Genotyping Analysis of Garlic Cultivars Grown in Egypt Using Simple Sequence Repeat (SSR) Markers with Post-Labeling Method

Haitham E. M. Zaki

Horticulture Department, Faculty of Agriculture, Minia University, El-Minia 61517, Egypt
haitham.zaki@mu.edu.eg

Abstract: The main objective of this study is to compare the productive performance and genetic relationships among three garlic genotypes which are common in Egyptian market. Cultivars with fewer cloves gave higher yields. Eggaseed-1 cv. and Sids-40 cv. produced between 9.7-11.9 ton/fed (fresh yield) and Egyptian cv. gave between 8.8-9.1 ton/fed (fresh yield) in both seasons of experiment. Simple sequence repeat (SSR) markers with post-labeling method have been used to reveal the genetic diversity among garlic cultivars. Ten SSR markers which showed high polymorphic content were tested for genetic characterization in 18 garlic selections. A total of 61 DNA fragments were amplified by the 10 markers with an average of about 6.1 fragments per marker. The genetic distances for SSR data using 18 garlic selections were done and relationships among selections were portrayed graphically in the form of a dendrogram. The value of genetic similarity ranging from 65.77 to 91.54% was observed among the garlic cultivars. The highest genetic similarity of 91.54% was among Eggaseed-1 and Sids-40 selections while, the highest dissimilarity was seen between Egyptian and Eggaseed-1 selections. These results allow identifying highly related garlic colored garlic; Eggaseed-1 cv. and Sids-40 cv. and separate them from Egyptian cv. that is characterized as white garlic. From the current study, it may be concluded that SSR markers with post-labeling method are efficient tool for investigation of genetic relationship in garlic therefore, this can help for selection in the breeding program.
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1. Introduction

Garlic (*Allium sativum* L.) belongs to the family of *Alliaceae* and genus *Allium*, which contains more than 600 species **(Osman *et al.*, 2007)**. It was pointed out that garlic was grown and consumed between 2780 and 2100BC, during the building of the pyramids in Egypt **(Yamaguchi, 1983)**. Commercial cultivars of garlic are apomyctic diploid ($2n=2x=16$), sterile, and obligate clonally propagated. Although male fertility in wild garlic has been observed in nature and genetic linkage maps from S1 (family of plants produced by self-pollination) are already available **(Ipek *et al.*, 2005; Zewdie *et al.*, 2005)**, the selection of spontaneous or induced mutations still plays an important role in garlic breeding. Breeding programs depend on genetic variability and identification of traits of interest, which require a rigorous characterization of accessions within germplasm collections.

A wide range of morphological variations has been observed in garlic; including flowering ability and traits, leaf traits, bulb traits, bulbing response to environment and plant maturity **(Senula and Keller, 2000)**. Common cultivars in Egypt have been preferably introduced from China; other genotypes have been selected through cultivation. This is the reason why there are many phenotypes that differ in number, weight, color and size of cloves and bulbs. Meanwhile, many studies have been conducted at the Fac. of Agriculture, Minia Univ., Egypt to study the relationship among these cultivars. **Osman *et al.*, 2007** have studied the cytological features of fourteen garlic genotypes; Eggaseed-1, Sids-40, Eggaseed-2, Egyptian and ten clonal selections. Chromosomal studies showed that all the numbering and adjustment of the homologous chromosome pairs were very difficult for many reasons; 1) high percentage of large fragment; 2) determining the centromere position is not easy; 3) great variations in satellite number and size among the genotypes. **Gadel-Hak *et al.*, 2010** have tried to find out the suitable way of selection. Meanwhile, two methods of selections were applied; whole bulb was in the first population and single clove was in the second population. Both populations exhibited considerable variations among their plants. The means, range, variances, standard deviation and coefficients of variability showed wide range in both the original and selection populations in bulb and neck diameter and fresh weight. **Abdel-Ghany, 2011** has screened some garlic cultivars by using random amplified polymorphic DNA (RAPD) and according to the morphological characteristics of the cultivars. The cluster analysis illustrated contrast results based on the morphological and RAPD markers.

Consequently, it was important to study the relationship of the garlic cultivars with more efficient method.

DNA-based molecular markers have been used previously in studies of genetic diversity and phylogenetic analysis in plants **(Savolainen and Chase, 2003 and Nybom, 2004)**. Among the different molecular markers, simple sequence repeat (SSRs) or microsatellites are the marker of choice for a broad number of genetic studies because of their high polymorphism, genomic abundance, and facility in lab usage **(Ma *et al.*, 2009 and Zaki *et al.*, 2010)**. Various types of DNA markers including simple sequence repeat (SSR), have been developed and used in a wide variety of genotyping studies with a fluorescent capillary DNA sequencer **(Nguyen and Xiaolei 2005)**. The post-labeling method using fluorescently labeled short oligonucleotides and nested PCR of the amplified product obtained from unlabeled primer pairs is a simple and inexpensive alternative. Post-labeling was useful for genotyping with SSR markers, with minimal modification of the PCR program used for prelabeled primers **(Shimizu and Yano, 2011)**.

