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ORIGINAL PAPER

Contents of micro- and macroelements and the biochemical composition of garlic (*Allium sativum* L.) genotypes

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Abstract

Garlic (*Allium sativum* L.) is a plant species of cultural and medicinal importance, in addition to which it is consumed globally as a very nutritious and healthy food ingredient. This study compared the micro- and macro-element content, phenolic compounds, and protein content in several local garlic genotypes from varied regions of Turkey, and from China and Iran. Mineral contents were analyzed including macro- and micro-elements, total phenolics, and proteins. In this regard, genotype 2 contributed significantly to the total phenolic content, having a value of 7359.05 mg kg^{-1}. Similarly, a high total phenolic content of 3685.44 mg kg^{-1} was recorded in genotype 5, which also had a high protein share, 4.20%, and was rich in potassium (K), 6078.09, and calcium (Ca), 1912.74 mg kg^{-1}. Both the hierarchical clustering analysis (HCA) and principal component analysis (PCA) displayed clear groupings among the genotypes with respect to biochemical and mineral compositions. The high amounts of nitrogen (N) and manganese (Mn) constituents in genotype 1 and balanced levels in the phenolic and protein content in genotype 3 proved their promising nutritional value. Genotype 4, with lesser calcium (Ca) and iron (Fe), had a moderate level of phenolics and protein. This work underlines the importance of genetic diversity for nutritional and bioactive compounds in garlic. These findings may be useful regarding farming technique improvements, aiming at the selection of high-quality genotypes to improve the value of harvested yield through breeding. These results must be confirmed by further studies in which more genotypes are tested in various environmental conditions. Such a critical evaluation underlines the importance of the conservation of genetic resources as well as improvement of farming techniques for obtaining high nutritional quality and yield.

Keywords: garlic, *Allium sativum* L., phenolic compounds, micro and macro elements, protein content

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INTRODUCTION

Garlic, *Allium sativum* L., is a crop valued for cultural reasons and medicinal purposes, and it is consumed globally owing to its immense nutritional value and healthfulness attributed to traditional medicine and confirmed by recent research (Suleria et al. 2015). Indeed, garlic contains a wide variety of bioactive compounds, indicating great potential for treating a wide variety of diseases (Shang et al. 2019). Major active substances identified in garlic include micro- and macro-elements, phenolic compounds, and proteins.

The general functions of nitrogen (N), phosphorus (P), and potassium (K), which are considered macroelements, without which the growth and development of the higher plants are impossible (Sinha, Tandon 2020) are as follows. N is used in protein synthesis (Leghari et al. 2016); P takes part in energy transfer and photosynthesis (Rao, Pessarakli 1996), while K is related to the osmotic balance in cellular and enzymatic matters (Sardans, Peñuelas 2021). Calcium (Ca) is involved in the formation of the cell wall structure (Hepler 1994); magnesium (Mg) has a central place in photosynthesis (Fiedor et al. 2008), while sodium (Na) maintains osmotic balance and water uptake (Hasegawa 2013). Major functions are also performed by microelements in the metabolism of plants and enzymes (Bhat et al. 2020). Organosulfur compounds are created from sulfur, which provides garlic with flavor and pharmacological properties. Other microelements include copper (Cu), manganese (Mn), iron (Fe), and nickel (Ni) – Hänsch, Mendel (2009).

The antioxidant effect of garlic is attributed to the phenolic compounds fighting against oxidative stress, which occurs in the human body due to free radicals. They include flavonoids, polyphenols, and saponins, which have been reported to possess anti-inflammatory activities against cancer (Asif 2015, Nouroz et al. 2015, Kovarovič 2019) and may have an immunomodulating effect, hence exerting cardiovascular protection (Lutz et al. 2019, Jiang, Dusting 2003). One of the most critical functions of proteins pertains to cell structure and function. It plays an important role in the Improvement of a plant that would be useful for human nutrition. Proteins from garlic have expressed antioxidant, anti-inflammatory, and antimicrobial activities (Trio et al. 2014, Subramanian et al. 2020).

