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ORIGINAL PAPER

CHARACTERIZATION OF MUTANT GARLIC GENOTYPES BASED ON VOLATILE SULFUR COMPOUNDS AND MINERAL CONTENT

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Abstract

This study was conducted to determine volatile sulfur compounds and mineral content of mutant garlic genotypes. The volatile sulfur compounds analysis was performed with a gas chromatography-mass spectrometer. The mineral content of mutant garlic genotypes was determined with an atomic absorption spectrometer and flame photometer. The results indicated that mutant garlic genotypes showed large variation in terms of their content of volatile sulfur compounds and minerals. A total of 16 volatile sulfur compounds were detected, which were mainly diallyl disulfide (68.09%), disulfide methyl propyl (52.34%), disulfide methyl 2-propenyl (29.81%), sulfide allyl methyl (25.72%), diallyl sulfide (22.38%) and disulfide dimethyl (9.88%). The macro- and micro-elements detected in mutant garlic genotypes were K (232.45-513.25 mg L^{-1}), Ca (2.19-7.35 mg L^{-1}), Mg (7.38-21.64 mg L^{-1}), Na (4.52-13.18 mg L^{-1}), Cu (0.01-0.15 mg L^{-1}), Fe (0.13-1.25 mg L^{-1}), Mn (0.10-0.27 mg L^{-1}), and Zn (0.12-0.69 mg L^{-1}). As a result, it was determined that the content of both volatile sulfur compounds and minerals in mutant garlic genotypes was higher than in the control (cv. 'Taşköprü 56'). The genotypes which had a higher content of both volatile sulfur compounds and minerals were GM1, GM8, GM18, GM20, GM21, GM22, GM33, GM35, GM36, GM37, GM39, GM40, GM46, and GM51. The research findings are extremely valuable in revealing the originality of mutant garlic genotypes obtained by mutation breeding, and establishing the foundation for an assessment of mutant garlic genotypes to be used in breeding programs.

Keywords: *Allium sativum* L., garlic, GC-MS, mineral content, mutation breeding, sulfur compounds.

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INTRODUCTION

Garlic (*Allium sativum* L.) is an economically important species of the *Allium* genus, cultivated as a vegetable, spice, and medicinal plant since ancient times across the world (Avato et al. 1998, Divya et al. 2017, Satyal et al. 2017, Petropoulos et al. 2018). And also it is known that garlic belongs to the group named "functional foods" (Turan et al. 2017). Recent studies have revealed that garlic has antioxidant, anti-inflammatory, and antimicrobial properties. These properties allow for successfully treatment and prevention of a wide range of diseases, for example reducing the impact of risk factors of cardiovascular disease, lowering blood pressure and glucose concentrations, stimulating the immune function, reducing the risk of some cancer types including the stomach, colon, and laryngeal cancers, and restoring physical strength (Calvo-Gomez et al. 2004, Gorinstein et al. 2005, Asdaq, Inamdar 2010, Dziri et al. 2014, Divya et al. 2017, Satyal et al. 2017, Turan et al. 2017, Sehitoglu et al. 2018, Yarali Karakan 2022). The positive effects of garlic on human health arise from its chemical composition (Martins et al. 2016, Turan et al. 2017). The biological activities of garlic can be ascribed partially to its volatile essential oils, and non-volatile compounds such as minerals (Wu et al. 2001, Martins et al. 2016, Petropoulos et al. 2018, Akan, Tuna Güneş 2021). Among the volatile sulfur compounds, such as diallyl disulfide, allyl methyl disulfide, and allyl methyl sulfide responsible for flavor, there are labile and reactive compounds, which are released only when cells are damaged by slicing or crushing (Brewster 2008, Ikram et al. 2019). After allicin is produced, it contributes to the formation of allyl methyl disulfide and diallyl disulfide (Ozcan Sinir, Barringer 2020).

Macro- and micro-elements in food are important not only because of their essential nutritional value but also because of their beneficial effects on human health. Mineral elements cannot be synthesized by the human body, thus, they must be supplied by food or else their deficits may cause an illness. Throughout the world, there has been an increasing interest in the use of supplementary foods, including mineral elements, to prevent various diseases, such as osteoporosis or cardiovascular disorders, as well as aging and mineral deficiency (Sharma et al. 2021). Therefore, analyses of the mineral composition of a variety of food samples have critical importance in designing diets for people (Turan et al. 2017). The content of valuable sulfur compounds and mineral elements, analysed in our study, depend on a specific plant genotype (Dziri et al. 2014, Turan et al. 2017, Petropoulos et al. 2018).

The garlic's chemical composition and content of bioactive compound, which determines its quality, strongly depends on a genotype and growing conditions (Martins et al. 2016). Although garlic is reproduced only vegetatively, it exhibits great diversity in morphological and agronomic traits because of various ecotypes grown in the same areas for a long time and undergoing natural mutations (Avato et al. 1998, Petropoulos et al. 2018). Genetic diversity among different garlic populations and ecotypes is advantageously exploited to select germplasms with higher content of bioactive compounds (Martins et al. 2016). However, creating a new variety from the breeding material in this way causes a problem for researchers. Therefore, chemical or physical mutagen applications are widely used to create variability. Mutation breeding is accepted as one of the techniques for creating a new genetic variety of garlic. In mutation breeding, varieties can be created by using different irradiation sources, like proton, electron or gamma ray sources, and they are perceived as physical mutagens. These varieties offer many advantages for plant genetics studies (Taner et al. 2004, Beşirli et al. 2006, Petropoulos et al. 2018, Mahmoud et al. 2020). According to the International Atomic Energy Agency Mutant Variety Database (MVD 2022), one mutant variety was created and registered in 1990 by Chinese researchers, distinguished by its high yield and drought tolerance.

In this paper, it was aimed to characterize mutant garlic genotypes in the context of their content of volatile sulfur compounds and minerals, in order to identify a promising genotype or genotypes that could be used in garlic breeding.

MATERIAL AND METHODS

Plant material

Fifty-one mutant garlic genotypes which had high yield traits at M1V6 stage (Anonymous 2022) and the garlic cv. 'Taşköprü 56' (control) were used as plant material (Table 1). Mutant garlic genotypes (GM) were obtained from Taşköprü 56 (T56), a garlic clone commercially cultivated in Turkey, through the application of gamma rays according to Taner et al. (2004) and Beşirli et al. (2006). Bulbs of the mutant garlic genotypes and cv. 'Taşköprü 56' were grown in Atatürk Horticultural Central Research Institute, Yalova. The climate and soil characteristics of the growing area are given in Table 2. Analyses of the content of volatile sulfur compounds and minerals in the garlic genotypes were performed in the Advanced Technology Application and Research Center of University of Kilis 7 Aralik.

