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Evaluation of Turkish shallot genetic resources for morphological, biochemical and sensory properties

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Abstract: Shallots, a subspecies of onion, are considered gourmet and are particularly preferred in French and Asian cooking. Their unique taste and aroma, especially after being caramelized through heat treatment, increases their importance for boutique restaurants and specialized cuisines. This study examined the morphological, biochemical, and sensory properties of seven generatively propagated shallot genotypes (GMSY-2, GMSY-3, GMSY-4, GMSY-5, GMSY-6, GMSY-7, GMSY-8) and one vegetatively propagated shallot genotype (GMSY-1) in 2021 and 2022. Sixteen morphological and 15 biometric features of the International Association for the Conservation of New Plant Varieties were used in characterization. Total phenolic content, total antioxidant capacity, total monomeric anthocyanin and pyruvic acid contents were determined as biochemical markers. The data were evaluated with hierarchical clustering (HCA) and principal component analysis (PCA) methods. With sensory analysis, genotypes were compared in terms of pungency, aroma, crunchiness, smell, consumption preferences, and perceptibility of the epidermis membrane. Results showed that leaf waxiness, bulb shape, shape of the bulb base, tendency to split into bulblets, main color of dry skin, and time of harvest maturity may be utilized to discriminate shallot genotypes. As biometric characteristics, head weight varied between 21.67 and 93.82 g, and the smallest heads were obtained from the GMSY-1 genotype while the largest heads were obtained from GMSY-7. The water-soluble solid contents of the genotypes showed significant variation (2.35%–9.50%), with the highest being determined in GMSY-2, GMSY-3, and GMSY-1, respectively, while the lowest was found in GMSY-8. According to the HCA results, four different main groups were formed in terms of biochemical and sensory characteristics. As a result of PCA, it was seen that the total variation consisted of the first two principal component axes, and the variation between biochemical contents and genotypes was 79.00%. The findings for the sensory analysis evaluation criteria highlighted the GMSY-8 genotype as promising.

Key words: *Allium ascalonicum* auct. hort., biometric parameters, shallot, genetic resource characterization

1. Introduction

Vegetables constitute a broad category encompassing the edible parts of horticultural plants, which usually comprise their leaves, roots, fruits, or seeds. They are staple foods around the world, essential for healthy diets and a fundamental part of modern agriculture (Kumlay and Ercisli, 2015; Nadeem et al., 2018; Maxim et al., 2023). They are highly beneficial for the maintenance of health and prevention of diseases. They contain valuable components such as vitamins, minerals, fiber, phytochemicals, and phytonutrients, which can be successfully utilized to build up and repair the body. Vegetables are valuable in maintaining the alkaline reserve of the body. There are different kinds of vegetables and each group contributes to the diet in its own way (Kumlay and Ercisli, 2015; Brezeanu et al., 2022; Kul, 2022; Sarker et al., 2022).

Edible *Allium* species constitute the most important vegetable group globally after tomatoes and potatoes in terms of production and consumption in the world.¹ These vegetables, which include onion, garlic, leek, and shallot varieties, are preferred for human nutrition and health due to their nutritive properties, biochemical contents, aroma, and taste (Fenwick and Hanley, 1990; Upadhyay, 2017). Shallot (*Allium ascalonicum* auct. hort.), a member of the genus *Allium*, is a subspecies in the Aggregatum group of *Allium cepa* L. of the family Alliaceae. This group includes genotypes propagated vegetatively as well as those propagated by seeds. The chromosome number of shallot is $2n = 16$, similar to that of onion (Fritsch and Friesen, 2002; Perkovic et al., 2020). Shallot can be grown in a wide range of climate zones on different continents, including Asia, Africa, northern Europe, and North America. It is

¹FAOSTAT (2023). Food and Agriculture Organization of the United Nations, Statistics [online]. Website <http://www.fao.org/faostat/en/#data/> [accessed 08 September 2023].

