f	3.4 d	1.2 d
Egyptian garlic	5.1 d	5.1 e	1.0 e	0.4 g	1.1 e	0.4 e
Elephant garlic	7.4 a	7.2 a	19.3 a	5.2 a	24.0 a	4.5 a

-Data are expressed as mean

-Means within the same column and followed by the same coefficient are not significant different from each other ($p \leq 0.05$).

Total soluble solid (TSS) content of cloves was highest in Egyptian cultivar (Balady) and Chinese line 5 (L5) (37.0 and 38.6 %) and (38.8 and 38.4 %) in both seasons respectively (Table 6). On the other hand, Elephant cultivar and Chinese line 6 (L6) gave the lowest total soluble solid content. Singh and Chand (2003) found that total soluble solid content of cloves was significantly differed among garlic cultivars and clones. Marketable yield (g/m^2) showed that Elephant cultivar and Chinese line 5 (L5) resulted in the highest yield (2352.1 and 2372.5 g/m^2) and (1373.2 and 1352.2 g/m^2) in both seasons, respectively (Table 6). While, Egyptian cultivar (Balady) and Chinese line 6 (L6) produced the lowest yield (729.8 and 723.2 g/m^2) and (743.3 and 714.9 g/m^2) in both seasons, respectively. Similar results were reported by Hussein, *et al*. (1995) who found that Balady cultivar produced the lowest garlic yield.

Table 6. T.S.S and marketable yield of garlic cultivars and lines during 2004/05 and 2005/06 seasons.

Cultivars & Lines	Cloves T.S.S %		Marketable yield g /m2	
	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

-Data are expressed as mean

-Means within the same column and followed by the same coefficient are not significant different from each other ($p \leq 0.05$).

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 7).

Table 7. RAPD primers with the number of amplified products, polymorphic fragments among garlic cultivars and lines, and polymorphic fragments among Chinese lines.

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

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 Fig.1.