Analysis of volatile sulfur compounds

The volatile oil content analysis was carried out by gas chromatography-mass spectrometry (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. The extraction process was carried out in a headspace unit (Agilent, PAL, RSI 85) with 10 mL, 20 mm aluminum capped vials and a temperature-adjustable oven system. For the volatile oil content analysis,

Table 1

Plant material

Number	Genotype	Number	Genotype	Number	Genotype	Number	Genotype
T56(52)	cv. 'Taşköprü 56'	GM13	1.51/10	GM26	2.65/5	GM39	3.25/2
GM1	1.7/9	GM14	1.55/5	GM27	2.68/7	GM40	3.25/8
GM2	1.14/230	GM15	1.66/6	GM28	2.73/8	GM41	3.25/9
GM3	1.15/5	GM16	2.13/3	GM29	2.78/5	GM42	3.35/1
GM4	1.15/72	GM17	2.13/8	GM30	2.78/11	GM43	3.34/2
GM5	1.20/18	GM18	2.20/4	GM31	2.09/2	GM44	3.39/1
GM6	1.21/2	GM19	2.20/9	GM32	2.92/3	GM45	3.40/50
GM7	1.24/4	GM20	2.22/6	GM33	20.16/2	GM46	3.42/3
GM8	1.27/6	GM21	2.32/3	GM34	3.16/2	GM47	3.48/5
GM9	1.34/3	GM22	2.33/2	GM35	3.21/1	GM48	3.52/4
GM10	1.35/2	GM23	2.34/9	GM36	3.21/9	GM49	3.52/5
GM11	1.44/6	GM24	2.39/6	GM37	3.21/13	GM50	3.55/1
GM12	1.51/2	GM25	2.56/2	GM38	3.24/4	GM51	3.61/1

Table 2

The soil and climate characteristics of growing area

Soil characteristics (sandy-loam)							
EC_{25} (ds m^{-1})	pH	lime (%)	organic matter (%)	phosphorus (mg kg^{-1})	potassium (mg kg^{-1})	calcium (mg kg^{-1})	magnesium (mg kg^{-1})
0.16	7.3	0.20	2.3	23	193	75.50	292

Climate characteristics (2020)				
temperature (°C)			humidity (%)	total precipitation (mm)
maximum	minimum	average		
20.06	12.06	16.06	72.95	51.33

5 g fresh garlic samples were sliced and transferred to 10 ml aluminum capped vials. The headspace unit extraction temperature was kept constant at 80°C for 45 minutes. Extracted samples were transferred from the vial to the GC-MS system by an autosampler.

Analysis conditions: The column temperature was increased from 50°C to 240°C with an increase of 10°C min^{-1}. The analysis was terminated after 6 min at 240°C. MS source temp. is 230°C, MS quad to temp. 150°C, and syringe temp. 80°C. The split ratio is 5/1, the injection volume 2.5 mL and the flow rate 3 ml min^{-1}. Total analysis time was 27 min and the conditions set for the analysis were maintained for 4 min to re-establish and equilibrate the initial conditions (Molina-Calle et al. 2017). Identification of essential oil components was achieved by comparing the GC retention times and MS degradation products with data from the NIST library. Results were evaluated as percentage components, and measurements were performed in three replicates.

Determination of mineral content

Calcium (Ca), magnesium (Mg), iron (Fe), manganese (Mn), zinc (Zn), copper (Cu), sodium (Na) and potassium (K) concentrations in garlic were determined using an atomic absorption spectrometer (Perkin Elmer brand, 240 FS AA model) and flame photometer (Jenway brand, PFP 7 model). Garlic samples were dried in a compressed air oven at 72°C, and the dried samples were pulverized in mortar and pestle. 0.25 g of sample were mixed with 9 mL of HNO_3 and 3 ml of H_2O_2 and burned in a Cem brand Mars 6 model microwave oven under 200W power for 30 minutes. The incinerated samples were filtered on Whatman filter paper and diluted by adding distilled water to a final volume of 25 mL. Calcium, magnesium, iron, manganese, zinc, and copper were determined on an atomic absorption spectrophotometer using the flame ionization method; sodium and potassium were determined on a flame photometer (Tefera, Chandravanshi 2018). All analyses were performed in triplicate.

Statistical analysis

JMP pro version 14 (SAS Institute, NC, USA) was used for all statistical analysis. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were performed to describe patterns of variation in volatile sulfur compounds as well as the mineral content of garlic genotypes. The distribution of volatile sulfur compounds and mineral content of genotypes was computed using the first two components (PC1 and PC2).

RESULTS AND DISCUSSION

The volatile sulfur compounds of mutant garlic genotypes

The volatile sulfur compounds of essential oils originating from the genotypes determined by GC-MS analysis were presented and graphed in Table 3. A total of 16 volatile sulfur compounds were detected, which were mainly diallyl disulfide (68.09%), disulfide methyl propyl (52.34%), disulfide methyl 2-propenyl (29.81%), sulfide allyl methyl (25.72%), diallyl sulfide (22.38%) and disulfide dimethyl (9.88%). Diallyl disulfide was the major component in all the mutant garlic genotypes and cv. 'Taşköprü 56' (Table 3). Its amount was particularly high in GM18 (68.09%), GM19 (62.26%) and GM42 (60.17%). Disulfide methyl propyl was the second major volatile component

