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Physiological and Biotechnological Studies on Some Local and Foreign Garlic Genotypes

El Nagar, M.M. Hort. Dep., Fac. of Agric., Benha Univ., Egypt and Fac. of Agric. Res. Park (Biotechnology Lab.), Benha University, Egypt

El-Zohiri, S.S.M Potato and Vegetatively Propagated Vegetables Dep., Hort. Res., Inst., A.R.C, Egypt.

ABSTRACT

Egyptian garlic cultivar and Sids-40 and three genotypes i.e., Chinese, Italian and Brazilian were evaluated for their productivity in two field experiments to determine the genetic diversity during winter seasons of 2012/2013 and 2013/2014. The results showed that the uppermost plant height was 100.0 and 96.3 cm for Egyptian cultivar (Balady) in both seasons, respectively. The maximum value of leaves number were detected with Sids-40 while the largest leaf area per plant were recorded to Chinese genotype. Moreover, Chinese genotype was also greater than other cultivars and genotypes in total plant and leaves fresh and dry weight. However, the highest chlorophyll content was obtained by Brazilian genotype. Whereas, the highest cloves number per bulb were produced by Egyptian and Brazilian genotype, respectively. The highest bulb diameter, average bulb weight and average clove weight were achieved by Chines genotype compared with other cultivars and genotypes tested. The greatest TSS and allicin contents in cloves and bulbs of garlic were found in cv. Egyptian (Balady) followed by Brazilian genotype in case of allicin content. Whereas, the marketable yield weight was observed by Chines and Sids-40.

Inter-simple sequence repeat (ISSR) was assayed to determine the genetic diversity of 5 garlic genotypes. Six ISSR primers were used in current investigation. A high level of polymorphism among garlic genotypes was found with ISSR marker number 6 that also showed amplicons with all garlic genotypes under study. Our results indicate that ISSR can be useful for genetic diversity studies, to provide practical information for parental selection and to assist breeding and conservation strategies Also, the present results along with those of other researchers show that ISSRs can be used for cultivar differentiation in garlic.

KEYWORDS Garlic genotypes, productive characters, genetic diversity, DNA fingerprinting, ISSR markers.

INTRODUCTION

Garlic is considered among the most important vegetables for local consumption and export. Garlic, was characterized by high yield with good quality in the past, but in the last few years its productivity deteriorated and the demands of garlic for the foreign markets became limited. Garlic deterioration may be due to changes in its genetic germplasm and appearing undesirable ones (Abd El-Hamed et al 2006), also, this deterioration may be attributed to attacking some pests to garlic plant. Several diseases attack garlic plant at all stages of growth causes great quantities and qualitative losses in yield production such diseases include soil borne, foliar and storage diseases. Many efforts were made for introducing high yielding garlic cultivars to Egypt for overcoming the problem of yield decline. In addition, unbalancing cropping of Balady garlic the dominant garlic cv grown under Egypt conditions encourage garlic workers to search about more new garlic cvs (Anwar and Gouda, 2012).

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 and Al-Otayk et al., 2008). 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; Al-Otayk et al., 2008 and Jappes et al., 2011), 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 evaluate garlic genotypes for the productivity and investigate the assessment of genetic diversity in garlic genotypes using ISSR markers.

MATERIALS AND METHODS

Site description and soil type:

The experiment was conducted on clay loam soil at the Experimental Farm of the Faculty of Agriculture, Moshtohor, Kalubia, Benha University during two successive winter seasons of 2012/13 and 2013/14, to investigate the physiological and biotechnological characteristics in two garlic cultivars, namely Egyptian (Balady) and Sids-40 and three foreign genotypes of Chinese, Italian and Brazilian garlic The soil of the experimental field was clay loam in texture with pH 7.5. The site is located at an altitude of 21.1 m above sea level, latitude 30°16' N and longitude 31°12' E. The mechanical and chemical analysis of the soil was determined according to the methods described by Jakson (1973), and are shown in Table (1).

Table (1): The main physical and chemical properties of the experimental soil during 2012/13and 2013/14 seasons.