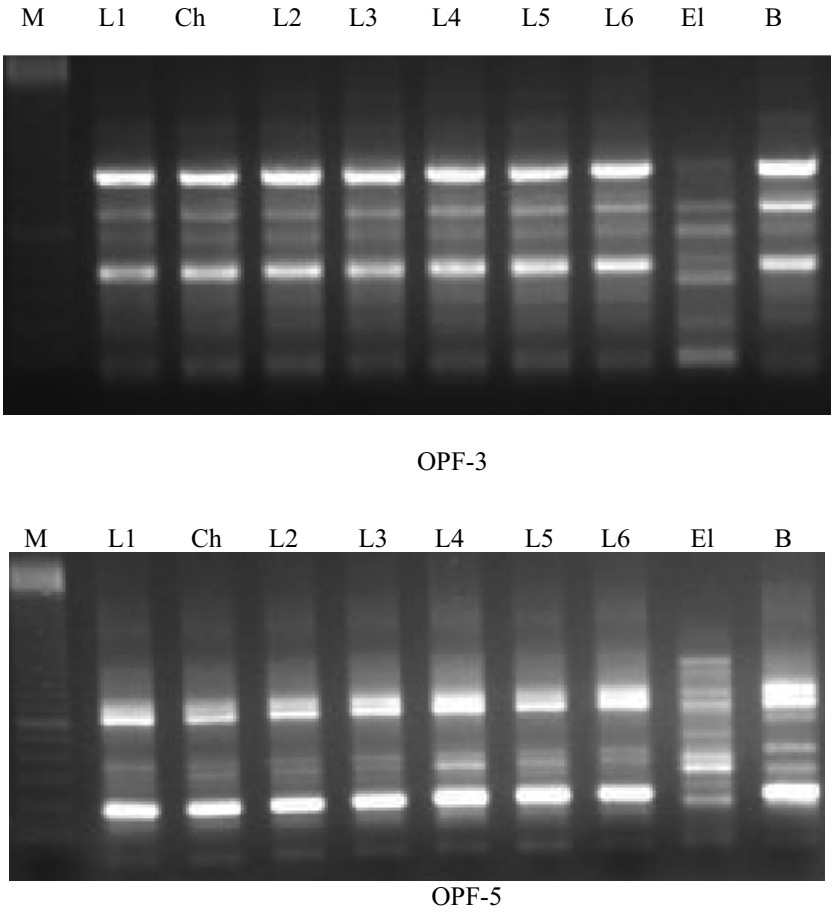

Fig. 1. Polymorphism revealed using primer OPF-3 and OPF-5 to amplify genomic DNA purified from the tested garlic cultivars and lines. M lane is 1 kbp ladder DNA marker.

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.

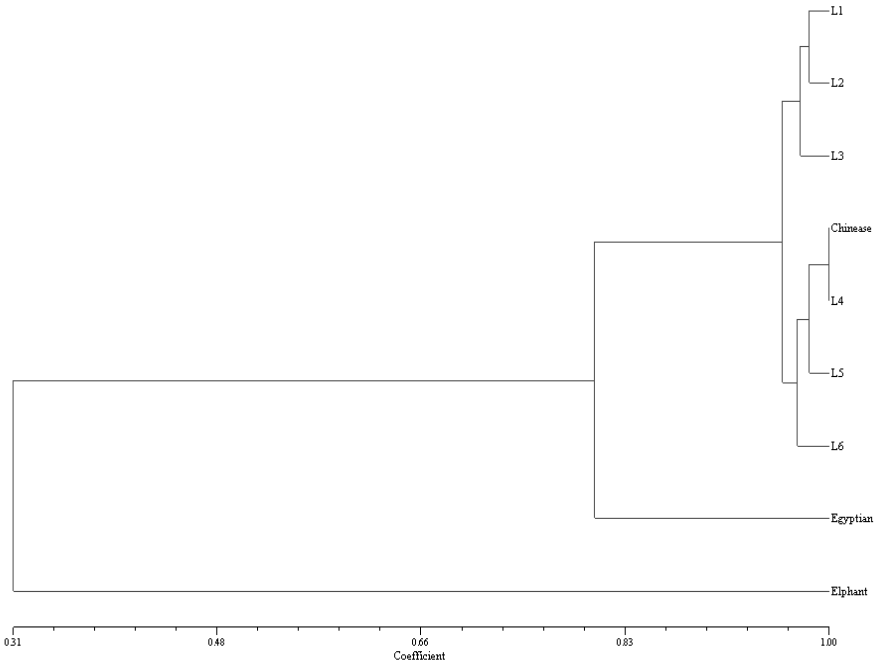

Fig. 2. Dendrogram constructed from similarity coefficients and showing the clustering of the tested garlic cultivars and lines using RAPD markers.

In ISSR analysis, the number of amplification bands per primer varied between 0 and 10. Examples of polymorphism are shown in Fig. 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 8).