Table 3

The mineral content of mutant garlic genotypes

Genotype	Sulfide, allyl methyl	Disulfide, dimethyl	Diallyl sulfide	Diallyl disulfide	Disulfide, methyl propyl	Disulfide, methyl 2-propenyl	Dimethyl trisulfide	Trisulfide-methyl 2-propenyl	Trisulfide, di-2-propenyl	1-Methyl-2-(prop-1-en-1-yl) disulfane	1-Allyl-2-isopropyldisulfane	1-Allyl-2-(prop-1-en-1-yl) disulfane	1-(Prop-1-en-1-yl)-2-propyldisulfane	1-((E)-Prop-1-en-1-yl)-2-((Z)-prop-1-en-1-yl) disulfane	1,2-Di((E)-prop-1-en-1-yl) disulfane	Disulfide, methyl (methylthio) methyl
T56 (52)	3.36±0.02	1.13±0.01	4.17±0.08	59.88±0.04	nd	19.06±0.03	nd	0.67±0.04	nd	1.06±0.04	0.45±0.02	7.08±0.04	nd	nd	nd	nd
GM1	16.47±0.12	9.69±0.06	8.47±0.03	35.31±0.01	0.26±0.01	26.54±0.03	0.12±0.01	0.51±0.02	nd	0.29±0.01	0.71±0.02	nd	0.04±0.01	nd	nd	nd
GM2	17.19±0.01	6.61±0.29	12.29±0.09	37.81±0.05	0.16±0.03	22.71±0.10	0.12±0.01	0.49±0.04	nd	0.32±0.03	0.64±0.02	0.05±0.01	nd	nd	nd	nd
GM3	15.83±0.06	9.88±0.41	8.04±0.24	35.96±0.09	0.25±0.02	26.99±0.47	0.10±0.03	0.44±0.04	nd	0.28±0.02	0.66±0.06	nd	nd	nd	nd	nd
GM4	11.3±0.94	5.2±0.38	9.20±0.57	47.41±0.94	nd	24.57±0.15	nd	0.29±0.05	nd	0.25±0.02	0.46±0.02	nd	nd	nd	nd	nd
GM5	5.93±0.05	2.36±0.03	8.97±0.13	53.05±0.40	nd	23.58±0.24	nd	nd	nd	0.29±0.03	0.62±0.04	1.27±0.07	nd	nd	nd	nd
GM6	8.37±0.03	7.05±0.05	5.51±0.05	42.40±.098	0.84±0.04	29.32±0.14	0.09±0.01	0.55±0.09	0.01±0.01	0.88±0.07	2.11±0.09	0.17±0.02	nd	nd	nd	nd
GM7	9.89±0.09	6.48±0.08	7.60±0.10	44.88±0.10	0.28±0.05	26.51±0.24	nd	0.29±0.02	nd	0.33±0.03	1.20±0.07	nd	nd	nd	nd	nd
GM8	10.43±0.09	1.03±0.03	15.96±0.05	49.32±0.16	nd	17.30±0.52	nd	0.24±0.01	nd	nd	0.52±0.01	nd	nd	nd	nd	nd
GM9	16.46±0.42	8.15±0.45	9.57±0.27	37.84±0.73	0.22±0.01	24.76±0.13	0.09±0.01	0.41±0.02	nd	0.27±0.01	0.77±0.03	nd	nd	nd	nd	nd
GM10	5.85±0.11	1.70±0.09	6.44±0.05	55.26±0.12	nd	22.85±0.02	nd	nd	nd	nd	nd	1.16±0.05	0.29±0.03	nd	nd	nd
GM11	14.62±0.05	8.36±0.06	9.18±0.06	37.85±0.19	0.28±0.03	25.38±0.04	0.21±0.03	0.67±0.01	0.11±0.03	0.77±0.05	0.93±0.04	0.10±0.01	nd	nd	nd	0.03±0.01
GM12	7.15±0.01	8.79±0.05	4.42±0.01	44.85±0.05	0.41±0.04	29.81±0.03	0.11±0.01	0.54±0.01	nd	0.62±0.02	1.18±0.02	0.13±0.01	nd	nd	nd	nd
GM13	4.76±0.02	1.47±0.01	4.75±0.02	57.46±0.09	nd	21.81±0.09	nd	nd	nd	0.18±0.22	0.31±0.04	1.36±0.06	nd	nd	nd	nd
GM14	8.16±0.01	2.84±0.09	10.11±0.04	48.07±0.45	nd	23.98±0.13	nd	0.17±0.01	nd	0.17±0.01	0.42±0.01	nd	nd	nd	nd	nd
GM15	7.79±0.10	4.41±0.09	7.41±0.10	48.94±0.19	nd	25.87±0.18	nd	0.29±0.02	nd	0.46±0.02	0.93±0.01	nd	0.10±0.1	nd	nd	nd
GM16	4.05±0.03	1.53±0.03	5.17±0.14	58.01±0.40	nd	20.74±0.14	nd	nd	nd	nd	0.32±0.02	0.65±0.04	2.58±0.05	nd	nd	nd
GM17	4.41±0.10	2.06±0.04	2.89±0.47	48.73±1.20	nd	23.22±0.43	nd	0.45±0.04	nd	1.17±0.04	nd	4.86±0.31	0.46±0.04	nd	0.22±0.03	nd
GM18	4.05±0.02	1.82±0.39	1.11±0.14	68.09±0.90	nd	11.06±0.14	nd	nd	nd	1.22±0.15	nd	9.07±0.47	0.54±0.08	nd	nd	nd
GM19	3.23±0.20	1.00±0.18	5.70±0.14	62.26±0.45	nd	0.25±0.01	nd	nd	nd	nd	0.46±0.05	1.95±0.24	0.11±0.02	nd	nd	nd
GM20	16.88±0.47	6.23±0.45	12.02±0.30	38.93±0.49	0.15±0.02	23.69±0.39	nd	0.30±0.03	nd	0.24±0.04	0.58±0.05	nd	nd	nd	nd	nd
GM21	5.59±0.04	0.90±0.05	8.68±0.03	59.39±0.01	nd	17.45±0.05	nd	nd	nd	0.37±0.01	1.28±0.02	nd	nd	nd	nd	nd
GM22	16.26±0.13	6.21±0.01	11.69±0.02	38.80±0.02	23.05±0.05	nd	nd	0.30±0.01	0.17±0.01	0.51±0.03	nd	nd	nd	nd	nd	nd
GM23	6.56±0.33	6.18±0.08	5.10±0.14	27.77±1.31	nd	nd	nd	0.34±0.06	nd	0.27±0.04	0.68±0.08	0.07±0.01	nd	nd	nd	nd
GM24	8.06±0.10	1.97±0.04	9.79±0.21	48.86±1.45	nd	23.06±0.49	nd	0.33±0.05	nd	0.52±0.09	0.34±0.06	2.15±0.39	nd	nd	nd	nd
GM25	4.03±0.47	5.77±0.05	3.84±0.17	51.66±1.69	nd	29.10±0.41	nd	0.38±0.01	nd	0.72±0.07	1.02±0.07	0.21±0.03	nd	nd	nd	nd
GM26	6.46±0.06	7.95±0.43	4.32±0.40	48.22±1.31	0.27±0.05	28.98±0.46	0.09±0.01	0.38±0.02	nd	0.45±0.06	0.92±0.06	0.15±0.03	nd	nd	nd	nd
GM27	3.44±0.10	1.28±0.12	5.60±0.31	56.14±1.95	nd	19.89±0.68	nd	0.31±0.05	nd	0.83±0.08	0.66±0.05	5.04±0.49	0.24±0.05	nd	nd	nd
GM28	5.67±0.32	1.32±0.16	8.21±0.10	54.48±0.71	nd	20.73±3.68	nd	0.25±0.03	nd	0.30±0.09	0.77±0.08	2.19±0.09	nd	nd	nd	nd
GM29	9.91±0.13	1.72±0.25	6.77±0.47	48.20±1.06	nd	19.67±0.74	nd	0.38±0.04	nd	0.27±0.03	0.49±0.01	1.71±0.10	nd	nd	nd	nd
GM30	10.09±0.47	1.87±0.14	10.41±0.86	48.56±1.28	nd	22.34±0.59	nd	0.38±0.04	nd	0.31±0.05	0.41±0.04	1.34±0.07	nd	nd	nd	nd