This study has compared the composition of micro- and macroelements, phenolics, and the protein contents of garlic genotypes taken from different regions of Türkiye. As a major player in garlic cultivation, Türkiye provides a rich genetic resource for this study. Samples were collected from the main garlic producing provinces, focusing on long-established local genotypes. The results will guide the conservation and use of garlic genetic resources and contribute to future research and characterization of local garlic genotypes.

MATERIALS AND METHODS

Experimental materials

Five garlic genotypes from different regions of Turkey, where garlic has been grown for many years, were investigated in this study. The genotypes include Iran from Balıkesir, China from Kırklareli, Purple from Aksaray and Karasarımsak from Kütahya, and Araban from Gaziantep. In addition, Iran and China genotypes were collected from garlic production areas. Iranian genotypes were collected from fields in Balıkesir and Chinese genotypes were collected from fields in Kırklareli (Table 1). Each local genotype was morphologically examined and selected to represent the regional diversity. Selection of these genotypes was done based on factors such as regional adaptation, cultivation history, and morphological differences between each other. Thus, this study represents the diversity of garlic genotypes originating from various regions of Turkey.

Table 1

Cultivated garlic genotypes and coding

Code	Genotype name
Genotype 1	Balıkesir (Irani)
Genotype 2	Kırklareli (China)
Genotype 3	Aksaray (Purple)
Genotype 4	Kütahya (Karasarımsak)
Genotype 5	Gaziantep (Araban)

Discussions with growers revealed that garlic cloves are generally planted in August-September, grown using standard agricultural practices and harvested in June-July. During the crop cultivation, the optimum growing conditions were maintained through adequate fertilization and irrigation management. The experiment was carried out under open field conditions. Soil preparation, planting, and field management practices followed the average local agricultural recommendations in order to maintain the same conditions for all genotypes. Standard routine agronomic care given to each plot included control of pests and diseases as necessary to promote good growth. The coordinates and altitude of the collection sites for the garlic genotypes are shown in Table 2, which gives an idea about the various geographical regions, altitudes, and climatic conditions of Turkey from where the samples were collected. These represent the normal conditions in which the genotypes are usually grown and therefore give a good indication of the potential and performance of each genotype in its native region.

Macro- and microelemental analyses, total phenolic and protein analyses of the garlic samples were carried out at Kilis 7 Aralik University Advanced Technology Application and Research Centre. The aim of these analyses was

Table 2

Coordinates and altitude information of the fields where garlic genotypes were collected

Provinces	Coordinates	Height (m)
Aksaray	38° 31′ 37″ N 33° 50′ 00″ E	944
Balıkesir	39° 30′ 40″ N 27° 50′ 53″ E	231
Gaziantep/Araban	37° 04′ 05″ N 37° 23′ 35″ E	850
Kütahya/Şaphane	38° 58′ 43″ N 29° 16′ 17″ E	781
Kırklareli/Babaeski	41° 34′ 33″ N 27° 50′ 43″ E	314

to detail the nutritional components and variations among the genotypes and to reveal differences in bioactive compounds and nutritional values.

Analysis of micro- and macroelements

Micro- and macroelemental analyses of the five garlic genotypes were carried out under controlled laboratory conditions. Samples were dried at 72°C, pulverized, and each 0.25 g sample was mixed with 9 mL of HNO_3 and 3 mL of H_2O_2 and then burned in a microwave oven at 200 W for 30 min (Erol, Arpaci 2023). The samples were filtered and diluted to 25 mL after burning.

Atomic absorption spectrometry was used for determination of Ca, Mg, Fe, Mn, Zn and Cu. A flame photometer was used in determination of Na and K, whereas a UV spectrophotometer was used for analyses of N and P (Tefera, Chandravanshi 2018). All the analyses were replicated thrice in order to ensure that the analyses are correct.