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also grown noncommercially using traditional methods in many countries such as Finland, France, Spain, Russia, Poland, Indonesia, and Argentina. Shallot production is generally more local than onion production and it has the potential to be produced in climate zones where larger onions cannot be propagated by direct seed sowing (Tendaj, 2005). Shallot onions have smaller heads than other onions. They can be propagated directly by seed, like other onions, or by bulblets and seedlings. In onions, the head is a single whole piece. In shallot bulbs, multiple heads of 2–22 pieces are formed, which are attached to a root and have individual peels. The head size is smaller compared to other onions (0.40–159.00 g) and yield is lower (0.13–0.38 t ha⁻¹) (Brewster, 1994; Tendaj and Mysiak, 2013; Beşirli et al., 2014). While the morphological development and flowering stages of shallot genotypes propagated by seeds are similar to those of common onion, the vegetation period is 65–100 days, while that of common onion is 120–150 days (Jones and Mann, 1963; Hanelt, 1990; Brewster, 1994; Perkovic et al., 2020).

Although the nutritional value of shallots varies according to climate, growing conditions, fertilization, and genotype, contents per 100 g of shallot bulbs are reported to include water (88.00 g), vitamin A (5 IU), vitamin B (10.03 mg), vitamin C (2.00 mg), protein (1.50 g), fat (0.30 g), carbohydrates (9.00 g), fiber (0.70 g), ash (0.60 g), Ca (36.00 mg), P (40.00 mg), and Fe (0.80 mg) with an energy value of 160 kJ/100 g (Francke and Klasa, 2009).

The criteria of the International Association for the Conservation of New Plant Varieties (UPOV) are widely used to determine morphological differences between genotypes of *Allium* species. Poulsen and Henriksen (2001) used a total of 161 types of shallots collected from Denmark, Finland, and Norway; Ahmed et al. (2013) collected 30 types of onions; Sharma et al. (2018) collected 131 types of garlic; and Çakmakçı et al. (2021) determined the morphological characteristics of wild garlic *Allium vineale* using the UPOV criteria.

Morphological characters are commonly used to elucidate genetic variations within and between populations. Such properties are also used to establish the genetic similarities and dissimilarities of populations (Hunter, 1993; Singh et al., 2022). In genetic diversity studies, morphological characters are commonly expressed in numerical values (Sneath and Sokal, 1973). However, in recent years, techniques have emerged with which multiple variables can be analyzed together (Özdamar, 2004; Tan, 2005). Multivariate analyses such as clustering, two-way hierarchical clustering analysis (HCA), and principal component analysis (PCA) are widely employed to reveal genetic variations (Hair et al., 1995; Akan, 2022).

In addition to morphological characterization, determining the biochemical properties of genotypes is

important in terms of identifying the existing genetic material and revealing its potential for use in future breeding studies (Akan, 2022). The taste, aroma, odor, flavor, and nutritional properties of *Allium* species arise from their biochemical contents, consisting of minerals, vitamins, and predominantly sulfur compounds. The most important biochemical components are antioxidants, anthocyanin, phenolics, and flavonoids (Fenwick and Hanley, 1990; Brewster, 1994; Fattorusso et al., 2002; Adeyemo et al., 2023). More recently there has been increasing interest in lesser known horticultural plants that contain these components intensively (De Sousa and Solberg, 2020; Abanoz and Okcu, 2022). Climate change and the incidence of chronic diseases such as cancer, diabetes, and obesity have inspired people to benefit from local biodiversity, including the adoption of sustainable living philosophies and the spread of organic agriculture and slow food movements (Signore et al., 2022).

In Turkish food culture, shallots are preferred in making meat stews, various roasted dishes, beans, and lahmacun (Turkish pizza), and they are currently demanded by famous gourmets and boutique or niche restaurants. The main reasons for choosing shallots are that they caramelize quickly after heat treatment and that their presence cannot be seen in dishes although their taste and aroma are noticed easily. There is no registered variety in the Turkish National Variety List for shallot in spite of the fact that they are widely used. Therefore, the aim of this study is to define the Turkish shallot gene pool morphologically and biochemically and to determine qualified lines that may form the basis for further variety development.