cont. Table 3

The mineral content of mutant garlic genotypes

Genotype	Sulfide, allyl methyl	Disulfide, dimethyl	Diallyl sulfide	Diallyl disulfide	Disulfide, methyl propyl	Disulfide, methyl 2-propenyl	Dimethyl trisulfide	Trisulfide-methyl 2-propenyl	Trisulfide, di-2-propenyl	1-Methyl-2-(prop-1-en-1-yl) disulfane	1-Allyl-2-isopropyldisulfane	1-Allyl-2-(prop-1-en-1-yl) disulfane	1-(Prop-1-en-1-yl)-2-propyldisulfane	1-((E)-Prop-1-en-1-yl)-2-((Z)-prop-1-en-1-yl) disulfane	1,2-Di((E)-prop-1-en-1-yl) disulfane	Disulfide, methyl (methylthio) methyl
GM31	8.04±0.40	3.21±0.05	7.52±0.12	48.68±1.68	nd	26.45±1.63	nd	0.24±0.43	nd	0.22±0.04	0.31±0.02	0.86±0.05	nd	nd	nd	nd
GM32	14.20±1.43	2.32±0.47	12.33±0.91	42.62±0.57	nd	21.85±4.71	nd	0.43±0.09	nd	0.33±0.10	0.35±0.02	1.13±0.12	nd	nd	nd	nd
GM33	4.68±0.33	2.81±0.47	6.35±0.27	57.21±0.48	nd	22.96±4.71	nd	0.24±0.25	0.08±0.02	0.61±0.10	0.88±0.02	0.32±0.04	nd	nd	nd	nd
GM34	3.49±0.43	1.81±0.15	3.74±0.47	58.47±1.18	nd	23.46±0.50	nd	nd	nd	0.52±0.05	0.55±0.05	2.83±0.15	nd	nd	nd	nd
GM35	11.57±0.26	8.24±0.29	6.76±0.33	43.18±0.39	0.18±0.01	26.70±0.62	0.09±	0.44±0.10	0.07±0.01	0.41±0.10	0.63±0.11	0.09±0.01	nd	nd	nd	nd
GM36	16.09±0.71	0.89±0.04	22.38±0.40	40.30±0.77	nd	13.02±0.47	nd	nd	nd	0.73±0.11	nd	nd	0.25±0.05	nd	nd	nd
GM37	5.23±0.07	2.17±0.33	7.34±0.42	51.08±0.46	nd	24.79±0.13	nd	0.24±0.05	nd	0.66±0.08	0.93±0.06	2.65±0.32	nd	nd	0.12±0.01	nd
GM38	5.00±0.47	0.91±0.05	5.00±0.47	58.53±1.15	nd	16.70±0.37	nd	nd	nd	0.32±0.04	nd	1.93±0.25	0.32±0.07	nd	nd	nd
GM39	6.66±0.08	1.92±0.24	3.63±0.09	nd	52.34±0.68	23.44±0.79	nd	0.50±0.05	nd	0.90±0.12	0.43±0.10	3.91±0.45	nd	nd	nd	nd
GM40	25.72±0.66	3.87±0.37	19.87±0.90	31.37±0.48	0.11±0.02	16.35±0.41	0.17±0.02	0.30±0.07	0.11±0.02	0.15±0.02	0.57±0.08	nd	nd	nd	nd	nd
GM41	11.76±0.55	4.79±0.38	9.64±0.29	44.38±0.55	nd	24.38±0.74	nd	0.21±0.04	nd	0.32±0.04	1.18±0.20	0.07±0.01	nd	nd	nd	nd
GM42	2.59±0.07	1.79±0.07	3.13±0.51	60.17±0.54	nd	23.20±0.38	nd	0.26±0.04	nd	0.39±0.02	0.61±0.06	2.28±0.37	nd	nd	nd	nd
GM43	14.79±0.21	2.81±0.16	13.84±0.40	41.01±1.13	nd	21.16±0.46	0.17±0.02	0.30±0.03	nd	0.27±0.02	0.51±0.07	nd	nd	nd	nd	nd
GM44	3.17±0.03	1.78±0.21	4.05±0.23	58.92±0.96	nd	24.63±0.50	nd	0.24±0.03	nd	0.26±0.06	0.42±0.04	1.48±0.10	nd	0.12±0.01	nd	nd
GM45	5.25±0.24	1.52±0.15	7.07±0.04	54.44±0.66	nd	20.88±0.45	nd	0.43±0.04	nd	0.24±0.05	0.66±0.08	1.98±0.04	0.14±0.01	nd	nd	nd
GM46	7.95±0.05	7.78±0.04	5.15±0.03	45.91±0.43	0.35±0.03	nd	0.10±0.03	0.40±0.09	0.10±0.03	0.42±0.03	1.13±0.07	0.11±0.01	nd	nd	nd	nd
GM47	13.05±0.07	1.91±0.05	13.52±0.05	42.26±0.03	nd	20.74±0.09	nd	nd	nd	0.22±0.02	0.49±0.04	0.94±0.02	nd	nd	nd	nd
GM48	6.22±0.10	1.42±0.03	7.36±0.02	52.23±0.11	nd	20.11±0.05	nd	0.28±0.04	nd	0.75±0.05	0.39±0.04	4.03±0.04	nd	nd	nd	nd
GM49	13.22±0.10	2.14±0.06	12.52±0.05	42.89±0.07	0.13±0.01	22.52±0.09	nd	0.34±0.04	nd	0.40±0.09	0.57±0.05	1.29±0.05	nd	nd	nd	nd
GM50	5.32±0.06	2.58±0.04	6.87±0.05	50.44±0.21	nd	25.21±0.03	nd	0.22±0.02	nd	0.35±0.03	0.75±0.03	1.47±0.06	nd	nd	nd	nd
GM51	5.46±0.03	2.13±0.06	5.08±0.05	51.03±0.07	nd	25.16±0.08	nd	0.40±0.09	nd	0.80±0.07	0.57±0.03	3.88±0.47	nd	nd	nd	nd

nd – not detected (p<0.05, n=3)

of the garlic genotypes, which even becomes the dominant constituent in GM39 (52.34%) and GM22 (23.05%) genotypes. Disulfide methyl 2-propenyl was the third major volatile components. Its amount was particularly high in GM12 (29.81%) and GM6 (29.32%). Similarly, Calvo-Gomez et al. (2004), Kim et al. (2004), Keles et al. (2014), Molina-Calle et al. (2016), Satyal et al. (2017), Ozcan Sinir, Barringer (2020) and Yarali Karakan (2022) reported that diallyl disulfide was the most abundant component of garlic essential oils. Similarly, diallyl trisulfide and diallyl disulfide were the two major volatiles found in the oils from all the selected garlic ecotypes collected in Southern Italy and other Mediterranean areas by Avato et al. (1998). On the other hand, Kozan (2012) identified that the major flavor components of Kastamonu and Denizli garlic were allyl trisulfide (42.52% and 45.22%, respectively) and allyl disulfide (24.48% and 32.60%, respectively). The differences in results could be related to the geographical location (soil contents, climate difference, etc.), genetic variation as well as extraction techniques (Sufer, Bozok 2019). Unlike the other sulfur compounds 1-((E)-prop-1-en-1-yl)-2-((Z)-prop-1-en-1-yl) disulfane was detected only in genotype GM44, 1,2-di((E)-prop-1-en-1-yl) disulfane was detected in genotype GM17 and genotype GM37, disulfide methyl (methylthio) methyl was detected in genotype GM6 and GM11. In the current study, the other main sulfides were disulfide methyl propyl, disulfide methyl 2-propenyl, and sulfide allyl methyl and diallyl sulfide.

In this study, relationships between genotypes and volatile sulfur compounds using principal components biplot analysis were determined and given in Figure 1. It was calculated by using the scatter biplot method that PC1 (25.8%) and PC2 (15.9%) constituted 41.7% of the total variation. When the relationships among genotypes and volatile sulfur compounds are interpreted using a biplot graph, the relationship between a genotype and volatile sulfur compounds can be clearly demonstrated. As a matter of fact, disulfide dimethyl and dimethyl trisulfide and, disulfide methyl 2- propenyl, disulfide methyl propyl, disulfide methyl (methylthio) methyl, trisulfide methyl-2--propenyl, 1-allyl-2-isopropyldisulfane which have a narrow angle, were positively related to each other. However, sulfide allyl methyl, trisulfide di-2-propenly and diallyl sulfide, which have a wide angle, were in another group. It was demonstrated that the correlation with these properties was negative and weak for 1-methyl-2-(prop-1-en-1-yl) disulfane, 1,2 di ((E)-prop--1-en-1-yl) disulfane, 1-allyl-2-(prop-1-en-1-yl) disulfane, and diallyl disulphide. The variation between genotypes increases as the vector moves away from the origin, while the variation between genotypes decreased as the vector approaches to the origin. The heat map of the principal component analysis was conducted to demonstrate the similarities and dissimilarities among garlic genotypes in terms of the content of detected volatile sulfur compounds (Figure 2). In a heat map, rows and columns are grouped by correlation distance and average linkage. Regarding the results of the principal component analysis (PCA), 29 mutant garlic genotypes (GM1, GM2, GM3, GM6, GM7,

GM8, GM9, GM11, GM12, GM17, GM18, GM20, GM21, GM22, GM26, GM27, GM32, GM33, GM35, GM36, GM37, GM39, GM40, GM41, GM43, GM46, GM48, GM49, and GM51) were promising in terms of volatile sulfur compounds (Figure 1 and Figure 2).