Therefore, in order to find out a suitable and an efficient way to differentiate between garlic genotypes which is very important for selection in the breeding program, three common garlic cultivars in Egypt were analyzed to assess their productive performance and genetic relationship using SSR markers with post-labeling technique.

2. Materials and Methods

Plant materials

Three garlic cultivars used in this study were local Egyptian, Eggaseed-1 and Sids-40 cultivars (Fig.1). The three cultivars were recognized as the most common commercial garlic cultivars in Egypt. Egyptian cv. which is Egyptian landrace was received from Horticulture Department, Fac. of Agric., Minia Univ., El-Minia, Egypt. It is a local cultivar grown in Egypt for its strong aroma which the mature cloves have white covering scale with relatively long storability (Fig.1A). While, Eggaseed1 cv. which was developed by **Metwally and El-Denary, 2003** was received from Egyptian Agriculture Company for Seed Production (EGAS). This cultivar has big cloves size and its mature cloves have colored skin (Fig.1B). Sids-40 cv. was obtained from Sids Research Station, Agric. Res. Center, Giza, Egypt. The main source of this cultivar is China, it has big cloves size, easy peer and its mature cloves have white skin with purple vertical stripes (Fig.1C).

Field evaluation

Garlic cultivars were planted in field during two successive winter seasons of 2010/2011 and 2011/2012 at the farm of Horticulture Dep., Fac. of Agric., Minia Univ., El-Minia, Egypt, to study the vegetative growth, plant yield and its components of each cultivar. Soil composition of the experimental field was clay loam. Soil chemical analysis was applied using the method described by **Page *et al.* (1982)**. Results of pH, organic matter % and available inorganic N, P and K were 8.12, 1.09 %, 45.13, 11.25 and 81.15 ppm.

For the two seasons of field experiment, uniform and healthy cloves of the cultivars were sown in the season time, namely on Oct. 20 of 2010 and Oct. 15 of 2011. Cultivars were arranged in a simple experiment with three replicates. Each experimental unit (plot) was 3 x 3.5m. Prior to planting, garlic bulbs were split into the individual cloves. Cloves of all cultivars were planted upright with apical tip exposed at 10 cm inter row spacing. After sowing directly and before irrigation, weeds were controlled by using pre-emergence of herbicide. All other agricultural practices were performed as recommended for the commercial production according to the Egyptian Ministry of Agriculture for garlic production, 1999. Plants of each plot were harvested when older leaves turned yellowish green and started withering. The harvested bulbs were spread in single layers under room temperature conditions (in Twenty-one day period of time) for curing process.

Data recorded

At harvesting time data of plant height, number of leaves, fresh weight of whole plant, bulb fresh weight, bulb dry weight (dried at 70^{o}C), number of cloves/bulb, single clove weight and fresh total yield were recorded. Bulbing ratio (neck diameter/bulb diameter) was measured as described by **Mann, 1952**. Twenty-one day after harvesting, cured bulb diameter, cured bulb weight, and total cured yield of bulbs were estimated.

Statistical analysis

Data obtained in the two seasons of study was subjected to analysis using MSTATC software version 4 (1996). Duncan mean separation test at 0.05 level was done to compare means of the collected data **(Duncan, 1995)**.

Lab experiment

This experiment was conducted at the Laboratory of Plant Breeding, Fac. of Agric., Iwate Univ., Morioka, Iwate, Japan during the period of November, 26th 2013 to May, 25th 2014.

DNA polymorphism analysis

The plant materials used for the study of the genetic relationship were comprised from 18 garlic individual plants selected from the three cultivars; Egyptian, Egaseed-1 and Sids-40. Total DNA was extracted from young leaves of the selections grown in a greenhouse using GenElute TM Plant Genomic DNA Miniprep kit (Sigma, Saint Louis, USA)

according to the manufacturer's instructions. The relative purity and concentration of extracted DNA was estimated with NanoDrop ND-1000. The final DNA concentration was adjusted to 20 ng/µL.

Figure 1. Bulb structure, shape and size of three common garlic cultivars in Egyptian market, Egyptian cv. **(A)**, Eggassed-1 cv. **(B)** and Sids-40 cv. **(C)**.