2. Materials and methods

2.1. Plant material

This study was conducted at the Atatürk Horticultural Central Research Institute in Yalova Province, Türkiye (40°28'N, 28°45'E with altitude 4 m above sea level) during 2021 and 2022. A total of eight shallot genotypes, one propagated vegetatively and seven by seeds, were used (Table 1; Figure 1).

The seeds of the generatively propagated genotypes were sown in peat medium in viols with 108 cells (46.00 × 34.00 cm) on 10 October 2021. The developing seedlings were planted in the field at the same time together with the vegetatively produced (GMSY-1) genotype bulblets (heads) on 15 December 2021. In the experiment, which was carried out with three replications according to a randomized block trial design, 30 plants were planted in each plot. Planting was performed with row spacing of 20.00 cm and in-row plant spacing of 10.00 cm. Soil physicochemical properties were determined in accordance with Chapman and Pratt (1961) and results are given in Table 2.

Table 1. Shallot genotypes used in this study.

Genotypes	Log name	Propagation method
GMSY-1	Yalova Şalot	Vegetative
GMSY-2	THASPAU-4	Seed
GMSY-3	19Y32	Seed
GMSY-4	PAU1	Seed
GMSY-5	PAU2	Seed
GMSY-6	PAU3	Seed
GMSY-7	PAU5	Seed
GMSY-8	PAU6	Seed

**Figure 1.** Eight Turkish shallot genotypes evaluated in this study.**Table 2.** Some physical and chemical soil properties of the experimental plot (0–30 cm).

Saturation (%)	EC25 (ds/m)	pH	Lime (%)	Organic solid (%)	Available (mg/kg)	
					P	
61	0.25	7.50	0.25	2.12	21.00	
Exchangeable (mg/kg)			Available (mg/kg)			
K	Ca	Mg	F	Cu	Mn	Zn
185	7250	286	12.00	1.97	8.53	0.93

From planting to harvest, the same cultural practices were applied for each genotype. Approximately 120.00 kg/ha N, 75.00 kg/ha K, and 40.00 kg/ha S were applied as fertilizer during the vegetation period. During bulb initiation, a Zn-based microelement was applied three times by drip irrigation with weekly intervals. However, external phosphorus (P) was not applied to the plants

since the soils were found to be sufficient in phosphorus (Table 2).

Weed control was carried out manually by hoeing 3 times during the growing season. Irrigation was done via drip irrigation system 1–2 times per week depending on rainfall conditions. The monthly average temperature and total precipitation amounts for Yalova during the growing

period (2021–2022) and as long-term averages (1991–2022) are given in Table 3.²

The harvest was carried out between 10 and 30 July 2022, when 80% of the green parts of the plants had turned yellow. After the harvest, the heads were dried in the field under natural conditions for 1 week and then they were moved into a shed and the drying process continued there.

2.2. Morphological characteristics

Morphological characteristics of the genotypes were investigated according to the main descriptions for onions/shallots developed by the UPOV.³

2.2.1. Quantitative characteristics

Sixteen quantitative characteristics were investigated with three replications that comprised 10 randomly chosen plants and bulbs for the examined genotypes (Table 4).

Leaf and plant characteristics were measured in the field when the plants reached the stage of 8–10 leaves. Bulb characteristics were determined 2 months after the harvest, after the drying was completed. The investigated morphological characteristics were leaf attitude (LA), leaf waxiness (LW), green color intensity (GCI), leaf cranking (LC), bulb shape (BS), shape of bulb top (SBT), shape of bulb base (SBB), position of maximum diameter (PMD), tendency to split into bulblets (TSB), main color of dry skin (MCDS), adherence of dry skin, flesh color (FC), firmness of flesh (FF), fleshy scale thickness (FST), coloration of epidermis of fleshy scales (CEFS), and time of harvest maturity (THM) (Table 4).