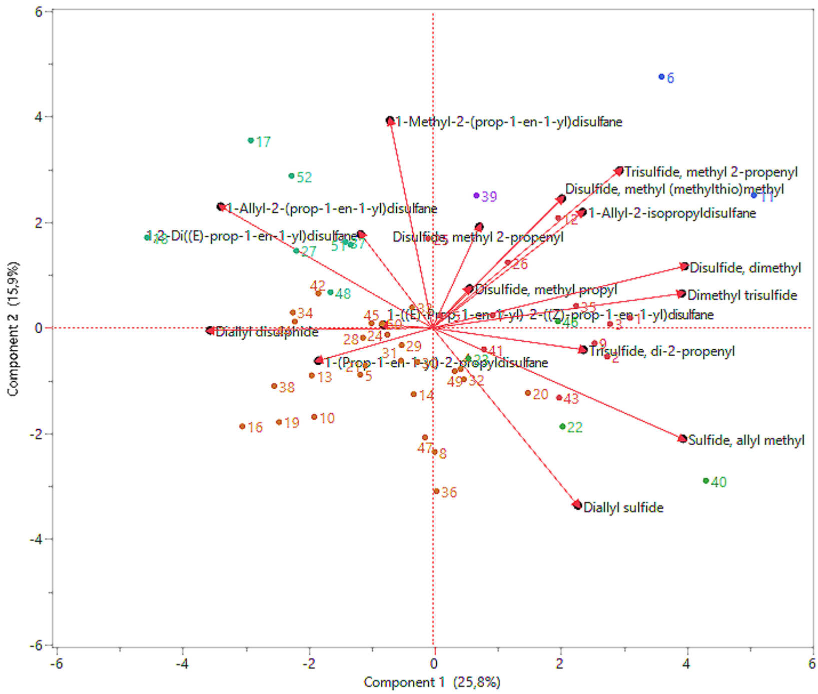

Fig. 1. Relationships among genotypes and volatile sulfur compounds obtained from the PCA biplot analysis

In addition, two-way hierarchical clustering analyses (HCA) were carried out, demonstrating that garlic genotypes could be separated from each other according to the volatile sulfur compounds of essential oils (Figure 3). The dendrogram and constellation plot revealed that there was a clear separation among 52 genotypes. It presented two major clusters, and in each cluster it showed the existence of differences and similarities among the genotypes based on the volatile sulfur compounds. As seen in Figure 3, cluster I comprised 18 garlic genotypes, and was divided into two subgroups; GM6 and GM11 and the other genotypes. Cluster II consisted of 34 genotypes, which were divided into two subgroups.

The mineral content of mutant garlic genotypes

The relationships among the garlic genotypes according to macro- and microelements (Ca, K, Mg, Na, Cu, Fe, Mn, Zn) determined on an atomic absorption spectrometer and flame photometer are presented in Table 4.

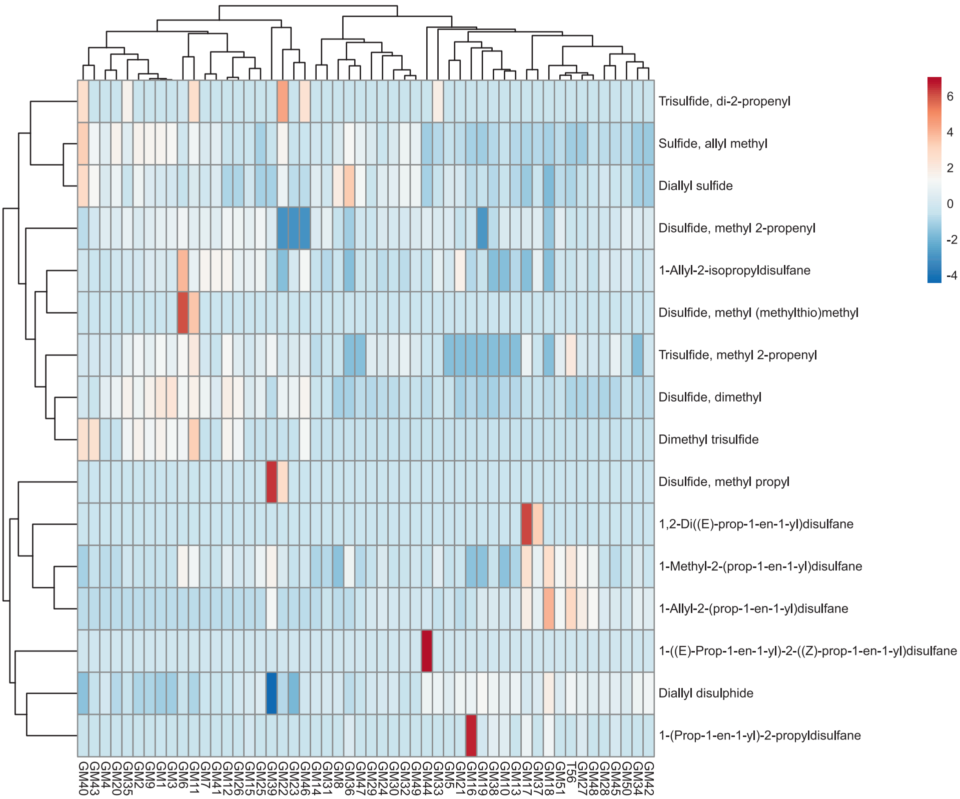

Fig. 2. The heat map of the principal component analysis on common sulfide volatiles in garlic genotypes

These minerals in garlic genotypes were K (232.45-513.25 mg L^{-1}), Ca (2.19-7.35 mg L^{-1}), Mg (7.38-21.64 mg L^{-1}), Na (4.52-13.18 mg L^{-1}), Cu (0.01-0.15 mg L^{-1}), Fe (0.13-1.25 mg L^{-1}), Mn (0.10-0.27 mg L^{-1}), and Zn (0.12-0.69 mg L^{-1}). Similarly, there was a high level of K in garlic bulbs, but also significant amounts of Mg, Na and Ca in the study of Hacıseferogulları et al. (2005). They determined the main minerals in garlic bulbs as K (21378.84 mg kg^{-1}), P (6009.37 mg kg^{-1}), Mg (1056.15 mg kg^{-1}), Na (532.78 ppm) and Ca (363.61 ppm). Petropoulos et al. (2018) reported that the main minerals were K (446-675 mg g^{-1}) and Ca (163-963 mg g^{-1}), while Fe and Zn were also detected in considerable amounts in local landraces/varieties, imported genotypes and commercial cultivars from Greece. In addition, Sajid et al. (2014), and Lee et al. (2016), Divya et al. (2017) and Yarali Karakan (2022) reported that potassium (K) was the major mineral in garlic. As a result, the data obtained from this study are in agreement with the aforementioned studies carried out on the mineral composition of garlic. Contrary to these findings, Akinwande and Olatunde (2015) found that garlic had a high content of P (4777.88 mg kg^{-1}) and Zn (66.08 mg kg^{-1}) but a low