Sulfur (S) analysis

S analysis of garlic genotypes was assayed using the turbidimetric method (AOAC 2016). Fresh garlic (0.5 g) was homogenized in 10 mL of distilled water, boiled, cooled, and filtered. A 5 mL filtrate was mixed with 10 mL of 5% $BaCl_2$ solution and incubated for 30 minutes in the dark. The resultant precipitates of barium sulphate were measured using a spectrophotometer at 420 nm, and the S content was calculated from a calibration curve done with standard solutions, expressed in g kg^{-1}. This method gives a very correct determination of the differences among different genotypes in their S content.

Analysis of total phenolic content

Extraction procedure

A 10 g garlic sample was homogenized with 80 mL of an 80% methanol solution, incubated in the dark at room temperature for 24 h, and centrifugation was carried out at 4000 rpm for 10 minutes. The supernatant was collected and filtered to increase the solubility of the phenolics and eliminate impurities (Erol et al. 2024*a*,*b*).

The total phenolic content was measured according to the method of Castro-Concha et al. (2014): Garlic extracts were mixed with Na_2CO_3 solution and Folin-Ciocalteu reagent, which turned blue in the presence of phenolic compounds. Then, the mixture was incubated for 90 min in the dark at room temperature. Then, the absorbance was read on a spectrophotometer at 765 nm.

The quantification was done by using gallic acid as the standard. Calibration curves with the standard solution of GA were carried out before the determination of phenolics content, which is expressed as milligrams gallic acid equivalent (GAE) per gram fresh weight (FW). It was also done through the calibration curve, realized by measuring the absorbance values of the solutions of GA for the different concentrations and providing the reference point for the determination of the content of phenolics. Total phenolic content in the samples was calculated using a calibration curve, and reported for each genotype.

Protein analysis

The protein analysis in garlic genotypes was determined using the Kjeldahl method as modified by Jung et al. (2003). A 0.25 g sample was added to a catalyst tablet and 15 mL of H_2SO_4, and combusted for 3.5 hours. The combusted samples were distilled using a VELP UDK 159 apparatus. The 40% NaOH solution provided an alkaline environment, while the 4% boric acid solution collected the distilled ammonia. Titrations were performed with standardized 0.1 N HCl, using methyl red and methylene blue indicators to determine the end point. The protein content was calculated according to AOAC (2016) procedures.

Statistical analysis

All data obtained were subjected to analysis of variance (ANOVA) and evaluated using the JMP 14 statistical analysis program (NC, USA). The Tukey’s HSD test was used for multiple comparisons. For the parameters in the analysis of variance, any *p*-value less than 0.05 was considered significant. Principal component analysis (PCA) and hierarchical cluster analysis (HCA) were calculated using Origin 2022 Pro software (OriginLab, Massachusetts, USA).

RESULTS AND DISCUSSION

Macro- and micro-element contents of garlic genotypes

The N content ranged from 0.65% to 0.83% among the genotypes, with genotype 5 having the highest N content at 0.83% (Table 3). These values are consistent with Petropoulos et al. (2018) (0.62% - 0.95%), indicating genetic diversity's impact on the N content. N is crucial for protein synthesis and plant growth (Leghari et al. 2018). The P content varied from 369.29 mg kg^{-1} to 503.35 mg kg^{-1}, with genotype 2 having the highest amount. These values align with Yusuf et al. (2018), underscoring the role of phosphorus in energy transfer and photosynthesis (Carstensen et al. 2018). The K content ranged from 4635.26 mg kg^{-1} to 6078.09 mg kg^{-1}, with genotype 5 having the highest. These results match Turan et al. (2017), highlighting the importance of potassium for osmotic balance and enzyme activation (Wang et al. 2013). The Ca content varied between 1281.82 mg kg^{-1} and 1912.74 mg kg^{-1}, with the highest value in genotype 5. The result from this study agreed with that obtained by Turan et al. (2017). Ca participates in maintaining cell wall structure and cell division. Mg varied from 204.97 mg kg^{-1} to 291.06 mg kg^{-1}; the highest values were recorded for genotype 5. This result supports the report of Polyakov et al. (2020) since Mg is the central atom of chlorophyll and thus plays an essential role in photosynthesis. The corresponding level of Na ranges from 115.93 mg kg^{-1} to 160.91 mg kg^{-1} and showed the maximum value in genotype 1. Garlic plants have been reported to contain Na within the range of 80.00 mg kg^{-1} to 160.00 mg kg^{-1} (Astaneh et al. 2018), thus suggesting the possibility of genotype 1 being effective in water uptake and osmotic balance. This is supported by the view of Grewal (2010).