Soil characteristics	2012/13	2013/14
	Values	Values
Clay %	40.82	41.66
Silt %	35.12	34.88
Fine sand %	15.58	15.92

Coarse sand %		8.48	7.54
Texture class		Clay-loam	
2- Chemical analysis as meq/100 g. soil:			
pH (1:2.5 suspension)		7.6	7.5
Organic matter %		1.77	1.82
HCO_3^-		1.92	1.90
Cl^-		1.38	1.41
SO_4^-		0.58	0.61
Ca^{++}		1.29	1.30
Mg^{++}		0.70	0.72
Na^+		1.68	1.66
Available	N	81.66	80.98
	P	19.00	20.80
	K	45.22	42.22

Data presented in Table (2) show average monthly temperature, relative humidity percentage and quantity of rainfall at Kalubia Governorate in the region surrounding the experimental site during the two seasons of experimental work.

Experimental design:
The layout of the experiments was completely randomized design with four replicates was adopted. Each experimental plot included five ridges 4.25 m length and 60 cm width with an area about 12.75 m^2, where, 4 ridges were planted and the fifth one was left without planting as a guard ridge between plots. All data collected were subjected to analysis of variance (ANOVA) to test treatment effects for significance using Statistix 9.0 software package. The means were compared using F-LSD.

Table (2): Average monthly temperature, relative humidity (%) at Kalubia Governorate in the region surrounding the experimental site through the two seasons of the experimental work.

Seasons	2012/13			
Month	Temperature (C°)			Relative humidity (%)
	Min.	Max.	Mean	
Oct. 2012	10.00	23.20	16.56	61.00
Nov. 2012	15.00	31.40	23.26	54.50
Dec. 2012	20.00	33.70	26.79	59.00
Jan. 2013	20.12	33.61	26.86	62.00
Feb. 2013	18.90	32.40	25.60	65.00
Mar. 2013	18.10	31.40	25.60	62.00
	2013/14			
Oct. 2013	13.40	29.70	21.50	57.00
Nov. 2013	17.40	32.46	24.93	55.33
Dec. 2013	19.53	34.53	27. 03	57.66
Jan. 2014	20.73	35.36	28.04	58.66
Feb. 2014	20.58	35.56	28.06	57.62
Mar. 2014	20.43	35.76	28.09	56.58

Planting technique and agricultural practices:
Garlic seed cloves for the two garlic cultivars and three foreign genotypes were soaked in tap water for 8 hours before planting then, manually planted on 1st and 8th October in 2012/13 and 2013/14 in the first and second seasons, respectively on one side of the ridge at the distance of 10 cm in between and 3-5 cm deep in the soil. Cloves were irrigated directly after planting then, 5 days later and at interval of 15 days from each one. The full amount of organic manure and phosphorus were applied at the time of final land preparation at rates of 25-m^3 farmyard manure and 64 kg P_2O_5/fed. calcium super phosphate (15.5% P_2O_5). 120 kg N/fed. as ammonium sulphate (20.5% N), and 96 kg K_2O/fed. as potassium sulphate (48% K_2O) were added at 3 equal portions the first portion was added one month after planting, while the second and third portions of N and K were added two and three months after planting, respectively. All other horticultural practices that were common applied in garlic management were followed as usual.

Data recorded:
At 150 days after planting fife 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), plant fresh and dry weight (g). In addition, chlorophyll content was measured of the fifth upper leaf using Minolta chlorophyll Meter SPAD –501.

At the harvesting stage (180 day after planting) fife plants were randomly chosen for recording yield parameters which included, average bulb fresh and dry weight (g), number of cloves/ bulb dry weight of cloves (g), bulb diameter (cm), total soluble solid (T.S.S) (%) and Allicin content of cloves. Also, marketable yield per square meter.

Estimated of Allicin:
Sample Preparation Garlic, outer skins of cultivars and genotypes garlic cloves were peeled and the cloves crushed in a mortar using a pestle. 1.0 g of the fine garlic mash was soaked in 10 ml of cold (refrigerated) distilled water and put in a refrigerator (< 5oC). After 24 h, the garlic solution was filtered through Whatman No. 42 filter papers under vacuum. The filtrate was preserved in a refrigerator (< 5oC).