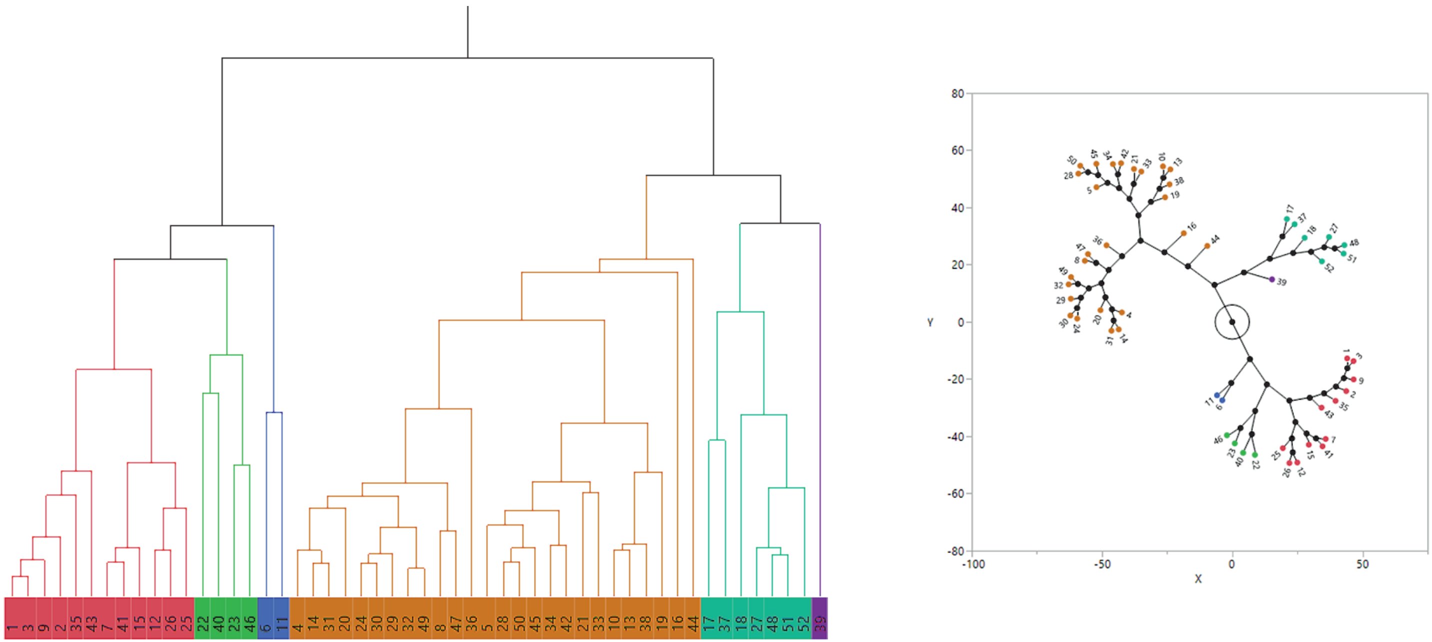

Fig. 3. Dendrogram and constellation plot obtained from the cluster analysis of 51 mutant garlic genotypes and cv. ‘Taşköprü 56’ according to essential oil compositions (individual clusters highlighted by different colored lines)

Table 4

The mineral content of mutant garlic genotypes

Genotype	Ca	K	Mg	Na	Cu	Fe	Mn	Zn
T56 (52)	3.95±0.18	429.46±0.93	18.89±2.16	7.62±0.36	0.12±0.02	0.66±	0.18±0.02	0.45±0.03
GM1	5.02±0.45	418.42±4.39	15.66±0.37	9.93±0.50	0.13±0.03	0.82±0.20	0.18±0.03	0.51±0.05
GM2	4.97±0.42	372.40±2.57	13.93±0.15	8.84±0.66	0.11±0.03	0.73±0.10	0.16±0.04	0.45±0.08
GM3	3.85±0.15	407.80±3.53	12.95±0.67	7.93±0.45	nd	0.23±0.04	nd	0.38±0.04
GM4	3.94±0.14	417.41±8.73	13.26±0.98	8.11±0.59	nd	0.24±0.03	nd	0.39±0.02
GM5	4.86±0.48	438.56±7.63	20.21±0.41	9.06±0.49	0.12±0.02	0.75±0.04	0.19±0.01	0.48±0.01
GM6	3.84±0.38	346.46±8.92	15.96±0.93	7.16±0.48	0.10±0.03	0.59±0.07	0.15±0.03	0.38±0.02
GM7	4.29±0.05	406.33±3.83	13.15±1.03	8.70±0.29	nd	0.44±0.10	nd	0.39±0.07
GM8	5.17±0.45	433.22±7.06	15.72±0.49	8.29±0.48	0.13±0.03	0.94±0.06	0.16±0.04	0.45±0.09
GM9	3.87±0.13	416.71±3.70	12.48±0.94	6.11±0.01	nd	0.48±0.09	0.12±0.01	0.52±0.13
GM10	4.24±0.12	423.46±7.08	13.87±0.83	9.52±0.51	0.11±0.01	0.76±0.08	0.17±0.02	0.53±0.10
GM11	3.99±0.33	378.13±2.83	12.24±1.03	8.09±0.45	0.01±0.01	0.41±0.07	nd	0.36±0.07
GM12	2.19±0.07	232.45±1.89	7.38±0.34	4.52±0.35	nd	0.13±0.01	nd	0.22±0.03
GM13	2.39±0.23	253.37±5.67	8.05±0.45	4.93±0.26	nd	0.15±0.03	nd	0.24±0.04
GM14	3.57±0.30	396.74±9.94	18.44±0.52	8.94±0.54	0.03±0.01	0.14±0.02	nd	0.37±0.06
GM15	5.11±0.43	428.97±2.91	15.57±1.28	8.21±0.32	0.13±0.03	0.93±0.04	0.16±0.02	0.44±0.05
GM16	4.74±0.41	414.05±4.52	19.67±0.84	8.82±0.41	0.12±0.02	0.73±0.09	0.19±0.02	0.47±0.01
GM17	4.55±0.34	409.87±4.77	18.88±1.00	8.47±0.48	0.11±0.02	0.70±0.09	0.18±0.02	0.45±0.08
GM18	4.98±0.44	426.90±1.19	18.77±0.97	8.14±0.32	0.14±0.02	0.77±0.09	0.19±0.02	0.46±0.07
GM19	4.93±0.33	422.59±6.64	18.58±1.15	8.05±0.45	0.14±0.01	0.76±0.11	0.19±0.04	0.46±0.09
GM20	4.71±0.05	423.15±5.71	18.47±1.37	9.16±0.52	0.15±0.01	0.70±0.14	0.21±0.01	0.39±0.04
GM21	3.79±0.38	422.07±8.62	19.62±1.99	9.51±0.52	0.04±0.01	0.15±0.05	nd	0.39±0.05
GM22	6.86±0.20	479.68±2.21	18.48±1.91	11.85±0.13	0.11±0.02	1.17±0.01	0.23±0.01	0.64±0.11
GM23	6.04±0.10	422.12±1.92	16.26±0.18	10.43±0.37	0.09±0.01	1.03±0.09	0.20±0.03	0.57±0.08
GM24	4.88±0.19	426.92±1.11	20.28±0.49	9.09±0.06	0.12±0.03	0.75±0.09	0.19±0.04	0.49±0.06
GM25	4.86±0.36	428.52±1.17	19.95±0.77	9.04±0.10	0.11±0.02	0.75±0.04	0.21±0.03	0.49±0.08
GM26	4.05±0.27	362.63±3.12	14.42±0.97	7.88±0.28	nd	0.36±0.04	nd	0.22±0.03
GM27	3.38±0.32	370.50±0.96	13.77±1.05	7.50±0.35	0.08±0.01	0.33±0.01	0.15±0,01	0.13±0.02
GM28	3.61±0.29	376.34±2.04	13.90±0.97	7.43±0.36	nd	0.34±0.04	0.17±0.02	0.12±0.03
GM29	4.31±0.42	407.14±3.80	15.82±0.44	8.73±0.37	0.06±0.01	0.37±0.04	0.10±0.01	0.21±0.03
GM30	4.45±0.36	398.49±8.60	15.84±0.18	8.66±0.56	nd	0.39±0.01	nd	0.24±0.03
GM31	5.21±0.37	455.45±7.27	21.64±0.29	9.70±0.41	0.13±0.02	0.80±0.08	0.21±0.01	0.52±0.05
GM32	5.05±0.39	421.14±1.92	17.85±0.66	8.32±0.21	0.09±0.01	0.61±0.04	0.19±0.02	0.38±0.06
GM33	7.07±0.44	494.51±1.76	19.05±0.93	12.21±0.48	0.11±0.01	1.21±0.21	0.23±0.03	0.66±0.09
GM34	6.95±0.09	490.18±1.93	17.48±0.84	12.22±0.87	0.13±0.02	1.17±0.07	0.23±0.05	0.66±0.08
GM35	6.76±0.18	474.40±1.36	18.17±0.95	13.09±0.11	0.11±0.01	1.07±0.03	0.26±0.03	0.58±0.04
GM36	6.77±0.20	475.63±3.10	18.22±0.51	13.12±0.26	0.11±0.03	1.07±0.04	0.26±0.04	0.58±0.03
GM37	6.81±0.11	477.79±1.45	18.30±0.23	13.18±0.41	0.11±0.02	1.08±0.08	0.27±0.03	0.58±0.12
GM38	7.34±0.50	513.25±8.85	19.77±0.64	12.68±0.27	nd	1.25±0.19	0.24±0.05	0.69±0.09
GM39	6.93±0.01	485.14±2.10	18.01±2.05	12.71±0.36	0.13±0.03	1.10±0.08	0.24±0.02	0.53±0.05
GM40	6.79±0.21	475.44±1.95	17.65±0.50	12.45±0.37	0.13±0.02	1.08±0.06	0.23±0.02	0.52±0.04
GM41	6.40±0.43	491.26±1.92	15.84±0.44	9.96±0.19	0.09±0.01	0.76±0.09	0.19±0.02	0.55±0.04
GM42	6.46±0.49	451.66±6.73	17.40±0.55	11.16±0.45	nd	1.10±0.08	0.21±0.04	0.61±0.08
GM43	5.75±0.11	401.98±3.31	15.49±0.59	9.93±0.34	nd	0.98±0.14	0.19±0.02	0.54±0.04
GM44	6.26±0.04	491.80±2.32	15.85±1.32	10.70±0.37	0.10±0.01	0.81±0.10	0.21±0.02	0.48±0.04
GM45	7.35±0.42	491.14±1.83	16.82±1.42	11.21±0.69	0.08±0.02	1.02±0.10	0.22±0.03	0.40±0.07
GM46	6.07±0.57	491.84±2.51	16.85±1.49	11.58±0.59	0.12±0.03	1.04±0.13	0.21±0.01	0.37±0.02
GM47	5.20±0.36	510.40±7.05	15.78±0.51	11.05±0.49	nd	0.35±0.03	0.19±0.01	0.24±0.03
GM48	5.74±0.37	484.53±12.98	15.03±0.48	10.98±0.62	nd	0.34±0.02	0.13±0.02	0.22±0.03
GM49	4.95±0.43	486.09±7.54	15.03±0.93	10.53±0.34	nd	0.33±0.04	0.18±0.04	0.23±0.03
GM50	5.35±0.32	474.30±8.67	17.93±2.24	12.44±0.59	nd	0.25±0.01	0.10±0.01	0.52±0.06
GM51	5.79±0.41	486.86±5.19	18.47±2.43	12.84±0.43	nd	0.22±0.03	0.15±0.04	0.40±0.05