The Cu content varied from 1.66 mg kg^{-1} to 2.46 mg kg^{-1}, with the highest value for genotype 2. From this viewpoint, the results in this investigation are in good agreement with Sałata et al. 2021, underlining the importance of Cu in plant metabolism until the process of lignin synthesis. The Mn content varied from 8.28 mg kg^{-1} to 9.71 mg kg^{-1} and is observed to be the highest in genotype 3. Although Chanchan et al. (2011) reported higher levels (50 mg kg^{-1} - 130 mg kg^{-1}), the essential role of Mn in photosynthesis and N metabolism is evident. Fe levels varied from 765 mg kg^{-1} to 3408 mg kg^{-1}, with genotype 5 having the highest. These results align with Gharehbaghli et al. (2022), highlighting the role of iron in chlorophyll synthesis and oxidative stress reduction. The Zn content ranged from 130.3 mg kg^{-1} to 181.5 mg kg^{-1}, with genotype 1 having the highest amount. This matches Hadji Sfaxi et al. (2012), underscoring the importance of zinc for enzyme activity and protein synthesis. The Ni content ranged from 0.06 mg kg^{-1} to 0.09 mg kg^{-1}, with the highest levels in genotype 3. These results align with Manna and Bandyopadhyay (2023), highlighting the role of nickel in enzyme functions and urea metabolism.

Table 3

Changes in macro- and microelements, total phenolics and protein content in garlic genotypes

Geno-types	N (%)	P (mg 100 g^{-1})	K (mg kg^{-1})	Ca (mg kg^{-1})	Cu (mg kg^{-1})	Mn (mg kg^{-1})	Fe (mg kg^{-1})	Zn (mg kg^{-1})	Mg (mg kg^{-1})	Na (mg kg^{-1})	Ni (mg kg^{-1})	S (g kg^{-1})	Total phenolics (mg kg^{-1} GAE)	Protein (%)
1	492.21*a*	5578.04*ab*	1766.28*b*	2.24*b*	9.73*a*	28.15*b*	18.15*a*	275.44*a*	160.30*a*	0.07*b*	8.50*c*	3634.33*b*	492.21*a*	5578.04*ab*
2	503.35*a*	5149.32*b*	1281.82*d*	2.46*a*	8.98*c*	7.65*e*	13.03*d*	215.88*c*	121.21*c*	0.06*c*	10.02*b*	7359.05*a*	503.35*a*	5149.32*b*
3	404.26*c*	5483.56*ab*	1365.03*c*	2.18*b*	9.71*a*	8.15*d*	13.87*c*	229.89*b*	129.08*b*	0.09*a*	10.88*b*	3556.12*c*	404.26*c*	5483.56*ab*
4	474.83*b*	4635.26*c*	1357.18*c*	1.66*c*	8.28*d*	20.52*c*	13.77*c*	281.83*a*	115.93*d*	0.08*a*	12.07*a*	3034.07*d*	474.83*b*	4635.26*c*
5	369.29*c*	6078.09*a*	1912.74*a*	2.38*a*	9.17*b*	34.08*a*	16.96*b*	291.06*a*	140.91*b*	0.07*b*	12.84*a*	3685.44*b*	369.29*c*	6078.09*a*
Mean	**0.75**	**448.39**	**5384**	**1536.61**	**2.18**	**9.174**	**19.71**	**15.956**	**258.82**	**133.49**	**0.074**	**10.86**	**4253.00**	**3.81**
CV (%)	**9.84**	**13.11**	**9.94**	**18.43**	**14.34**	**6.53**	**59.89**	**14.87**	**12.99**	**13.25**	**15.41**	**15.72**	**38.46**	**10.39**
***F* value**	**11.53**	**20.34**	**11.75**	**39.68**	**24.27**	**5.09**	**334.49**	**26.07**	**19.99**	**20.79**	**27.95**	**29.07**	**179.82**	**12.85**

Different letters in the same column indicate that there is a statistical difference (p<0.05) between the means. Values mean ± standard deviation (SD).