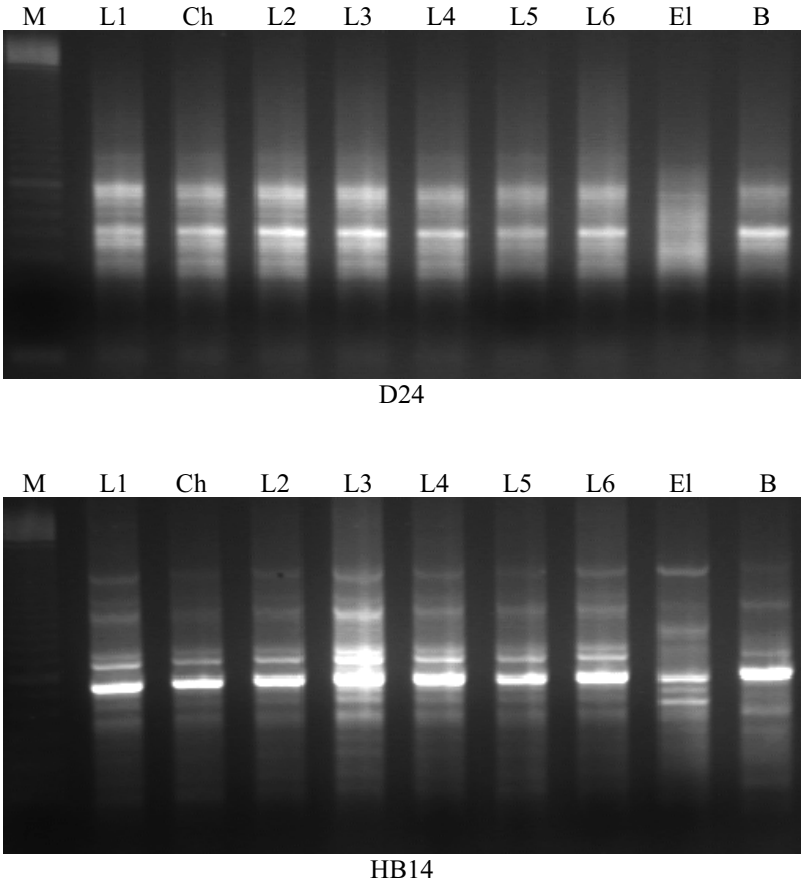

Fig. 3. Polymorphism revealed using primer D24 and HB14 to amplify genomic DNA purified from the tested garlic cultivars and lines. M lane is 1 kbp ladder DNA marker.

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. Mondal, *et al*. (2008) found that a total of 17 selected RAPD and 21 ISSR primers produced 119 and 153 bands respectively, of which 56 and 114 were polymorphic correspondingly. Of the two markers, ISSR revealed higher polymorphism (74.5%) than RAPD (47.1%) in peanut genotypes.

Table 8. ISSR primers with the number of amplified products and polymorphic fragments among garlic cultivars and lines, and polymorphic fragments among Chinese lines.

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

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)

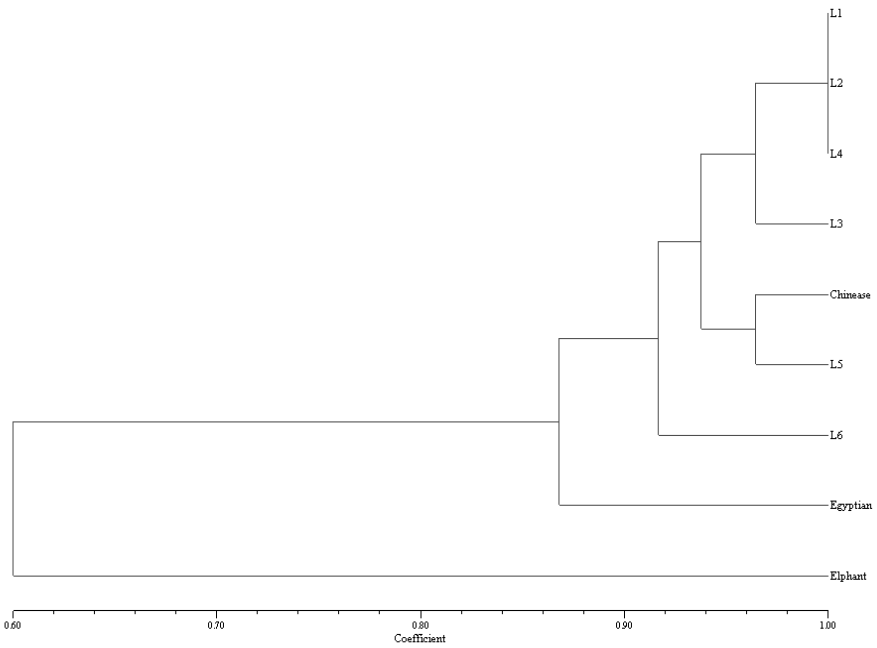

Fig. 4. Dendrogram constructed from similarity coefficients and showing the clustering of the tested garlic cultivars and lines using ISSR markers.