Solid Phase Extraction (SPE) Optimization and UV Absorbance
Measurements
C18 Sep pak cartridges were conditioned by rinsing with 5 ml methanol and then equilibrated with 10 ml of water. Approximately 1 ml of the garlic extract solution was introduced to the top of a cartridge and eluted with varying volumes of cold distilled water, ethanol, methanol, methanol/ water (2:3 v/v), methanol/ water (1:1v/v) and methanol/ water (3:2 v/v) after adjusting the flow rate to about 1-2 ml min-1. The fractions were collected in test tubes placed in an ice bath and their absorbances against eluting solvent was measured at 240 nm and 254 nm in a 1cm quartz cuvette using a UV–VIS Spectrophotometer (Shimadzu model UV – 1601 PC).

DNA extraction, ISSR–PCR, data scoring
Bulk leaf samples from garlic genotypes 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 °C 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 H_2O. 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 H_2O and stored at 4°C.

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 MgC_{l2}) and 1 unit of Taq polymerase (Pharmacia Biotech, Germany) supplemented with 0.01% gelatin, 0.2 mM of each dNTPs (Pharmacia Biotech, Germany), 50 pmol of ISSR primers (Table 1), 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 at 50 °C to 58 °C depending on the primer, and 1.3 min at 72˚C; followed by 20 min at 72˚C. Replicate accessions were tested in separate experiments to verify repeatability of results. Negative controls, with genomic DNA omitted, were run with every PCR to check for DNA contamination.

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. ISSR bands were visualized on a UV tran-silluminator, documented digitally using a Kodak imaging system (Alpha Innotech Corporation). Data of ISSR analysis were scored for computer analysis on the basis of the presence or absence of the amplified products for each ISSR primer using ImageJ software. Bands were scored as diallelic for each assigned locus (1 = band present; 0 = band absent). Fragment sizes were estimated based on ladder 123 bp standards (Gibco BRL).

Table 3: ISSR primers

Primer		Annealing T (°C)
1	CCA(TGA)5TG	54
2	(ACC)6CC	54
3	GCA(AC)7	58
4	AG(CA)8	58
5	(AG)8YT	50
6	(AC)8YT	50

Primers were from the Biotechnology and Agriculture Division, ENEA C.R. Casaccia, Rome, Italy.

RESULTS AND DISCUSSION

Growth characters:

Data presented in Table (4) show that there were significant differences in plant height, number of leaves per plant and leaf area among two cultivars (Balady and Sids-40) and genotypes. In this respect, the highest plant height recorded to Egyptian garlic (Balady) cultivar (96.3 and 100 cm) and Brazilian genotype (96.3 and 98.3 cm), in both seasons, respectively. However, cv. Sids-40 produced the shortest plants (79.7 and 79.3 cm), respectively. This result was harmony with results reported by Omer and Abou Hadid (1992) reported approved approximately 105.5 cm for Egyptian cultivar. Hussein, et al. (1995) found that the maximum plant height for Egyptian cultivar was 74.0 cm. whereas; Al-Otayk et al. (2008) indicated that the maximum plant height was 70.5 and 70.8 cm for Egyptian cultivar in both seasons, respectively. Anwar and Gouda (2012) stated the same result when compared cv Balady with cvs Sids-40, Egaseed-2 and Balady El-Wady.

Regarding the leaves number, it can be noted that Sids-40 cultivar produced the maximum leaves number per plant (11.0 and 11.0) in both seasons respectively, compared with other tested cultivars and /or other genotypes. The same trend reported by Anwar and Gouda (2012) found that, the highest values were recorded in garlic cvs Sids-40 followed by Chinese cv. The lowest number of leaves per plant was appeared in garlic cvs Balady and Balady El-Wady. Chines genotype was superior to the other cultivars and genotypes in leaves area (308.6 – 301.0 cm^2) in both seasons respectively. Chines genotype produced the widest leaf area followed by Italian genotype, whereas, the narrower leaf area was obtained from Brazilian genotype.

Table (4): Plant height, number of leaves and leaf area of garlic cultivars and genotypes during 2012/13 and 2013/14 seasons.