nd – not detected (p<0.05, n=3).

content of calcium, magnesium, iron and aluminum. Turan et al. (2017) found high variability in the composition of the minerals (N, P, K, Ca, Mg, S) in garlic samples. They determined the mineral concentrations of garlic bulb samples to be 23.81 g kg^{-1} of N, 3.90 g kg^{-1} of P, 12.33 g kg^{-1} of K, 0.42 g kg^{-1} of Ca, 10.50 g kg^{-1} of Mg and 10.23 g kg^{-1} of S, 2.49 g kg^{-1} of Zn, 11.46 g kg^{-1} of Fe, 0.72 g kg^{-1} of Mn, 1.00 g kg^{-1} of B and 2.34 g kg^{-1} of Na. Variations in the mineral composition reported by different researchers may be attributed to the effect of many factors, like climate conditions, genotype and analytical procedures (Martins et al. 2016, Turan et al. 2017).

Relationships between the genotypes and mineral content were computed by using the scatter biplot method, and PC1 (65.8%) and PC2 (14.3%) constituted 80.1% of the total variation (Figure 4). Cu, Fe, Mn, Mg, Zn and Ca, Na, K were positively related to each other having with a narrow angle. The variation between genotypes increases as the vector moves away from the origin, while the variation between genotypes decreases as the vector approaches the origin. The heat map of the principal component analysis was conducted to discriminate the similarities and dissimilarities among garlic genotypes in terms of the detected mineral content (Figure 5). In a heat map, rows and columns are grouped by using correlation distance and average

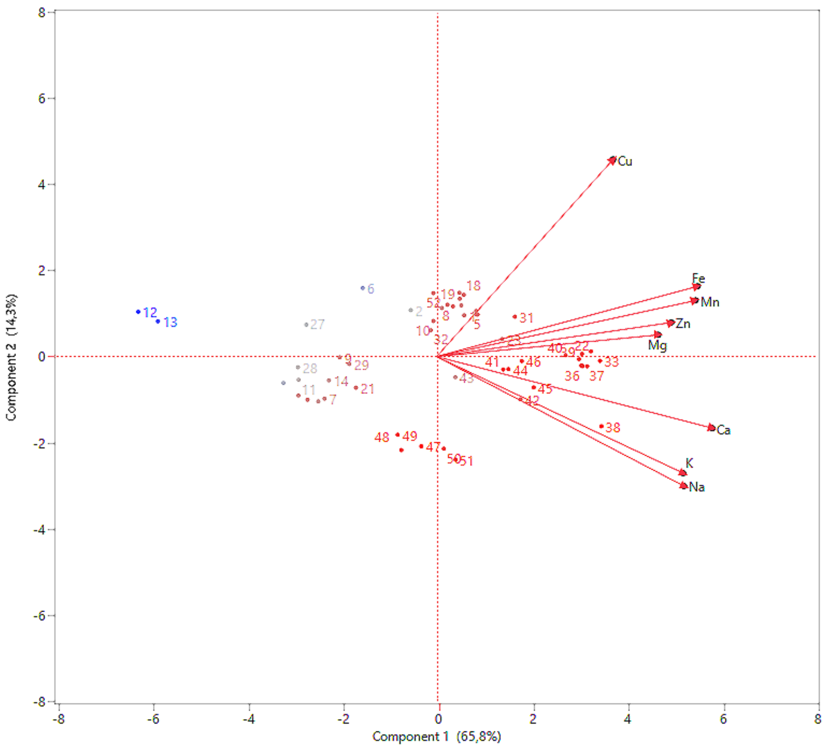

Fig. 4. Relationships among genotypes and mineral content according to the PCA biplot analysis

