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RELATIONSHIP BETWEEN VOLATILE SULFUR COMPOUNDS, MINERAL CONTENT, MORPHOLOGICAL AND MOLECULAR CHARACTERIZATION OF LOCAL GARLIC GENOTYPES

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Abstract

This study was conducted to determine the relationship between local garlic genotypes and Taskopru garlic, cultivated in Turkey, by using volatile sulfur compounds, mineral content, molecular and morphological characterizations. Results indicated that morphological features, mineral content and volatile sulfur compounds exhibited a great diversity among the garlic genotypes. Jaccard's similarity matrix prepared using 13 SSR markers also revealed the genetic diversity among genotypes. This genetic diversity may contribute significantly to the development of new garlic cultivars. Mineral content of the garlic leaves could be used as chemotaxonomic tool for classification of the local garlic genotypes.

Introduction

Garlic (*Allium sativum* L.) is an economically important species of the *Allium* genus and cultivated as a vegetable, spice and medicinal plant since ancient times throughout the world (Avato *et al.* 1998, Petropoulos *et al.* 2018). Although garlic exclusively reproduces by vegetative division, it exhibits great diversity in morphological and agronomic characters because various ecotypes have been grown in the same areas for a long time, and natural mutations, this diversity offers advantages for plant genetics studies (Bradley *et al.* 1996, Petropoulos *et al.* 2018). Garlic has been considered as a good source of nutrient densities. It is known to be part of a group of functional food which have antimicrobial, antioxidant, anti-cholesterol, anticancer and anti-aging properties. In addition, scientific studies have confirmed and recognized the medicinal effects of garlic, including the reduction of risk factors for cardiovascular disease, lowering of blood pressure and glucose concentrations, the stimulation of immune function, and the restoration of physical strength. The above mentioned positive effects of garlic on human health are due to their chemical composition (Turan *et al.* 2017). *Allium* vegetables are grown primarily for their distinct flavors. The chemistry of *Allium* flavor is complex because the sulfur-containing compounds responsible for flavor are labile and reactive. They are released only when cells are damaged by slicing or crushing (Brewster 2008). The content of these valuable components depends on the specific plant genotype (Dziri *et al.* 2014, Petropoulos *et al.* 2018). Recently SSR-based genetic diversity of local garlic accessions were analyzed from Brasil (Da Cunha *et al.* 2014), China (Wang *et al.* 2016), Czech Republic (Ovesna *et al.* 2014) and India (Kumar *et al.* 2019). Zhao *et al.* (2011) have developed a core set from garlic accessions and its relatives originated from 36 different countries using SSR markers. Flavonols can be used as chemotaxonomic markers and local onion genotypes can different in terms of the amount of genetic controlled polyphenols (Riggi *et al.* 2013). Ikram *et al.* (2019) have characterized different *Allium* species using their sulfur-compounds as chemotaxonomic markers. El-Fiki and Adly (2020) have compared two different irradiated garlic genotypes related to their morphological, molecular, and voiatile sulphur

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compounds characterization. The present study was aimed to investigate the relationship among local garlic genotypes by using volatile sulfur compounds, mineral content, morphological and molecular characterization.