SSR markers were used for the polymorphism analysis, a total of 10 SSR markers were screened for genotyping analysis **(Cunha *et al.*, 2012)**. Post-labeling method for multicolored genotyping analysis of SSR has been used as described by **(Shimizu and Yano, 2011)**. The name, sequences, annealing temperature of each primer and labeling tags are listed in Table 1. To amplify the microsatellite loci, the PCR was performed in a 20 µl containing 20 ng of genomic DNA, 1 µl of each primer (10 mM), 2 µl of dNTPs (10 mM), 1 µl of 10 x reaction buffer (Takara, Japan), and 1 unit of *Taq* polymerase (Takara, Japan). The polymorphism of the markers was checked using program of the thermal cycling as described in **Ma *et al.* (2009)**, consisting of an initial denaturing step at 94° C for 3min; followed by 30 cycles at 94° C for 30s, at the specific annealing temperature of each pair of primers (Table 1) for 45s, and at 72° C for 1min; 10 cycles at 94° C for 30s, at 2° C below the specific annealing temperature of each pair of primers for 45s, and at 72° C for 1min; and a final elongation step at 72° C for 10 min.

Scoring and analysis of SSRs polymorphism

Amplification products were confirmed by a fluorescent capillary DNA sequencer with fragment analysis. Fragment data was entered on a spreadsheet to form a binary matrix, where (1) represented fragment presence and (0) fragment absence for each fragment-selection combination. Cluster analysis was conducted by converting the data matrix into a similarity matrix using a simple matching coefficient. This coefficient was calculated by dividing the number of the match (0-0 and 1-1) by the total number of comparison **(Nei and Li, 1979)**. A cluster analysis was then conducted using unweighted pair group method, with arithmetical average (UPGMA) process using the S-Professional Plus 2000 program.

3. Results and Discussion

Field evaluation

The morphological results indicated that the three garlic cultivars reflected significant differences with respect to plant height, fresh weight of whole plant, bulb fresh weight and bulb dry weight percentage in both seasons (Fig. 2). Whereas, Egyptian cv. recorded the longest plants followed by Eggaseed-1cv. and Sids-40 cv. (Fig. 2A), however, Eggaseed-1 and Sids-40 plants showed the heaviest fresh weight of whole plant, bulb fresh weight and bulb dry weight percentage. The highest values of these traits were obtained from growing Eggaseed-1 plants as shown in Fig. 2B, C and D, respectively. This data was in accordance with **Al-Otayk *et al.* (2008)** who found that bulb fresh weight of Egyptian cultivar was the lowest among the tested cultivars and clones of both Elephant and Chinese types tested. These results might be expected based on the genetic background of each cultivar and the variations between the genotypes. These results were also in agreement with those reported by **Omer and Abou-Hadid (1992)**. On the other hand, Eggaseed-1 and Sids-40 cultivars gave significantly higher number of leaves compared with Egyptian cv. however; no significant differences were noticed between Eggaseed-1 and Sids-40 for this trait. Results are true in both seasons (Fig. 2E).

Growing temperature, day length and solar reaction differ from season to season. Thus some traits remain consistent and some others may change **(Waterer and Schmitz, 1994)**. Bulbing ratio character recorded significant differences between Eggaseed-1 and Sids-40 cultivars and Egyptian one in the first season but not in the second season (Fig. 2F). In general, Egyptian cv. gave the lower bulbing ratio than Sids-40 and Eggaseed-1. This result indicated that Egyptian cv. requires more time for bulb maturation compared with the other cultivars. The photoperiod, temperature and light are the factors which control bulbing ratio in garlic **(Brewester, 2008)**.

One of the challenges of garlic production for exportation in Upper Egypt especially, is to reach the maturation stage within relatively short season. For that, garlic farmers and producers seek the fast shifting from vegetative to bulbing phase in a suitable time to

assure enough time to develop larger bulbs. Genetic background of the genotypes controls nutrients shifting to develop bulbs.

Table 1. Characteristics of 10 SSR markers; forward and reverse sequence, labeling tags, annealing temperature T_a (°C) and no. of alleles (size-bp). All values are based on the screening of 18 garlic individual plants selected from the three cultivars; Egyptian, Eggaseed-1and Sids-40.

Loci	Primer sequence	Tag	T_a (°C)	No. of alleles (size-bp)		
				Egyptian	Eggaseed-1	Sids-40
Asa07	F: CTCGGAACCAACCAGCATA	6-FAM	58	2(244, 253)	3(244, 246, 253)	2(246,253)
	R: CCCAAACAAGGTAGGTCAGC					
Asa08	F: TGATTGAAACGAATCCCACA	NED	56	2(228, 230)	2(224,226)	2(222, 232)
	R: GGGGGTTACCTGAACCTGTTA					
Asa17	F: TCCACGACACACACACACAC	PET	56	5(149,151,153,155,157)	1(145)	5(149,151,153,155,157)
	R: ATGCAGAGAATTTGGCATCC					
Asa18	F: TCAAGCTCCTCCAAGTGTCC	VIC	45	3(210,216,221)	1(210)	3(210,216,221)
	R: TCGGGATATGACAGCATTTG					
Asa25	F: GCACTTCACTTTCCCCATTC	6-FAM	51	2(174,175)	2(174,175)	2(174,175)
	R: GGCGACGGTGAAGAGAGAG					
Asa31	F: CAGAGACTAGGGCGAATGG	NED	50	2(157-160)	2(154,163)	12(146,148,150,152,154,155, 157, 159,160,162,163,164)
	R: ATGATGATGACGACGACGAG					
Asa10	F: TTGTTGTTCTGCCATTTT	6-FAM	48	1(244)	1(240)	2(238,248)
	R: GATCTAAGCCGAGAGAAA					
Asa14	F: TCTATCTCGCTTCTCAGGGG	NED	48	2(235,236, 239)	2(222,240,244,247)	1(237)
	R: GCTGACAGAAGTAGTCTTTCC					
Asa16	F: CACGACTTTTCCTCCCATTT	PET	48	2(175,178)	4(171,178,179,189)	2(178,189)
	R: GCTAATGTTCATGTCCCCAGT					
Asa24	F: TTGTTGTGCCGAGTTCCATA	VIC	48	7(163, 164,168-172)	8(163,164, 169-172,176,177)	8(163,164,166,168,170-173)
	R: CAGCAATTTACCAAAGCCAAG					