²Turkish State Meteorological Service (2023). Forecasts [online]. Website <https://www.mgm.gov.tr/eng/forecast-cities.aspx> [accessed 08 September 2023].

³UPOV (2007). The International Union for the Protection of New Varieties of Plants [online]. Website <https://www.upov.int/portal/index.html.en> [accessed 19 July 2023].

2.2.2. Biometric parameters

Measurements of biometric characteristics were performed for 15 traits belonging to the plants, leaves, and bulbs. They included plant height (PH) (cm), pseudostem length (PH) (cm), pseudostem diameter (PD) (mm), number of leaves (NL), leaf length (LL) (cm), leaf diameter (LD) (mm), bulb weight (BW) (g), bulb height (BH) (mm), bulb diameter (BD) (mm), neck width of bulb (NWB) (mm), dry skin thickness (DST) (mm), root disc diameter (RDD) (mm), bulblet number per plant (BN), water-soluble solid contents (WSM) (%), and yield (Y) (kg/m²).

2.3. Biochemical properties

Biochemical properties of shallot genotypes were determined in juice obtained by centrifuging shallots at 5000 rpm for 5 min. The determined biochemical properties were pyruvic acid (PC) (µmol/mL), total antioxidants (TA) (mmol TE/L), total anthocyanins (TAC) (mg/100 mL as cyanidin-3-glycoside), total phenolics (TP) (mg GAE/100 mL), and total flavonoids (TF) (mg CE/100 mL).

2.3.1. Total antioxidant capacity (Trolox equivalent)

Extract (100 µL) was obtained from the juice samples and 3.90 mL of DPPH (2,2-diphenyl-1-picrylhydrazyl) solution (6×10^{-5} M) was added. For the control, 100 µL of methanol was taken and 3.90 mL of DPPH solution (6×10^{-5} M) was added. The absorbance values of the samples and that of the blank sample as measured at a wavelength of 515 nm were determined with a spectrophotometer after 30 min. The absorbance of the samples was subtracted from

Table 3. Monthly climatic data of Yalova Province during the experiment and long-term data (1991–2022).

Months	Year					
	2021		2022		1991-2020	
	Mean temperature (°C)	Total rainfall (mm)	Mean temperature (°C)	Total rainfall (mm)	Mean temperature (°C)	Total rainfall (mm)
January	9.10	164.00	6.30	95.30	6.80	84.60
February	7.90	59.70	7.70	11.70	7.20	68.70
March	7.50	117.50	5.60	49.60	9.00	73.90
April	11.90	59.10	13.30	41.50	12.60	51.30
May	18.00	31.10	16.80	29.30	17.40	39.00
June	20.50	98.80	22.30	54.30	21.90	47.40
July	24.90	27.50	23.40	13.30	24.30	22.00
August	25.00	7.30	25.20	68.30	24.50	34.50
September	20.30	16.90	20.80	39.30	20.80	52.90
October	15.40	44.90	16.20	40.40	16.50	93.70
November	13.00	60.70	14.10	16.60	12.00	75.90
December	10.50	159.20	11.80	34.40	8.60	105.00
Average	15.33	70.56	15.60	41.12	15.10	62.41

Table 4. Morphological characteristics of shallot genotypes.

Traits	Genotypes							
	GMSY-1	GMSY-2	GMSY-3	GMSY-4	GMSY-5	GMSY-6	GMSY-7	GMSY-8
Leaf								
Leaf attitude (LA) ¹	1	1	1	1	1	1	1	1
Leaf waxiness (LW) ²	7	7	9	7	5	9	7	9
Green color intensity (GCI) ³	7	7	7	7	5	7	7	7
Leaf cranking (LC) ⁴	1	1	1	1	1	1	1	1
Bulb								
Bulb shape (BS) ⁵	7	4	3	4	4	3	4	4
Shape of bulb top (SBT) ⁶	3	4	4	4	4	4	4	4
Shape of bulb base (SBB) ⁷	2	2	4	3	3	3	3	3
Position of maximum diameter (PMD) ⁸	2	2	2	2	2	2	2	2
Tendency to split into bulblet (TSB) ⁹	9	3	1	5	3	5	7	3
Main color of dry skin (MCDS) ¹⁰	6	6	4	6	6	8	4	1
Adherence of dry skin ¹¹	7	7	7	7	7	7	5	7
Flesh color (FC) ¹²	3	3	3	3	3	3	1	1
Firmness of flesh (FF) ¹³	7	7	7	7	7	7	7	5
Fleshy scales thickness (FST) ¹⁴	3	3	3	5	5	5	5	5
Coloration of epidermis of fleshy scales (CEFS) ¹⁵	3	3	1	3	3	3	1	1
Time of harvest maturity (THM) ¹⁶	3	5	7	5	5	5	5	3