Materials and Methods

Three different local garlic genotypes, cultivated in Kilis, province of Turkey, and Taskopru garlic as a control, a clone of garlic widely grown and consumed in Turkey, were used as plant material. Garlic samples were planted at the Agricultural Application and Research Center of Kilis 7 Aralik University. Hand-harvesting was accomplished when all the plants for each genotype and plots were well senescent. Approximately two weeks later, when the bulbs were well dried, samples selected from each garlic genotype were divided into three replicates, consisting of 15 bulbs each, and taken to the laboratory for morphological observations, mineral and essential oil analyses. Morphological features were determined for each genotype with three replicates, consisting of 15 bulbs. The measured features were plant height (cm), leaves number (number/plant), leaf width (mm), bulb height (mm), bulb diameter (mm), and bulb weight (g), number of cloves per bulb, and clove weight (g). Mineral content analysis was carried out according to Petropoulos *et al.* (2018). Calcium (Ca), Magnesium (Mg), Iron (Fe), Mn (Manganese), Zinc (Zn), Copper (Cu), Sodium (Na) and Potassium (K) content were determined by atomic absorption spectrophotometry (Perkin Elmer, Model 240 FS AA). Essential oil content analyzes were performed with a Gas Chromatography-Mass Spectrometer (GC-MS, Agilent, 7890B GC-5977MSD). HP-5 MS (30 m × 0.25 mm i.d., film thickness 0.25 m; Hewlett-Packard) was used as a capillary column. Analysis was carried out according to Calvo-Gomez *et al.* (2004). The identification of the essential oil constituents was based on comparison of their GC retention times and MS fragmentation patterns with authentic reference compounds and with spectral data from NIST library. Significant differences between morphological features and mineral content were evaluated JMP pro version 14 (SAS Institute, NC, USA). When significant effects were detected, the Tukey test was performed to compare within each genoype at the 0.05 significance level. Principal component analysis (PCA) was also performed for morphological features, mineral and volatile sulfur compounds of garlic genotypes.

Cetyltrimethyl ammonium bromide (CTAB) DNA isolation method was used for the isolation of total genomic DNA from fresh young garlic leaves then an RNase treatment was performed on the eluted DNA samples (Untergasser 2008). The genomic DNA was amplified using 13 primers (Table 1). PCR reactions were optimized in a volume of 20 µl using a method by Koc *et al.* (2019) with modifications. The dendrogram was constructed from the resultant matrices via the unweighted pair-group (UPGMA) method using an online tool (Garcia-Vallve and Puigbo 2009). As another way to test the relationship between genotypes, a three-dimensional principal component analysis (PCA) was performed with the EIGEN program NTSYS-pc version 2 (Rohlf 1989).

Results and Discussion

There was a great diversity among the garlic genotypes in terms of the morphological features under studied (Table 2). The dendrogram gave the clustering pattern for the garlic genotypes (Fig. 1B). Cluster analysis showed that there were two distinct major clusters in all morphological features in relation to genotypes. There were two main clusters; the genotype Kilis 2 cluster and the other genotypes. The Taskopru garlic and genotype Kilis 3 were classified into the same group, but genotype Kilis 1 was separated into a minor group. As shown in Table 2, the bulb weight ranged from 18.05 g (genotype Kilis 2) to 31.51 g (genotype Kilis 1), while the number of

cloves per bulb ranged from 10.50 (genotype Kilis 2) to 12.90 (genotype Kilis 1). The leaf number and clove weight varied from 8.00 to 9.37 and 3.45 g to 2.17 g in genotype Kilis 2 and Taskopru, respectively. Leaf width , bulb height and bulb diameter were varied from 6.06 mm to 8.69 mm, 34.23 mm to 37.44 mm and 36.97 mm to 43.76 in genotypes Kilis 2 and Kilis 1, respectively. Plant height was between 26.20 cm in genotype Kilis 3 to 33.96 cm in Taskopru. As a result, genotype Kilis 2 was found to exhibit distinct features from Taskopru garlic used as a control in the research. Similarly, Ragas *et al.* (2019) stated that four major local garlic cultivars from the Philippines displayed significant bulb traits. Petropoulos *et al*. (2018) found that there was significant diversity in bulb morphology not only among the genotypes from different growing regions, but also between those of the same region in different garlic genotypes such as local landraces/varieties, imported genotypes, commercial cultivars. Panthee *et al*. (2006) found that the level of variation found in garlic germplasm from various parts of Nepal showed the great potentiality of improving agronomic characters.

Table 1. Primer sequences used in the experiment.