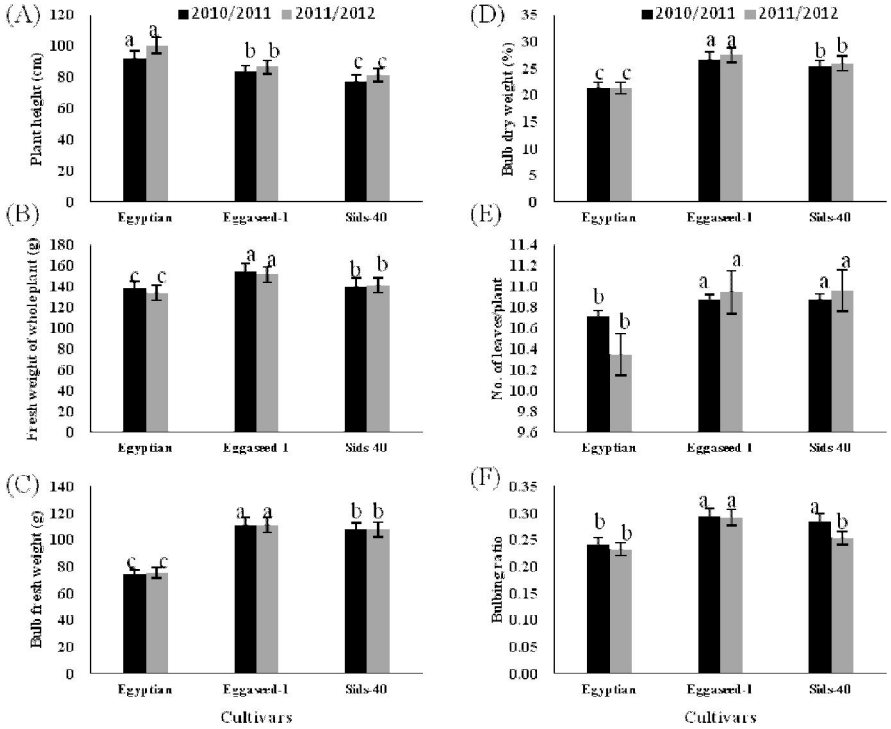

Figure 2. Plant height **(A)**, fresh weight of whole plant **(B)**, bulb fresh weight **(C)**, bulb dry weight percentage **(D)**, number of leaves/plant **(E)** and bulbing ratio **(F)** of Egyptian cv., Eggassed-1 cv. and Sids-40 cv. during two seasons of 2010/2011 and 2011/2012, respectively. Means within each season followed by the same letter are not statistically significant at 0.05 level (Duncan`s range test).

The analysis of the clove number per bulb, clove weight, fresh total yield, cured bulb diameter, cured bulb weight and cured yield indicated that the three garlic cultivars reflected significant differences of these traits in both seasons (Fig.3). Plants showing a smaller number of bulb cloves appeared to have greater clove weight (Fig. 1 and Fig. 3A). Egyptian cv. produced more cloves number per bulb (40.937 cloves / bulb) compared with Eggaseed-1 and Sids-40 plants. On the other hand, Eggaseed-1 cv. and Sids-40 cv. gave the highest mean value of clove weight over than Egyptian one (Fig. 3B). Similar

results were obtained by **Al-Otayk *et al.* (2008)** and **Zaki *et al.* (2014)** who found that Egyptian cv. produced the highest number of cloves followed by Sids-40 one.

Total fresh yield significantly differed among the three cultivars (Fig. 3C). The fresh yield of Egyptian cv. was lower than the fresh yield of both Eggaseed-1 and Sids-40 and ranged from 8.827 to 9.103 ton/fed. Eggaseed-1 cv. gave the highest fresh yield which ranged from 11.601 to 11.867 ton fed. These results also are in agreement with those of **Noorbakhshian *et al.* (2008)**, who evaluated some agronomic traits that related to yield and its components for several garlic cultivars and reported that clove weight had the maximum positive effect on the yield parameter.