¹Erect (1), erect to semierect (2), semierect (3), semierect to horizontal (4), horizontal (5); ²Absent or very weak (1), weak (3), medium (5), strong (7), very strong (9); ³Very light (1), light (3), medium (5), dark (7); ⁴Weak (1), intermediate (2), Strong (3); ⁵Elliptic (1), ovate (2), broad elliptic (3), circular (4), broad ovate (5), broad obovate (6), rhombic (7), transverse medium elliptic (8), transverse narrow elliptic (9); ⁶Depressed (1), flat (2), slightly raised (3), rounded (4), slightly sloping (5) strongly sloping (6); ⁷Depressed (1), flat (2), round (3), weakly tapered (4), strongly tapered (5); ⁸Towards top (1), at middle (2), towards base (3); ⁹Absent or very weak (1), weak (3), medium (5), strong (7), very strong (9); ¹⁰White (1), grey (2), green (3), yellow (4), brown (5), pink (6), red (7), purple (8); ¹¹Weak (3), medium (5), strong (7); ¹²White (1), reddish (2), purplish (3); ¹³Loos (3), medium (5), firm (7); ¹⁴Thin (3), medium (5), thick (7); ¹⁵Absent (1), greenish (2), reddish (3); ¹⁶Early (3), medium (5), late (7)

the absorbance of the control and the total antioxidant activity value of the samples was calculated according to a calibration chart drawn with Trolox. The results were given as mmol TE/L (Sanchez-Moreno et al., 1998; Akbulut and Coklar, 2015).

2.3.2. Total phenolic content (mg/L)

A juice sample (2.00 mL) was taken and 8.00 mL of 80% methanol was added, and this mixture was centrifuged at 5000 rpm for 5 min. Subsequently, 50 µL of the clear part obtained as a centrifugate was taken, 100 µL of Folin-Ciocalteu solution and 1500 µL of pure water were added, and the mixture was left standing for 10 min. As the next step, 50 µL of 20% sodium carbonate (Na₂CO₃) solution was added to the mixture and the mixture was held in the dark for 2 h, and absorbance values were determined against a blank sample on a spectrophotometer at a wavelength of

765 nm. Pure water was used instead of juice for the blank sample. The total amount of phenolic substances was calculated as mg/L gallic acid equivalent using the curve obtained from the prepared standard graph (Abdulkasim et al., 2007).

2.3.3. Total flavonoid content

A juice sample (1.00 mL) was taken and 4.00 mL of distilled water was added. By adding 0.30 mL of 5% sodium nitrite (NaNO₂) at the 0th minute, 0.30 mL of 10% aluminum chloride (AlCl₃) at the 5th minute, 2 mL of 1 M NaOH at the 6th minute, and then 2.40 mL of distilled water, a total volume of 10 mL was obtained. The absorbance value of the mixture was determined with a spectrophotometer at a wavelength of 510 nm. The total amount of flavonoids was calculated as catechin equivalent (CE)/100 mL from the curve obtained from the prepared standard graph (Zhishen et al., 1999).