Primer	Sequence		References
	Forward	Reverse	
ACM013	CAACCTCGAAGAACTCACCG	GCGAATCTTGTTTTTGGGAA	
ACM004	TCGTTCTTTAGAACACGTTAGGAA	TGTCGGCGGATATAGTGACA	Tetiana *et al.* 2017
ACM091	TCTCCTCCTCTAACCAGCCA	GGTGCTCCAGTTGAGCTTTC	
ACM101	CCTTTGCTAACCAAATCCGA	CTTGTTGAGAAGGAGGACGC	McCallum *et al.* 2008
AFA01A08	AGATAAGTGCTCATGGAGCAAGGG	ACATCCACAGCAAACATAGCAAGC	
AFA10A08	GTTTAGGGCGTAAAATCTAAACGCT	GTGCTTTTGACTAACCTCGCATCC	
AFS006	GTGACCTTATGTAGGGGTTAGGATT	TCGCTCCATTCAAATTAAAAA	Tsukazaki *et al.* 2010
AFS015	ATCTCACTGTCCTTGTACCTGAAAG	CATCTTGACTTTGTGATATTTGTGC	
AFS149	AACCAATTGATTACCTCTCATCTGC	TGCGGACCTTCCATAGTCTGTATAA	
AMS10	TTCATGTTGTATTGAGATTTGG	GAAGGAATGGAAGCAGTTC	
AMS14	CCCCTGAGTAAATTCAAAATCC	TCCTTAGTATAATTTCGGGGTAAC	Fischer and Bachmann 2000
AMS25	GAGGGCAGTGTTAGCATTCC	GAGCTCCACTTCTTCCAAACTAG	
AMS30	CACTAATGGGGTAAATAATGTTCTAC	TTGCCTTGAAATCCAGAC	

Table 2. Morphological features of garlic genotypes.

Genotype	Plant height (cm)	Leaf width (mm)	Leaf no. per plant	Bulb height (mm)	Bulb diameter (mm)	Bulb weight (g)	No of cloves per bulb	Clove weight (g)
Taskopru	33.96 a	7.28 ab	9.37 a	35.06 a	40.63 ab	25.33 b	12.10 ab	2.17 a
Kilis 1	33.09 a	8.69 a	9.00 a	37.44 a	43.76 a	31.51 a	12.90 a	2.49 a
Kilis 2	26.51 b	6.06 b	8.00 a	34.23 a	36.97 b	18.05 c	10.50 b	3.45 a
Kilis 3	26.20 b	6.67 b	8.83 a	36.96 a	38.27 b	23.50 b	11.80 ab	2.32 a

In each column, different letters mean significant differences among the genotypes ($p < 0.05$).

In the study, the mineral content of both garlic leaves and bulbs was determined by analyzing Ca, Mg, Fe, Mn, Zn, Cu, Na and K (Table 3). The main minerals were K (ranging from 3792 to 5844 mg/kg), Ca (ranging from 1994 to 8732 mg/kg) and Mg (ranging from 760 to 2174 mg/kg).

Considerable amounts of Cu (ranging from 4 to 12 mg kg^{-1}), Mn (ranging from 8 to 44 mg/kg), Fe (ranging from 106 to 840 mg/kg), Na (ranging from 136 to 208 mg/kg), and Zn (ranging from 30 to 68 mg/kg) were detected. Apart from the differences between the bulbs and the leaves in terms of mineral content, there were also differences among the genotypes as shown in Fig. 1C and D. According to cluster analyses, garlic genotypes separated from each other on the mineral content of garlic leaves and bulbs. Two obtained main clusters showed that genotypes differed from each other in terms of mineral composition of garlic leaves. There were two main clusters; the genotype Kilis 1 cluster and the other genotypes. Kilis 1 and Kilis 3 were found to exhibit distinct features from Taskopru garlic used as a control in the research. The Taskopru garlic and genotype Kilis 2 were classified into the same group, but genotype Kilis 3 was separated into a minor group (Fig. 1C). Similarly, two obtained major clusters showed that genotypes differ from each other in terms of mineral composition of garlic bulbs.There were two main clusters; the Taskopru garlic and genotype Kilis 3, genotype Kilis 1 and Kilis 2 were classified into the same group (Fig. 1D). Petropoulos *et al.* (2018) studied with local landraces/varieties, imported genotypes and commercial cultivars from Greek to determine for their mineral composition. They found the main minerals were K (ranging from 446 to 675 mg/g) and Ca (ranging from 163 to 963 mg/g), while Fe and Zn were also detected in considerable amounts. Turan *et al.* (2017) collected garlic bulb samples from eight different regions that have been recognized as important regions for cultivating and growing garlic in Kastamonu, Karaman, Kahramanmaraş, Kırklareli, Balıkesir, Hatay, Antalya and Muğla, Turkey. They evaluated the concentration of major (N, P, K, Na, Ca, Mg, S), and minor elements (Zn, Fe, Mn and B) in garlic bulbs and found high variability in the composition of the macro elements (N, P, K, Ca, Mg, S) of garlic samples. The results obtained from this study are in concordance with previous works regarding the mineral composition of garlic (Turan *et al.* 2017, Petropoulos *et al.* 2018).