Cultivars/ genotypes	Plant height (cm)		No. of leaves/plant		Leaf area /plant (cm^2)	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Cv. Sids-40	79.7	79.3	11.0	11.0	238.7	238.6
Italian genotype	82.0	87.0	8.7	9.3	254.6	260.0
Chines genotype	80.3	84.0	9.7	9.7	308.6	301.0
Brazilian genotype	96.3	98.3	8.3	8.0	235.0	236.3
Cv. Egyptian (Balady)	96.3	100.0	8.3	7.7	242.0	239.7
L.S.D at 0.05	12.32	9.19	1.87	1.15	9.83	14.8

For leaves fresh weight, leaves dry weight and total plant fresh weight, it is apparent in Table 5 that Chines genotype produced the heaviest leaves fresh weight, leaves dry weight and total plant fresh weight followed by Italian genotype. Whereas, Brazilian gave the lightest weight in this characters in both seasons. The remained cultivars (Egyptian and Sids-40) occupied an intermediate position between the aforementioned genotypes in both seasons. The previous significant differences on growth characters among various as garlic cvs were confirmed by the results of Shalaby (1973), Hussain et al, (1995), Mohamed (2004), Moustafa et al, (2009), Aly (2010) and Anwar and Gouda (2012).

The characters of plant dry weight, number of cloves per bulb and total chlorophyll illustrated in Table 6 show that the maximum value of total plant dry weight (73.00 and 73.06 g) in the first and second seasons respectively, were observed with Chines genotype followed by Italian genotype in the first season only. Whereas the Brazilian genotype gave the lowest result in both seasons compared with others cultivars and genotypes. Egyptian cultivar (Balady) produced more cloves number (38.66 and 38.67) compared with the other cultivar (Sids-40) and genotypes of chines, Italian and Brazilian in both seasons, respectively. These results are in agreement with Anwar and Gouda (2012) who reported that number of cloves per bulb was maximized in garlic Balady El-Wady followed by Balady garlic cvs. Bulbs of cvs Sids-40 and Egaseed-2 clone contained the lowest number of clovers in both seasons. On the other hand, Egyptian and Brazilian garlic had the highest chlorophyll content in the tested cultivars and other genotypes. The chlorophyll result was harmony with Al-Otayk et al. (2008) reported that the last Line (L6) and Egyptian garlic had the highest chlorophyll values.

Table (5): leaves fresh weight, leaves dry weight and total plant fresh weight of garlic cultivars and genotypes during 2012/13 and 2013/14 seasons.

Cultivars	Leaves F.W (g)		Leaves D.W (g)		Total plant F.W (g)	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Cv. Sids-40	105.0	112.6	32.55	34.93	238	238
Italian genotype	108.1	112.4	34.60	33.72	254	260
Chines genotype	138.0	134.3	44.16	42.98	308	301
Brazilian genotype	84.0	74.7	25.20	22.40	235	236
Cv. Egyptian (Balady)	90.8	89.5	27.25	26.85	242	239
L.S.D at 0.05	18.26	19.83	5.56	5.98	9.83	14.08

Table (6): Total plant dry weight, number of cloves per bulb and total chlorophyll of garlic cultivars and genotypes during 2012/13 and 2013/14 seasons.

Cultivars	Total plant D.W (g)		No. of cloves /bulb		Total chlorophyll (Value)	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Cv. Sids-40	55.70	57.40	14.07	13.80	63.76	65.82
Italian genotype	58.45	55.69	10.83	11.36	64.23	63.60
Chines genotype	73.00	73.06	11.96	12.33	69.36	70.53
Brazilian genotype	39.18	35.78	34.33	35.00	73.64	71.40
Cv. Egyptian (Balady)	42.15	41.62	38.66	38.67	71.83	69.70
L.S.D at 0.05	5.21	5.88	3.52	4.04	3.15	4.24

Yield and its components:

The obtained data in Table (7) reveal that there were significant findings in bulb diameter, average bulb and clove weight among cultivars and other genotypes. In this concern, bulb diameter, average bulb fresh and clove weight were significantly increased in case of Chines genotype compared with cv Egyptian garlic and Brazilian. However, the different between chines genotype and cv Sids-40 was not reach to significant level in both seasons. The same trend reported by Anwar and Gouda (2012) they found that the minimum bulb and cloves weight was obtained in garlic cv Balady El-Wady and Balady cv.