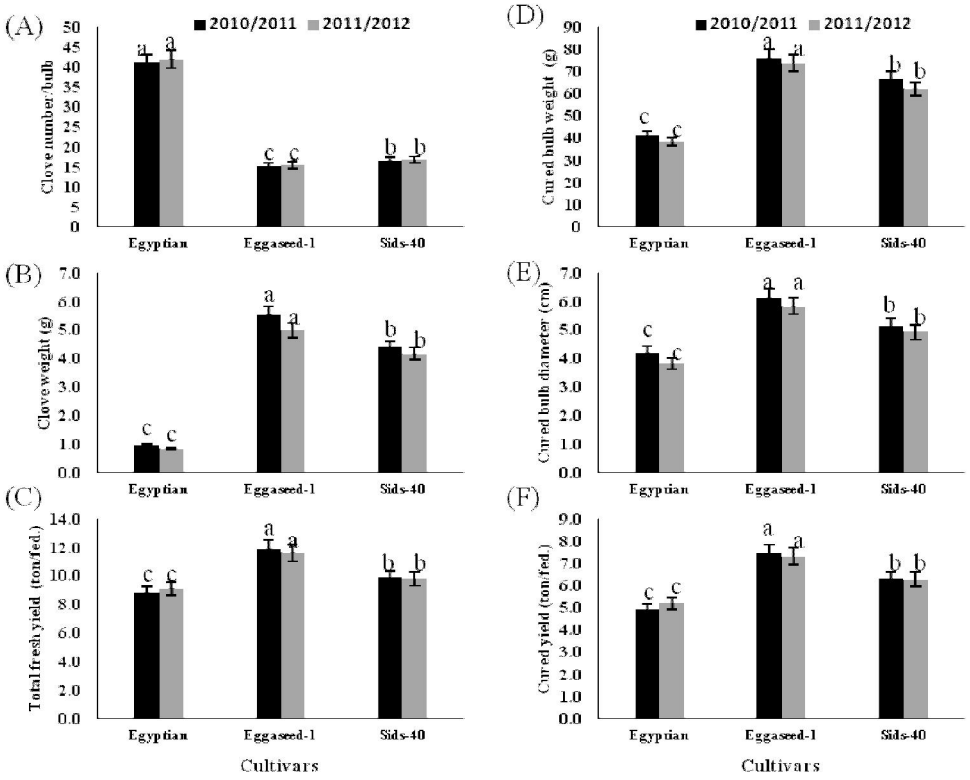

Figure 3. Clove number/plant **(A)**, clove weight **(B)**, total fresh yield **(C)**, cured bulb weight **(D)**, cured bulb diameter **(E)** and cured yield **(F)** of Egyptian cv., Eggassed-1 cv. and Sids-40 cv. during two seasons of 2010/2011 and 2011/2012, respectively. Means within each season followed by the same letter are not statistically significant at 0.05 level (Duncan`s range test).

Regarding the cured bulb weight, cured bulb diameter and cured weight Eggaseed-1 significantly surpassed those of cultivar Sids-40 cv. and Egyptian cv. in both seasons, respectively. The obtained results confirmed that there is a difficulty in determining whether genetic and/or environment has a key role in the growth behavior of garlic cultivars. These variations among cultivars in later characters could be referring to the genetical divergence which led to differences in tissues of garlic bulbs.

Lab experiment

Genetic markers are efficient tools for genetic analysis of populations and selections. According to this concept, molecular characterization of eighteen individuals selected from three common garlic cultivars in Egypt; Egyptian, Eggaseed-1 and Sids-40 was performed through simple sequence repeats (SSR) markers with post-labeling method. Twenty SSR primer pairs showing distinct polymorphic fingerprint were selected to reveal genetic variation among the garlic genotypes (Table 1). SSR markers with post-labeling method were used to detect polymorphism in three garlic cultivars with six selections each. The PCR products were observed on fragments analysis to study polymorphism, most of primers were polymorphic except one primer which was monomorphic (Fig. 4). All primers were found to give reproducible bands. A total of 61 DNA fragments were generated by the 10 markers with an average of about 6.1 bands per primer. In all selections, it was possible to identify from 1 to 12 bands. In this study the primers used were of the size ranging from 145-247bp (Table 1). Reactions were duplicated form to check the consistency of the amplified products.