Table 3. Mineral content of the studied garlic genotypes (mg kg^{-1}).

Genotype	K	Ca	Cu	Mn	Fe	Zn	Mg	Na
					Bulb			
Kilis 1	4090 c	8368 c	4 c	34 b	152 a	68 a	1730 d	146 c
Kilis 2	3792 d	7618 d	12 a	26 c	120 b	40 b	1738 c	136 d
Kilis 3	4392 a	8502 b	6 b	44 a	106 d	34 c	2174 a	156 a
Taskopru	4160 b	8732 a	4 c	14 d	108 c	30 d	2122 b	148 b
					Leaf			
Kilis 1	5844 a	1994 d	8 a	10 a	676 b	46 a	964 a	208 a
Kilis 2	5086 c	2044 c	4 b	10 a	338 d	44 b	826 b	182 b
Kilis 3	5150 b	2390 a	4 b	8 b	840 a	34 d	760 d	166 d
Taskopru	5030 d	2184 b	4 b	8 b	352 c	38 c	810 c	178 c

In each column different Latin letters mean significant differences between the samples ($p < 0.05$).

Table 4 described the volatile sulfur compounds in essential oils originating from the studied genotypes determined by GC-MS analysis. Volatile sulfur components were the main flavoring constituents in all the extracts analyzed in this study. A total of 10 compounds were detected which mainly of diallyl disulfide (22.93-34.75%), trisulfide, di-2-propenyl (3.93- 8.37%), and 1-allyl-2- proplenlyl disulfane (4.63 - 6.20%). Diallyl disulfide was the major compound in all the