These results show that there is much variation in the nutrient composition of different garlic genotypes due to genetic diversity. Some genotypes could be better for agricultural productivity and nutritional value. The PCA biplot analysis (Figure 1) of the elemental content of 5 garlic genotypes gives us valuable insights into their nutritional and agronomic profiles. Principal Component 1 (PC1) and Principal Component 2 (PC2) explain 45.03% and 24.79% of the variance, respectively, and together capture 69.82% of the total variance. Genotype 1 and genotype 5 are positioned at the extremes of PC1, indicating a high differentiation in their K, Ca and Mg levels. Specifically, genotype 5 has a higher K content, while genotype 1 has increased levels of Ca and Mg. S and Na significantly influence PC2, with higher concentrations observed in certain genotypes. The clustering of P, Fe, Cu and Zn around the origin suggests less variability between genotypes for these elements. This study is vital for breeding efforts to boost garlic's nutritional benefits, refine soil and fertilization techniques. The PCA biplot effectively highlights the elemental variations among the genotypes, offering practical insights for tailored agricultural methods.

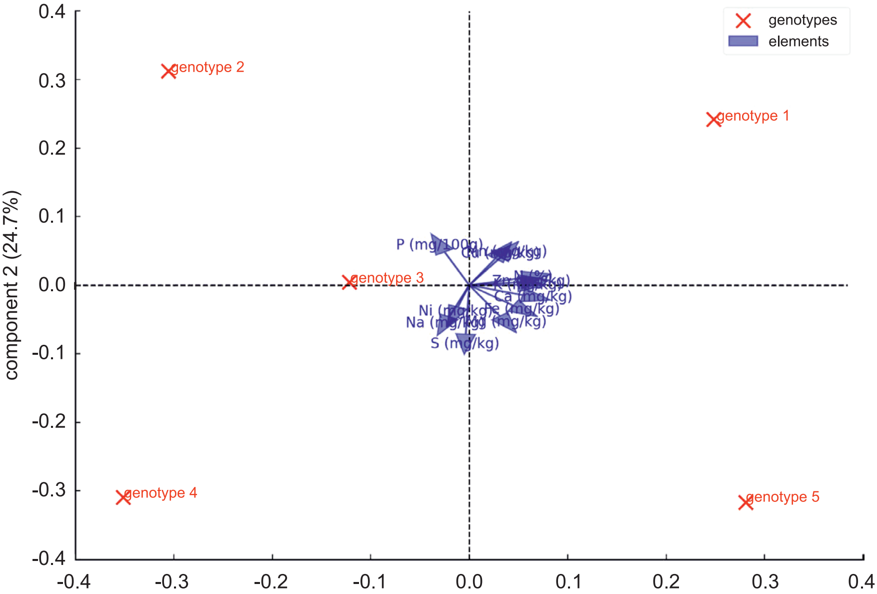

Fig. 1. PCA biplot of elemental analysis

The results of hierarchical clustering analysis (HCA) are shown for the genotypes and elements, which are divided into groups representative of their similarity relationships within this dataset. The dendrogram (Figure 2*a*) for the genotypes shows that genotype 2 and genotype 3 cluster closely together, indicating similar elemental profiles, whereas genotype 1 and genotype 5 are more distinct, reflecting their unique elemental compositions. This clustering makes the existence of genetic or environmental factors acting on