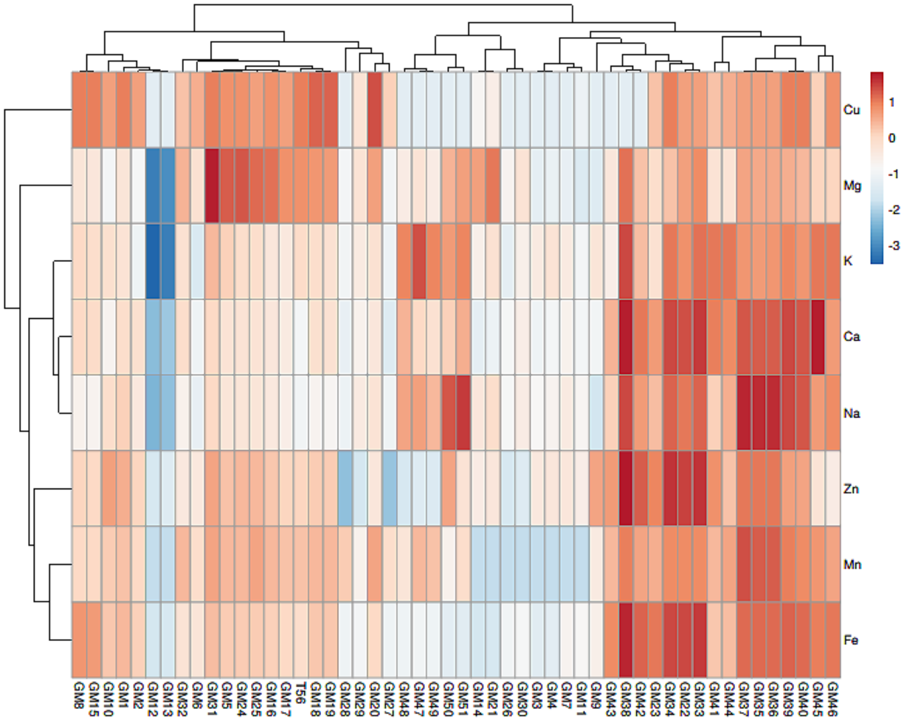

Fig. 5. The heat map of the principal component analyses on the mineral content in garlic genotypes

linkage. According to the principal component analysis (PCA), genotypes GM1, GM5, GM8, GM15, GM16, GM18, GM19, GM20, GM22, GM23, GM24, GM25, GM31, GM33, GM34, GM35, GM36, GM37, GM38, GM39, GM40, GM41, GM42, GM43, GM44, GM45, GM46, GM50, and GM51 had a higher content of Ca, Cu, Fe, K, Na, Mn, Mg, and Zn (Figures 4, 5).

According to two-way hierarchical clustering analyses (HCA), garlic genotypes were also separated from each other according to the mineral composition of garlic bulbs (Figure 6). The dendrogram and constellation plot revealed that there was a clear separation among 52 genotypes. It presented two major clusters, and in each cluster there were differences and similarities among the genotypes based on the mineral content. As seen in Figure 6, cluster I comprised 33 garlic genotypes, and was divided into two subgroups. A high degree of similarity was observed between genotypes GM35 and GM36. The other cluster (cluster II) consisted of 19 genotypes, which were divided into subgroups.

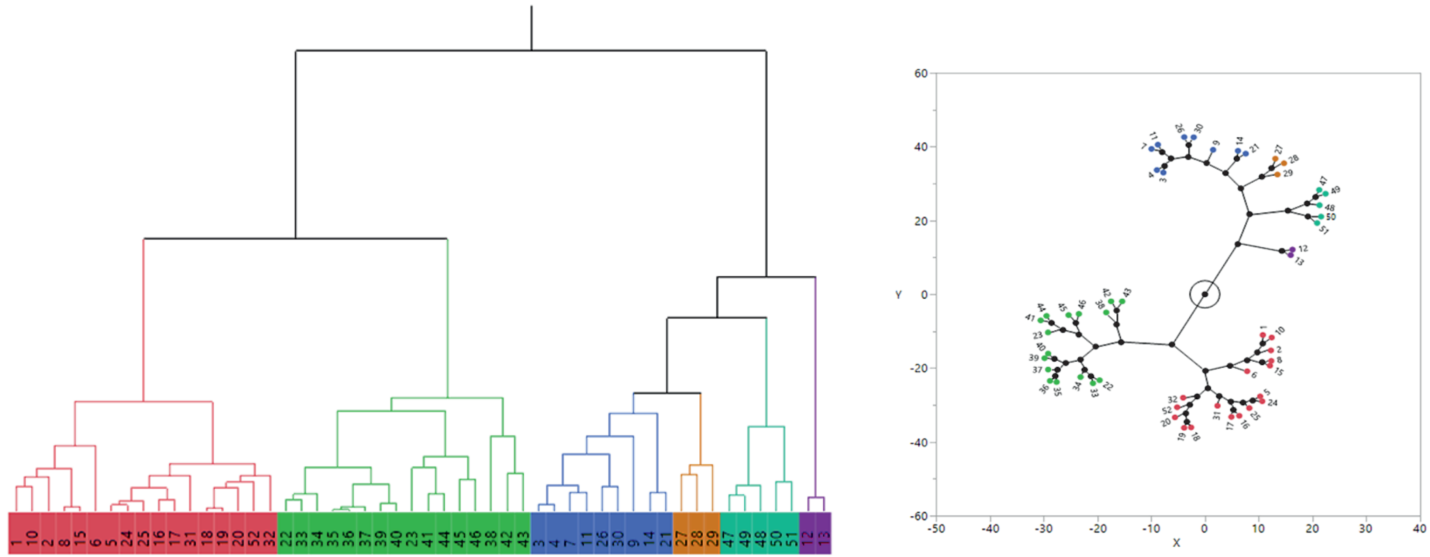

Fig. 6. Dendrogram and constellation plot obtained from the cluster analysis of 51 mutant garlic genotypes and cv. ‘ Taşköprü 56’ according to the mineral content (individual clusters were highlighted by different colour lines)

CONCLUSIONS

The chemical composition and bioactive compounds content of garlic, which determines its quality, highly depends on the genotype and growing conditions. Moreover, genetic diversity among different garlic populations and ecotypes is advantageously exploited to select germplasms with higher content of bioactive compounds. Based on the findings from the study, it was concluded that mutant garlic genotypes had a large variation in volatile sulfur compounds and mineral contents. As a result, it was determined that both the volatile sulfur compounds and mineral content of mutant genotypes were higher than cv. 'Taşköprü 56' (control). Regarding to results, 29 mutant garlic genotypes (GM1, GM2, GM3, GM6, GM7, GM8, GM9, GM11, GM12, GM17, GM18, GM20, GM21, GM22, GM26, GM27, GM32, GM33, GM35, GM36, GM37, GM39, GM40, GM41, GM43, GM46, GM48, GM49, and GM51) were promising in terms of volatile sulfur compounds. It was also determined that 27 garlic genotypes (GM1, GM5, GM8, GM15, GM16, GM18, GM19, GM20, GM21, GM22, GM24, GM25, GM31, GM33, GM34, GM35, GM36, GM37, GM38, GM39, GM40, GM42, GM45, GM46, GM47, GM50, and GM51) were also promising in terms of mineral contents. Mutant genotypes, came into prominence both volatile sulfur compounds and mineral contents, were defined as GM1, GM8, GM18, GM20, GM21, GM22, GM33, GM35, GM36, GM37, GM39, GM40, GM46 and GM51. In conclusion, research findings are extremely valuable in revealing the originality of mutant garlic genotypes obtained by mutation breeding. The current study established a foundation for the assessment of mutant garlic genotypes for using in breeding programs.

Ethics approval – not applicable.

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Conflict of interest – on behalf of all authors, the corresponding author states that there is no conflict of interest.

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