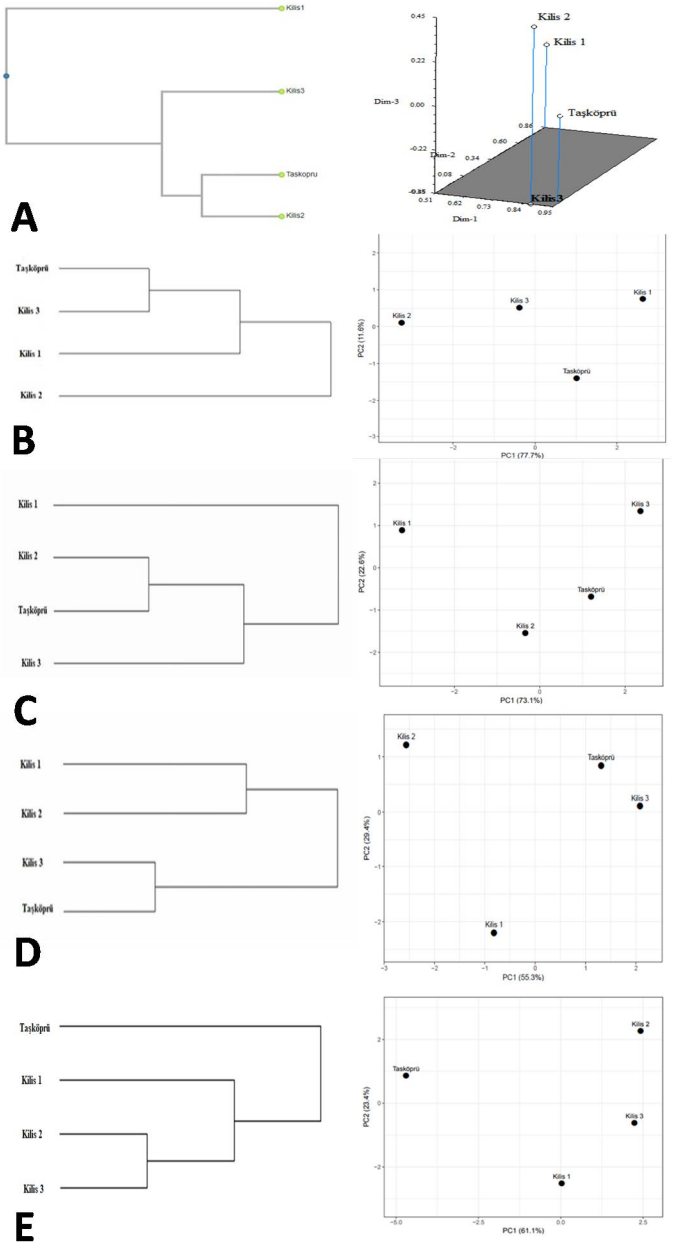

Fig. 1. A- Dendrogram depicting the genetic relationship among the garlic genotypes based on the SSR markers (left), and Scatterplot illustration drawn using SSR data (right), B- Dendrogram depicting the morphological features among the garlic genotypes based on cluster analyses (left) and principal component analysis- PCA (right), C- Dendrogram depicting the mineral content of garlic leaves among the garlic genotypes based on cluster analyses (left) and principal component analysis- PCA (right), D- Dendrogram depicting the mineral content of garlic bulbs among the garlic genotypes based on cluster analyses (left) and principal component analysis- PCA (right), E- Dendrogram depicting the volatile sulfur compounds among the garlic genotypes based on cluster analyses (left) and principal component analysis- PCA (right).

garlic genotypes (Table 4). Its amount was particularly high in Kilis 3 (34.75%) followed by Kilis 2 (31.99%), and Kilis 1 (31.03%). Similarly, Calvo-Gomez *et al.* (2004) stated that diallyl disulfide was the most abundant component of garlic oil. Trisulfide, di-2-propenyl represented the second major volatile, which became the dominant constituent in Taskopru garlic (8.37%), followed by genotypes Kilis 3 (4.90%), and Kilis 1 (4.01%). 1-Allyl-2- proplenlyl disulfane represented the third major volatile compounds and it was particularly high in Taskopru garlic (6.20%). According to cluster analyses and principal component analysis garlic genotypes separated from each other on volatile sulfur compounds of essential oils (Fig. 1E). As seen in Fig. 1E, there were two main clusters; the Taskopru garlic cluster and the other genotypes. The genotype Kilis 2 and genotype Kilis 3 were classified into the same group, but genotype Kilis 1 was separated into a minor group. Kozan (2012) reported that the major flavor compounds of Kastamonu garlic and Denizli garlic were allyl trisulfide, 42.52 and 45.22%, and allyl disulfide, 24.48 and 32.60%, respectively. Avato *et al.* (1998) used 36 different ecotypes of garlic, *Allium sativum*, formerly collected in Southern Italy and in other Mediterranean areas. They also reported that diallyl trisulfide and diallyl disulfide were the two major volatiles found in the oils from all the garlic ecotypes.

Table 4. Identified volatile sulfur compounds in garlic essential oil.