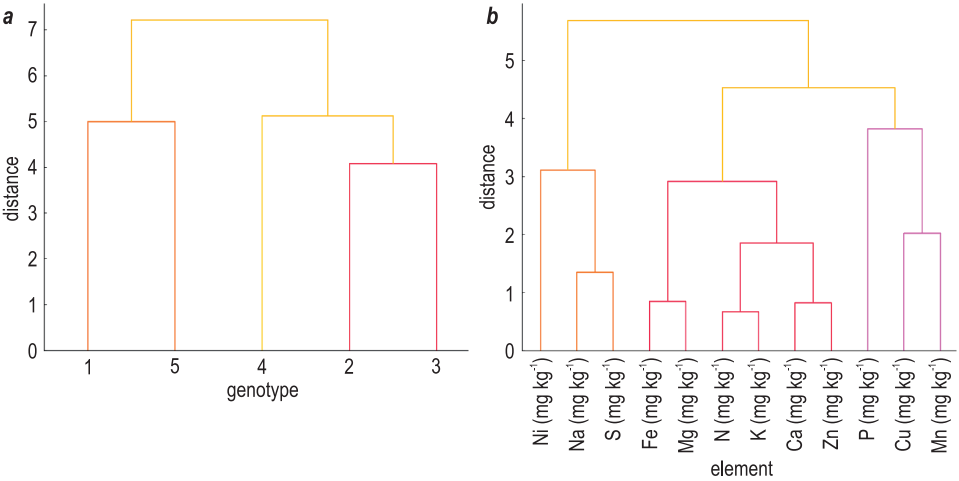

Fig. 2. Dendrograms for hierarchical clustering of (*a*) genotypes and (*b*) elements on elemental analysis

these profiles plausible. Similarly, in the elemental dendrogram (Figure 2*b*), certain elements, i.e. Na and S, have clustered together, which may indicate that Na/S co-occur or are metabolically linked across genotypes representing a similar mechanism of uptake/metabolic pathway. Ca, Mg and Fe also cluster together, suggesting a related pattern of variation that may be related to their roles in plant structural integrity and photosynthesis. On the other hand, K is relatively distant from other elements, highlighting its distinct distribution pattern among genotypes, consistent with its critical role in plant stress responses and osmoregulation. The specific elemental pattern of whole and fillet clustering provides useful information for the study of nutrient interactions that require the development of fertilization strategies through a proper balance between macro (K, P) and micronutrients (Fe, Zn). It can also help in the design of fertilizer management for sustainable agriculture, which makes the promotion of healthy garlic crops especially targeting nutritional content.

Total Phenolic Contents and Protein Percentages of Garlic Genotypes

Total phenolic content and protein analysis showed significant biochemical differences among the genotypes studied (Table 3). Genotype 2 stands out with the highest total phenolic content (7359.05 mg kg^{-1}) and a remarkable protein content (3.57%). Genotypes 5 and 3 also have remarkable phenolic contents of 368 544 mg kg^{-1} and 355 612 mg kg^{-1}, respectively. Genotype 4, with a phenolic content of 3034.07 mg kg^{-1} and a protein content of 3.28%, appears to be a candidate with a balanced nutritional value. Conversely, genotype 1 has comparatively lower values, with a phenolic content of 3634.33 mg kg^{-1} and a protein content of 4.18%.

Comparing the literature, the content of phenolics is highly increased in processed garlic products. The total phenolic contents in black garlic are about 1.5 to 11-fold higher than in raw garlic, whose protein content has been reported to vary between 6.8% and 14.5% (Choi et al. 2011, Moreno-Ortega et al. 2020, Lu et al. 2023). Improvement in the intake of phenolic compounds in black garlic is attributed to the Maillard reaction and other thermal processes. Dufoo-Hurtado et al. (2013), Zhang et al. (2016), and Ríos-Ríos et al. (2019), stated that a number of temperatures were used in the research, which indicated that the content of phenolics in garlic extracts strongly depends on temperature; therefore, methods for the optimization of thermal processing should be developed with the view of achieving a maximum phenolic content when enhancing garlic products nutritionally and therapeutically. Herrera et al. (2021), in turn, referred to antioxidant properties associated with phenolic compounds, strengthening the health value of the consumption of garlic. Due to the fact that high phenolic content was determined in varieties 2, 3, and 5, these could be consumed to yield greater health benefits. The protein content is another main aspect of garlic's nutritional value, according to Santhosha et al. 2013. The protein levels found in this paper ranged from 3.28% to 4.20%, and they agreed with those reported by Griffiths et al. (2002) in different cultivars of garlic. The relatively high protein content of genotype 5 (4.20%) probably indicates that this genotype could be of potential value as a source of food, especially in countries with a high incidence of protein malnutrition.