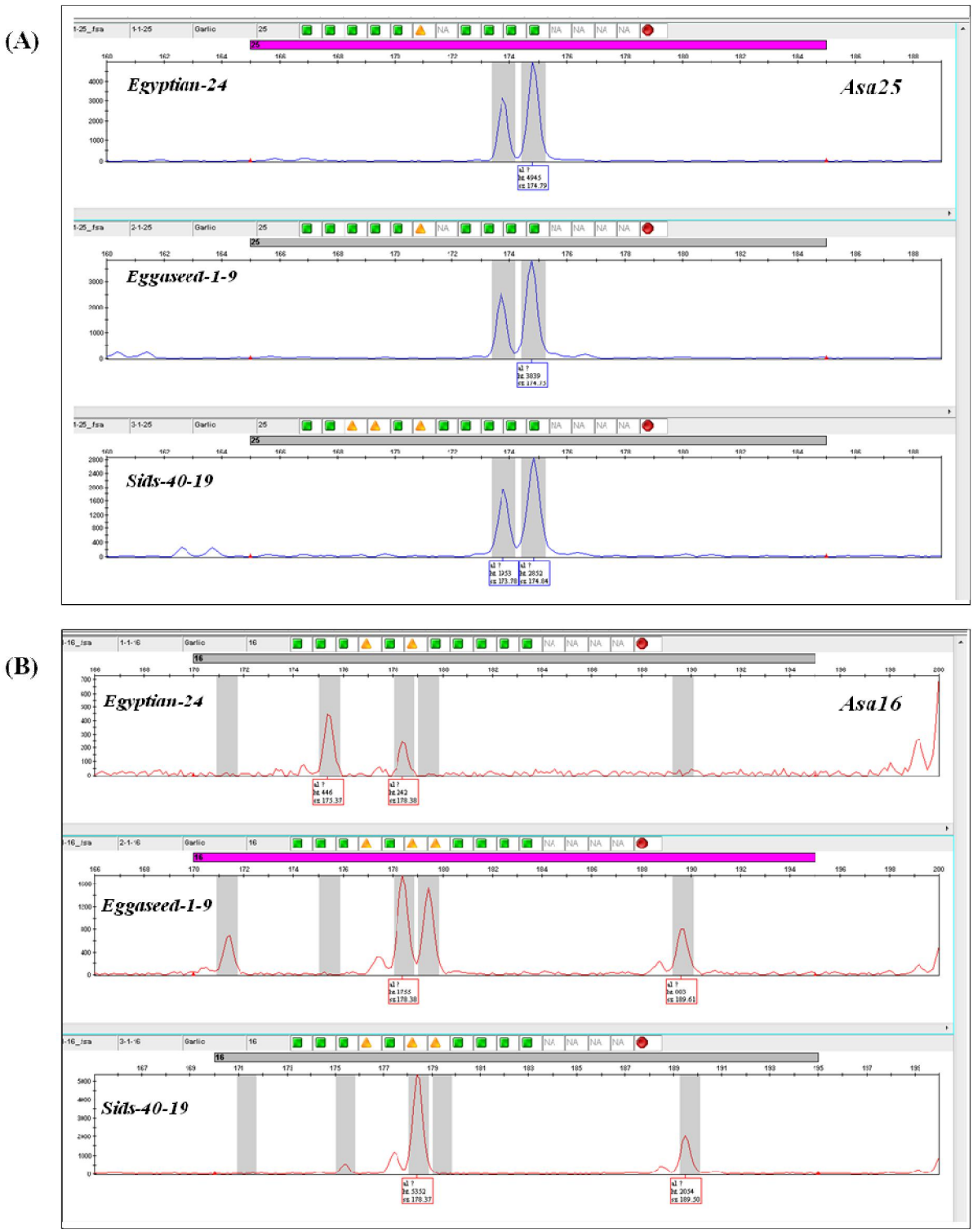

Figure 4. Electropherogram for SSR marker analysis with post-labeling method for the three garlic cultivars; Egyptian, Eggaseed-1 and Sids-40. Electropherograms of SSR marker Asa25 **(A)** and SSR marker Asa16 **(B)** as examples of monomorphic and polymorphic markers obtained using a post-PCR labeling with 6-FAM and PET, respectively.