Table (7) bulb diameter, average bulb and clove weight of garlic cultivars and genotypes during 2012/13 and 2013/14 seasons.

Cultivars / genotypes	Bulb diameter (cm)		Average bulb weight (g)		Average clove weight (g)	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Cv.Sids-40	6.06	5.96	56.47	56.18	3.40	3.29
Italian genotype	4.10	3.66	55.46	56.33	2.13	2.17
Chines genotype	6.63	6.52	73.96	73.36	5.13	4.54
Brazilian genotype	4.46	4.37	35.83	36.10	1.25	1.19
Cv. Egyptian (Balady)	4.73	4.30	39.23	38.87	1.02	0.97
L.S.D at 0.05	0.52	0.77	4.72	4.91	0.56	0.60

Data illustrated in Table 8 show that total soluble solids and Allicin contents of cloves were highest in cv. Egyptian garlic (Balady) (37.36 and 37.77 %) and (4.45 and 4.31 mg/g F.W) in the first and second seasons respectively, compared with others cultivars and genotypes. Where, Chinese and Brazilian genotypes were ranked the second order in cases of total soluble solids and Allicin contents of cloves (36.73 and 37.57 %) and (4.33 and 4.26 mg/g), in both characters and genotypes, respectively. However, TSS was medium in cv. Sids-40. On the other hand, Italian genotype produced the lowest total soluble solids percentage (34.30 and 34.93 %) and Allicin content of cloves (3.65 and 3.87 mg/g) in both seasons, respectively. (Singh and Chand (2003) found that total soluble solid content of cloves was significantly differed among garlic cultivars and clones. Al-Otayk et al. (2008) found that TSS was highest in Egyptian garlic compared with other cultivars and Lines genotype produced the lowest total soluble solids percentage (34.30 and 34.93 %), respectively. (Singh and Chand (2003) found that total soluble solid content of cloves was significantly differed among garlic cultivars and clones. Al-Otayk et al. (2008) found that TSS was highest in Egyptian garlic compared with other cultivars and Lines. Contra result on Allicin content was obtained by Mai et al. (2015) on garlic found that Sids-40 had a higher allicin content than the cv. Balady.

It is evident from Table 8 that significantly maximum marketable fresh yield was recorded in chines genotype (1.967 and 2.110 kg/m^2 bulb respectively), followed by cv Sids-40 (1.653 and 1.640 kg /m^2 respectively), while it was minimum in Egyptian garlic cultivars (1.213 and 1.196 kg / m^2 bulb) in both seasons respectively, and Brazilian genotype (1.213 and 1.216 kg /m^2 respectively). The remained Italian genotype occupied an intermediate position between the maximum and minimum marketable bulb yield. Similar results were reported by Hussein, et al. (1995), Al-Otayk et al. (2008) and Anwar and Gouda (2012) they found that Balady cultivar produced the lowest garlic yield.

Table (8) Total soluble solids of cloves and clove fresh weight of garlic cultivars and genotypes during 2012/13 and 2013/14 seasons.

Cultivars/ genotypes	Cloves T.S.S %		Allicin (mg/g F.W)		Marketable yield kg /m^2	
	2012/13	2013/14	2012/13	2013/14	2012/13	2013/14
Cv. Sids-40	35.92	35.47	4.27	4.21	1.653	1.640
Italian genotype	34.30	34.93	3.65	3.87	1.366	1.413
Chines genotype	36.73	37.57	4.13	4.19	1.967	2.110
Brazilian genotype	34.96	35.17	4.33	4.26	1.213	1.216
Cv. Egyptian (Balady)	37.36	37.77	4.45	4.31	1.213	1.196
L.S.D at 0.05	1.92	1.78	0.21	0.10	0.12	0.17

Genetic diversity

In the present study, molecular fingerprinting of garlic genotypes using six ISSRs were tested to explore the genetic diversity among different foreign and local garlic genotypes based on the clear scrabble band pattern and of good quality. The number of amplification bands per ISSR primer varied between 0 and 6. Examples of polymorphism are shown in Fig. 1. All garlic genotypes showed amplicons with primer number 6 (Fig. 1 and Table, 8). Primers number 1, 2, 4 and 5 showed bands with 3 different garlic genotypes while primer number 3 gave only bands with two garlic genotypes. Also, among six ISSR primers, primer number 6 accounted 25.0 % of total polymorphic bands among garlic cultivars. Analysis of ISSR primers among present garlic genotypes generated 4 polymorphic bands.