The HCA dendrogram of garlic genotypes based on the phenolic content (Figure 3*a*) shows that there is a significant biochemical difference among garlic genotypes. The dendrogram showed that genotype 2 was distinctly separated due to its highest phenolic content, hence it can depict its unique properties valuable in functional food or nutraceuticals. At the other extreme, genotype 4 had lower phenolic and protein content; it formed an independent cluster that could show a balanced profile with lower potency. Genotypes 1 and 5 cluster closely together with similar high phenolic and protein

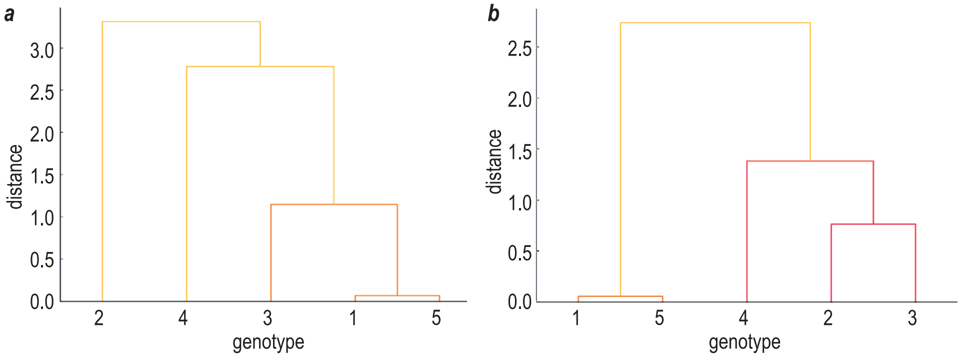

Fig. 3. Dendrograms for hierarchical clustering of genotypes on (*a*) total phenolic and (*b*) total protein

content, making them suitable for interchangeable use in breeding or product development. Genotype 3, with moderate similarity to genotypes 1 and 5, clusters somewhat separately, reflecting its distinct but related biochemical characteristics. In addition, HCA based on protein content (Figure 3*b*) alone shows that genotype 4 is clearly separated due to its lowest protein content, while genotype 2 also stands out with a relatively low protein content. Genotype 3, with a protein content of 3.84%, shows moderate similarity, while genotypes 1 and 5 cluster closely together, indicating very similar protein profiles.

According to the heat map analysis (Figure 4), the biochemical and mineral contents among the genotypes showed significant differences. Genotype 5 had a high total phenolic content (3685.44 mg kg^{-1}), protein content (4.20%) and mineral abundance, making it a preferred choice for nutritional purposes. While the extract of sample 2 had the highest total phenolic content (7359.05 mg kg^{-1}), it contained less protein (3.57%). Genotype 1 had a total phenolic content of 3634.33 mg kg^{-1} and its protein content was 4.18%. It has high levels of the elements N, P, Mn, Zn, Na in its composition. On the other hand, genotype 4 is low in calcium and iron, moderate in total phenolics (3034.07 mg kg^{-1}) and low in protein (3.28%).