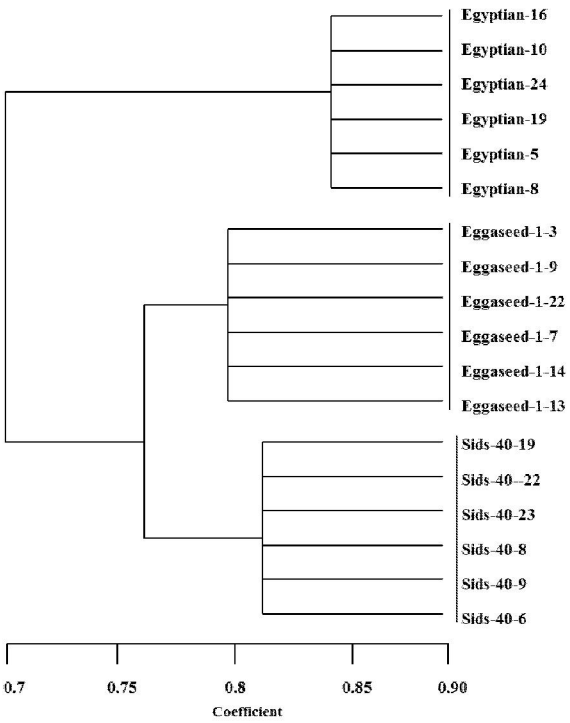

Figure 5. Dendrogram obtained by SSR markers with post-labeling technique and general description according to morphological and genetic diversity in garlic cultivars grown in Egypt. Eighteen garlic individual plants selected from the three cultivars; Egyptian cv. (Egyptian-16, 10, 24, 19, 5 and 8), Eggaseed-1 cv. (Eggaseed-1-3, 9, 22, 7, 14 and 13) and Sids-40 cv. (Sids-40-19, 22, 23, 8, 9 and 6) were used for analysis.

About 77.049% polymorphism was estimated, as 47 out of 61 fragments were polymorphic with 10 markers used among the 18 garlic selections and the rest of 14 bands were monomorphic. In the current experiment, the 18 garlic selections appeared to show variability with the SSR markers used. Many of the primers individually were as informative as to differentiate all the selections; highly polymorphic content (PIC value) were obtained with of the marker Asa31 (Table 1). Only one primer pairs; Asa17 was found to be monomorphic. The level of polymorphism indicates that distinction between any two varieties is possible with appropriate SSR primer pair. Therefore, it may be concluded from the present results can used for identification of genetic diversity and the relationship between the members of the complex genotypes such as garlic. **Cunha *et al.* (2012)** studied diversity in 75 *Allium sativum* accessions with these 10 markers and reported high level of heterozygosity.

The genetic distance for SSR data using 18 garlic selections, was constructed based on **Nei (1978)** and diversity or relationship were portrayed graphically in the form of dendrogram as shown in Figure 5. The value of genetic similarity ranging from 65.77 to 91.54% was observed among the garlic cultivars. The lowest dissimilarity corresponded to the selections within the cultivars. The highest dissimilarity was between Egyptian and Eggaseed-1 selections.

A dendrogram was generated from the similarity values (Fig. 5), and two groups were identified. The first group was formed by Egyptian cultivar selections (Egyptian-16, 10, 24, 19, 5 and 8) which characterized morphologically by a lower production (smaller clove weight and/or greater number of cloves: Fig. 3), require more time for bulbing. The second group was constituted by colored garlic Eggaseed-1 selections (Eggaseed-1-3, 9, 22, 7, 14 and 13) and Sids-40 individuals (Sids-40-19, 22, 23, 8, 9 and 6). This group was morphologically characterized by better bulb and clove weight, lower clove numbers/bulb and less time for bulb formation. The results agree with those of **Garcia *et al.* (2003)**. The most productive variety has the inconvenient of having a larger number of cloves/bulb.

Through the SSRs with post-labeling technique used in this study, Eggaseed-1 and Sids-40 selections were grouped as these two cultivars gave higher yield production compared with Egyptian cv. However, Eggaseed-1 and Sids-40 selections showed a band of 189 bp with Asa16 and could thus be identified as a possible molecular marker.

Conclusion

The investigation of variations in SSR fragments with post-labeling method provides an efficient tool for examining diversity which will help to develop plant breeding strategies. Finding good SSRs is important but in garlic this can be lengthy and difficult process due to abundance and complexity of garlic genome. Not much information is available on the genetic diversity within and between garlic cultivars which has been based mainly on morphological traits. Meanwhile, it can be concluded that evaluation of genetic diversity based on molecular markers and post-labeling method may give more accurate information to plant breeder. This data will support the exploitation of garlic germplasm on molecular basis. SSRs with post-labeling used in this study may also be used for genetic mapping and gene tagging in garlic. These results allow identifying highly related garlic colored garlic, better bulb and clove weight and lower clove numbers/bulb cultivars (Eggaseed-1 and Sids-40) and separate them from Egyptian cv. that is characterized by white garlic, lower production, smaller clove weight and/or greater number of cloves.