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.

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الاختلافات في الصفات الإنتاجية، وتقدير الاختلافات الوراثية بين أصناف وسلالات الثوم، باستخدام دلائل الحامض النووي (الـ DNA)

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المستخلص. تم تقييم الإنتاجية لصنف الثوم المصري، والصيني، وصنف الثوم الفيل، وستة سلالات من الثوم الصيني، في تجربتين حقليتين في الموسم الشتوي لعامي ٢٠٠٤/ ٢٠٠٥ م و ٢٠٠٥ / ٢٠٠٦م، وذلك في مزرعة محطة البحوث والتجارب الزراعية بكلية الزراعة والطب البيطري – جامعة القصيم – المملكة العربية السعودية. أظهرت النتائج أن صنف الثوم المصري كان أعلى في طول النبات، حيث كان طول النبات ٧٠,٥ و ٧٠,٨ سم في الموسمين على الترتيب، وسجل صنف ثوم الفيل أعلى قيمة لمساحة الأوراق للنبات، تبعه صنف الثوم الصيني السلالة ٢. وكان أيضا صنف ثوم الفيل متفوقا في الوزن الطازج للنبات، عند مقارنته بباقي أصناف الثوم، وكذلك السلالات، بينما سجلت السلالة رقم ٤ أعلى وزن جاف للنبات، وكذلك في محتوى الكلوروفيل بأوراقه. وكان الصنف البلدي المصري أقلهم في وزن الرأس طازجاً، أو جافاً، بمقارنته بالأصناف تحت الدراسة، ومن ناحية أخرى كان الصنف البلدي المصري أعلى الأصناف في عدد الفصوص،

بمقارنته بباقي الأصناف أو السلالات. وقد أعطى كـــلا الـــصنفان الفيل والصيني، أعلى قيم متوسطات لقطر البصلة، مقارنة بالصنف المصري. أيضا السلالة (٢ ،٥) التابعة للصنف الصيني، أعطـــت أعلى قيم متوسطات لقطر البصلة، مقارنة بالسلالات الأخرى. وقد لوحظ نفس الاتجاه في وزن الفص. وقد سجل الصنف المـــصري والصنف الصيني السلالة رقم ٥، أعلى محتـــوى مـــن المـــواد الصلبة الذائبة في الفص. وأعطى صنف الفيل والـــسلالة رقـــم ٥، أعلى محصول قابل للتسويق (جرام/ م^{٢})، بينما الصنف المـــصري (البلدي) والصنف الصيني السلالة رقم ٦ سجل أقل إنتاجية.

واستخدم نوعان من الدلائل الجزيئية همـــا RAPD وISSR لتقدير الاختلافات الوراثية بين ستة سلالات ثوم، وثلاثة أصـــناف ثوم. ووجدت نسبة كبيرة من الاختلافات بين أصـــناف وســـلالات الثوم، باستخدام كل من RAPD و ISSR، بينما أظهرت الدلائل الجزيئية ISSR اختلافات بين سلالات الثوم الصيني بنسبة أكبـــر من الدلائل الجزيئية RAPD. وأوضح التحليل التجميعـــي لنتـــائج RAPD أنه تم تقسيم أصناف وسلالات الثوم إلى أربع مجموعات، بينما قسمت إلى خمس مجموعات بتحليل نتائج ISSR . وكانـــت سلالة الثوم الصيني رقم ٦ منفصلة عن باقي السلالات، كما أنهـــا أقل السلالات في المحصول ونسبة المواد الصلبة الذائبة. وكانـــت الاختلافات الناتجة من الدلائل الجزيئية ISSR متوفرة في الثـــوم، وأظهرت الاختلافات الوراثية بين سلالات و أصناف الثوم، ولذلك تعتبر هذه الطريقة إضافية لعمل البصمة الوراثية وتقدير العلاقـــات الوراثية في الثوم.