Compound (Nist)	Taskopru		Kilis 1		Kilis 2		Kilis 3	
	RT	% Area	RT	% Area	RT	% Area	RT	% Area
Diallyl sulfide	7.870	1.23	7.866	1.74	7.865	2.40	7.876	2.71
Diallyl disulphide	14.574	22.93	14.571	31.03	14.611	31.99	14.653	34.75
Disulfide, dimethyl	5.284	0.67	5.240	0.51	5.239	0.93	5.262	0.46
Disulfide, methyl 2-propenyl	9.537	5.66	9.528	6.88	9.553	9.51	9.542	5.98
Dimethyl trisulfide	11.108	0.46	11.107	0.19	11.107	0.28	11.107	0.13
Trisulfide, methyl 2-propenyl	16.237	4.70	16.210	1.97	16.241	2.21	16.215	1.71
Trisulfide, di-2-propenyl	20.839	8.37	20,789	4,01	20.797	3.93	20.820	4.90
1-Allyl-2- proplenlyl disulfane	15.115	6.20	15.102	5.74	15.109	4.81	15.125	4.63
1-Allyl-3- proplenlyl trisulfane	21.457	0.69	21.431	0.39	21.443	0.27	21.453	0.33
1-Methyl-2- proplenlyl disulfane	9.923	0.38	10.187	0.84	10.184	0.69	10.182	0.36

RT: Retention time.

Jaccard's similarity matrix was prepared using 13 SSR markers (Table 5). Taskopru garlic and genotype Kilis 1 formed a separate cluster with a similarity of 36%. However, genotypes Kilis 2, Kilis 3 and Taskopru formed a cluster with a similarity of 80%. In principal coordinate scatter plot the differences of genotype Kilis 1 and other genotypes were also quite distinctive (Fig. 1A). Previously Ipek *et al.* (2005) reported that garlic has a heterogeneous genome. (Similarly, Zhao *et al.* (2011) observed higher values of genetic diversity in a total of 613 accessions of garlic and its relatives. Ipek *et al.* (2008) evaluated the genetic relationship between Kastamonu garlic and 20 garlic clones collected from different parts of the world using AFLP and locus specific DNA markers. According to the dendrogram, Taskopru garlic has clustered closely over 97% similarity with other non-bolting garlic clones, PI493112, PI493118, and PI383824. It has been reported that genetic diversity is related to the geographical region and there may have been genetic differences due to adaptation to different geographical conditions with local selection pressure (Jo *et al.* 2012).

Comparison of the dendogram showed that the best fit was observed from depicting the mineral composition of garlic leaves to genetic relationship based on the SSR markers (Fig. 1A and Fig 1C). Genotype Kilis 2 and Taskopru garlic were in the same group and Kilis 3 was near

this group while Kilis 1 was a distinct genotype both genetically and in terms of mineral composition of the leaves. Many researcher reported that chemotaxonomic classification can be usefull to separate genotypes (Riggi *et al.* 2013, Ikram *et al.* 2019, El-Fiki and Adly 2020). Despite the chemical compound of *Allium cepa* L. genotypes have been affected by environmental conditions, the pathway of the bio compounds correspond the genotypes was under the effect of both environment and genetic background (Mogren *et al.* 2006).

Table 5. Similarity matrix computed with Jaccard's coefficient among the garlic genotypes based on SSR markers.

Genotype	Kilis 1	Kilis 2	Kilis 3	Taskopru
Kilis 1	1	0.286	0.286	0.357
Kilis 2		1	0.600	0.800
Kilis 3			1	0.800
Taskopru				1

It is believed that genetic diversity increases with the cultivation of different ecotypes for a long time in the same areas and as a result of the accumulation of natural mutations. The genetic variations of different garlic genotypes should be determined and the genetic relationships between them should be revealed for breeding studies. Studied garlic genotypes in this research, cultivated in Kilis province, showed great diversity in their morphological features, mineral, and volatile sulfur compounds. The SSR markers used for molecular characterization revealed distinctive groups. The results indicated genetic diversity among the genotypes. It seems that this genetic diversity may contribute significantly to the development of new garlic cultivars. Mineral content of the garlic leaves could be used as chemotaxonomic tool for classification of the local garlic genotypes.

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