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References

1. Abdel-Ghany, G.M.A. (2011). Genetical and molecular studies on some garlic clones. Ph.D. thesis, Genetics, Fac. of Agric., Minia Univ. El-Minia, Egypt.
2. Al-Otayk, S., El-Shinawy, M.Z. and Motawei, M.I. (2008). Variation in productive characteristics and diversity assessment of garlic cultivars and lines using DNA markers. Met. Env. Arid Land Agric. Sci., 20:63-79.
3. Brewester, J.L. (2008). Crop production science in horticulture onions and other vegetable Alliums. CABI is a trading name of CAB international CABI North American office 875 massachustts avenue 7th floor.
4. Cunha, C.P., Hoogerheide, E.S.S., Zucchi, M.I., Monteiro, M. and Pinheiro, J.B. (2012). New microsatellite markers for garlic, *Allium sativum* (Alliaceae). American Journal of Botany: e17–e19.
5. Duncan, D.B. (1995). Multiple range and multiple F test. Biometrics, 11:1-42.
6. Gadel-Hak, S.H., Moustafa, Y.M.M. and Abdel-Wahab, I.A. (2010). Selection of new promising white garlic (*Allium sativum* L.) clones under the drip irrigation system. The Sixth Inter. Conf. of Sustain. Agric. And Develop. Fac. of Agric., Fayoum Univ., 27-29, December, 2010.
7. Garcia, L.S., Martinez, L. and Burba, J.L. (2003). Genetic diversity among Argentinean garlic clones (*Allium satvium* L.) using AFLP (Amplified Fragment Length Polymorphism). Euphytica 132:115-119.
8. Ipek, M., Ipek, E.A., Alquist, E.S.G. and Simon, E.P.W. (2005). Demonstration of linkage and development of the first low-density genetic map of garlic, based on AFLP markers. Theoretical and Applied Genetics 110:228-236.
9. Ma, K.H., Kwag, J.G., Zhao, W., Dixit, A., Lee, G.A., Kim, H.H., Chung, I.M., Et, A.L. (2009). Isolation and characteristics of eight novel polymorphic microsatellite loci from the genome of garlic (*Allium sativum* L.). Scientia Horticulturae 122: 355-361.
10. Mann, L.K. (1952). Garlic bulb studies. Calif. Agric. Vol. 6: 13.
11. Metwally, E.I. and El-Denary, M.E. (2003). Elevation of (AVRDC) international garlic collection under Egyptian conditions. Acta Hort. 604, ISHS.
12. Nei, M. (1978). Estimation of average heterozygosity and genetic distance from a small number of individual. Genetics 89:583-590.
13. Nei, M. and Li, W. (1979). Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Natl. Acad. Sci. USA. 79:5256-5273.
14. Nguyen, H.T. and Xiaolei, W.u. (2005). Molecular marker systems for genetic mapping. In the handbook of plant genome mapping. Edited by: Khalid Meksem GK. Weinheim: WILLEY-VCH GmbH:23-52.
15. Noorbakhshian, S.G.J., Mousavi, S.A. and Bagheri, H.R. (2008). Evaluation of agronomic traits and path coefficient analysis of yield for garlic cultivars. Paiouhes Sazadegi, 77:10-18.
16. Nybom, H. (2004). Comparison of different nuclear DNA markers for estimating intraspecific genetic diversity in plants. Molecular Ecology 13: 1143–1155.
17. Omer, E.A. and Abou-Hadid, A.F. (1992). Evaluation of some lines of Sids-40 garlic comparing with Balady cultivar. Egypt J. Hort., 19: 17-20.
18. Osman, S.A.M., Ata, A.M. and Gad El-Hak, S.H. (2007). Morphological, germination, bolting and cytogenetical characteristics of fourteen promising garlic genotypes. African Crop Science Conference Proceedings, 8:2005-2012.
19. Page, A.L., Miller, R.H. and Keeney, D.R. (1982). Methods of soil analysis part 2: chemical and microbiological properties. 2nd Edn. ASA and SSSA, Madison, WI., USA., pages 1159.
20. Savolainen, V. and Chase, M.W. (2003). A decade of progress in plant molecular phylogenetics. Trends in Genetics 19: 717–724.
21. Senula, A. and Keller, R.J. (2000). Morphological characterization of garlic core collection and establishment of a virus-free in vitro gene bank. Allium Improv. Newsletters, 10:3-5.
22. Shimizu, T. and Yano, K. (2011). A post-labeling method for multiplexed and multicolored genotyping analysis of SSR, indel and SNP markers in single tube with bar-coded split tag (BStag). BMC Research Notes, 4:161.
23. Warterer, D. and Schmitz (1994). Influence of variety and cultural practices on garlic yields in Saktchewan. Canadian J.P. Scineca. 74:611-614.
24. Yamaguchi, M. (1983). World vegetables (principles, production, and Nutritive value). AVI Publishing Company Inc; Westport CT.
25. Zaki, H.E.M., Yokoi S., Takahata, Y. (2010). Molecular genetic analysis of root traits in brassicaceae root crops. Ph.D. thesis, Science of bioresources, functional genomics and biotechnology, Lab of Plant Breeding, Fac. of Agric., Iwate Univ., Iwate, Japan.
26. Zaki, H.E.M., Toney, H.S.H. and Abd Elraouf, R.M. (2014). Response of two garlic cultivars (*Allium sativum* L.) to inorganic and organic fertilization. Nature and Science, 12(10): 52-60.
27. Zewdie, Y., Havey, M.J., Prince, J. P. and Jenderek, M. M. (2005). The first genetic linkages among expressed regions of the garlic genome. Journal of the American Society for Horticultural Science 130: 569 – 574.

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