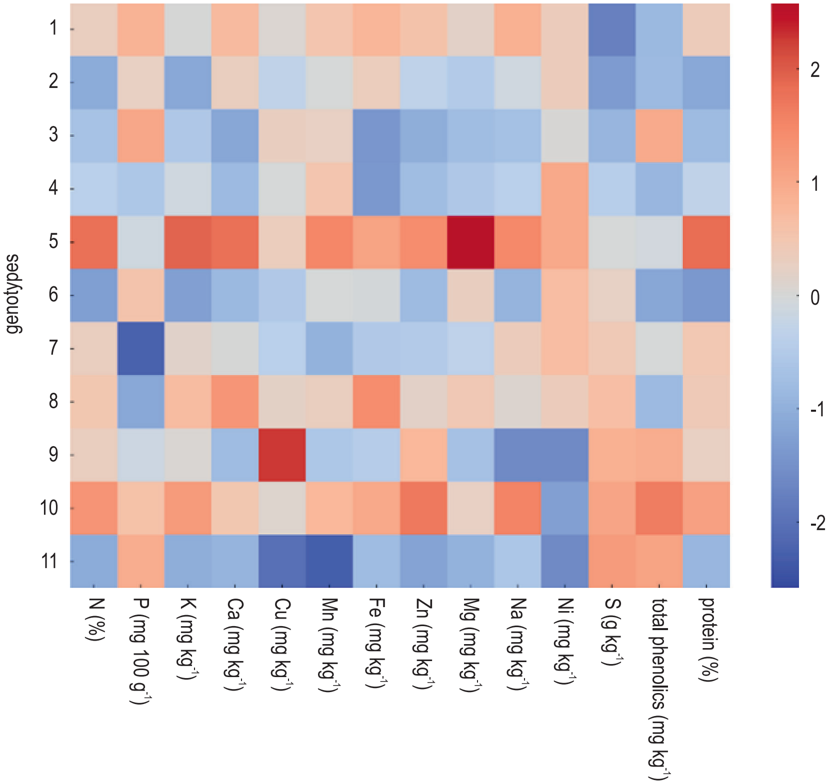

Fig 4. Heatmap of elemental analysis, total phenolics, and protein contents

With its total phenolic content of 3556.12 mg kg^{-1} and a protein content of 3.84%, genotype 3 occupies an intermediate position in terms of the elemental composition. The breakdown allows a thorough examination of what the genotypes are made up of. The results proved that genotypes 5 and 2 are rich in phenolic and mineral content and are therefore highly rich nutritionally and recommendable for agricultural production. The analyses of genotypes provide the necessary information for genotype breeding programs. The combined results can form a database for strategic decisions on plant improvement and genetic research for future studies. Future research is expected to examine this performance in more detail, taking into account variations in weather conditions. The results of the current study were presented in this article, and the above assertion was validated when considering the effects of genotypes on yields under different trials. It is therefore crucial to have a well-informed assessment of the morphological, biochemical and mineral footprint of given genotypes in order to make a decision which one or ones to select for production.

CONCLUSIONS

We considered the biochemical composition and mineral contents of different garlic genotypes in the present study and pointed out some important differences. Among these, genotype 2 excelled in the total phenolic composition, while genotype 5 was outstanding regarding the high amount of phenolics and protein. Heatmap and clustering analyses demonstrated clear differences among these genotypes. The highest and lowest values for the variables evaluated were obtained for the total phenolic content in genotype 2 and for the protein content in genotype 4. Genotype 5 had the highest content of potassium and calcium, indicating that it is highly nutritious. In general, these genotypes, 2 and 5, are recommended for breeding purposes in the region due to their high nutritional value and productivity. We further observed that the Iranian and Chinese garlics, represented by genotype 1 and genotype 2, respectively, when cultivated in Turkey, presented biochemical profiles different from the typical Turkish genotypes. The Chinese genotype, i.e. genotype 2, had the highest phenolic content, whereas the Iranian variety had a higher protein content. This means that these foreign genotypes have very good adaptability to Turkish agricultural conditions and provide wholesome nutritional and health benefits, hence contributing to the genetic diversity of local garlic. The present study is of great importance in efforts to preserve and enhance garlic genetic resources, thus contributing to the selection of varieties with high nutritional value and productivity. Further studies should be done to confirm the present findings on larger groups of genotypes and under diverse environmental conditions.

Conflicts of interest

The authors declare that they have no conflict of interest.

Author contributions

U.H.E. – analyzed the data and wrote the manuscript, I.S. and O.K.S. – contributed to the collection of genotypes, the reading, review, and revision of the manuscript.

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