Table 9. ISSR primers with the number of amplified products and polymorphic fragments among garlic genotypes.

Primers	Amplified products of garlic genotypes					Polymorphic fragments among garlic genotypes
	Cv. Sids-40	Italian genotype	Chinese genotype	Brazilian genotype	Cv. Egyptian (Balady)	
1	2	3	4	0	0	2
2	0	3	5	3	0	3
3	0	3	4	0	0	1
4	4	6	4	0	0	3
5	2	5	5	0	0	3
6	2	5	6	4	3	4

Al-Otayk et al., (2008) reported that ISSR technology is a useful tool for analysis of genetic diversity of garlic along with productive characters. ISSR markers can provide a better approximation to true variation among garlic lines. They showed that in ISSR analysis, the number of amplification bands per primer varied between 0 and 10. Also, among seven ISSR primers, poly (CTC) based primers accounted 37.5% of total polymorphic bands among garlic cultivars and lines. Mondal, et al. (2008) found that a total of 21 selected ISSR primers produced 153 bands, of which 114 were polymorphic correspondingly and ISSR revealed higher polymorphism (74.5%) than RAPD (47.1%) in peanut genotypes. Jabbes et al., (2011) reported that the availability of a relatively high number of polymorphic ISSR markers reflects the heterozygous genome and that ISSR technique is able to detect as much polymorphism in a vegetative as in sexually propagated species.

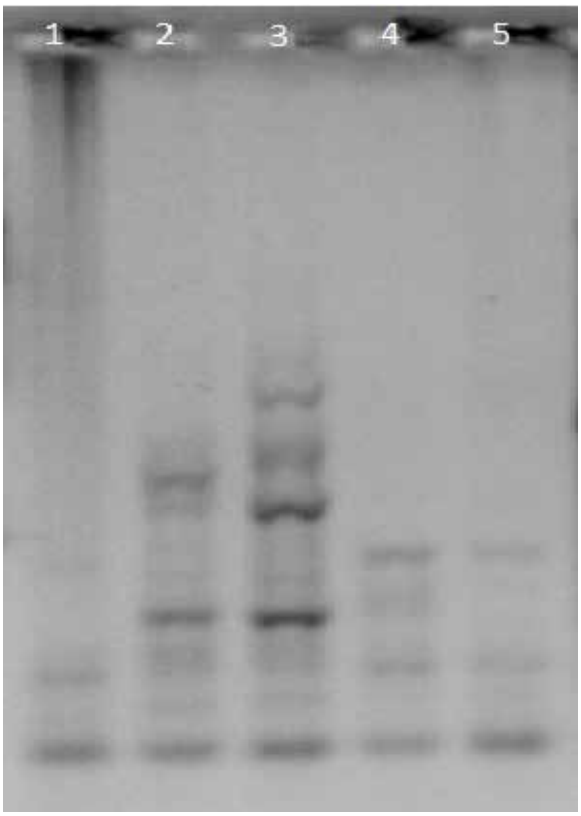

Fig. 1: Polymorphism revealed using primer number 6 to amplify genomic DNA purified from the tested garlic genotypes.

In fact, the ISSR amplification of garlic DNA revealed a rather high degree of genetic variability among and within accessions not expected in vegetatively propagated specie. This variability is consistent with the phenotypic variability observed confirming the multi clonal composition of the analyzed landraces. Most of the primers produced a different banding pattern for each individual belonging to the same accession. Cultivar specific bands were not observed. The variation within accessions makes it difficult to define unique ISSR profiles for the groups of individuals that belong to the same landrace. The diversity within garlic populations might also be due to mutations that occurred over time due to non-reduction in grower's selection pressure (Simon and Jenderek, 2003).

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