2.3.4. Total anthocyanin content

A juice sample (1.00 mL) was taken and 24.00 mL of 0.03 M potassium chloride (KCl) buffer solution with pH of 1.00 was added. Another 1.00 mL of the same juice sample was taken and 24.00 mL of 0.40 M sodium acetate buffer solution with pH of 4.50 was added. The absorbance values of the resulting mixtures were determined with a spectrophotometer at wavelengths of 520 and 700 nm. The total anthocyanin amount of the samples was calculated as mg/100 mL in cyanidin-3-glycoside using the equation given below (Giusti and Wrolstad, 2001):

$$\text{Total monomeric anthocyanin content } \left(\frac{\text{mg}}{100 \text{ mL}} \right) = \frac{A \times MW \times Sf \times 100}{\epsilon \times L}$$

A (absorbance value): (A520 nm – A700 nm) pH 1.00 – (A520 nm – A700 nm) pH 4.50

MW: Molecular weight of anthocyanin to be taken as the base (cyanidin-3-glucoside molecular weight: 449.20 g/mol)

Sf: Dilution factor

ϵ : Molar absorption coefficient (molar absorbance value of cyanidin-3-glucoside: 26,900)

L: Layer thickness of the spectrophotometer cuvette (cm)

2.3.5. Pyruvic acid content ($\mu\text{mol/mL}$)

Extract (100 μL) was taken from juice and 3 mL of dinitrophenylhydrazine (DNPH) was added. The mixture was kept in a water bath at 40 °C for 10 min, and then 8.00 mL of 0.60 N NaOH was added to the samples taken out of the water bath and absorbance values were determined at a wavelength of 420 nm with a spectrophotometer. The pyruvic acid concentration was calculated from the sodium pyruvate standard curve obtained from the prepared standard chart (Schwimmer and Weston, 1961).

2.4. Sensory analysis

Sensory analysis was conducted by modifying the method used by Gündüz (2007) for grapes. Ten bulbs of each genotype were peeled, cut into rounds with a thickness of 2.00 mm, and presented for tasting. Twelve previously trained panelists evaluated the genotypes on a scale of 0–5 (Table 5). The Akgün 12/1, Akgün/2,

and Kantartopu 3 onion varieties were used as controls. As sensory properties, pungency, aroma, crunchiness, smell, consumption preference, and perceptibility of the epidermis membrane were investigated.

2.5. Statistical analysis

Student's t-test (least significant difference) was used in the analysis of agromorphological and biochemical data. Experiments were carried out according to a randomized plot design with 3 replications and 15 plants in each replication. SAS Version 9.1 (SAS Institute Inc., Cary, NC, USA) was used to evaluate the data. When the bidirectional F-test was significant (p^*), the means were compared with Tukey's post hoc test. Heatmap analysis was performed with the Bioconductor R package (Gentleman et al., 2004).

3. Results

3.1. Morphological characteristics

Findings regarding the morphological characterization of the tested shallot genotypes are summarized and presented in Table 6. There were differences in morphological characteristics among the eight shallot genotypes. Genotypes were compared in terms of a total of 16 morphological characters. While they were similar in terms of LA, LC, and PMD, they differed in terms of the other 13 features. While the LW trait was strong in half of the genotypes (GMSY-1, GMSY-2, GMSY-4, and GMSY-7), it was moderate in one (12.50%) (GMSY-5) and very strong in three (37.50%) (GMSY-3, GMSY-6, and GMSY-8).

Green color intensity was moderate in one genotype (GMSY-5) and strong in the other genotypes. The shallot genotypes formed three different groups in terms of head shape: 12.50% (GMSY-1) were considered rhombic, 62.50% (GMSY-2, GMSY-4, GMSY-5, GMSY-7, and GMSY-8) were circular, and 25.00% (GMSY-3, GMSY-6) were broadly elliptical.

The upper part of the head (SBT) was determined to be slightly raised in one genotype (GMSY-1) and rounded in the others (87.50%). The shape of the bulb base was flat in 25.50% (GMSY-1 and GMSY-2), weakly tapered in 12.50%

Table 5. Sensory analysis criteria.

Scala	Sensory analysis criteria					
	Pungency	Aroma	Crunchy	Smell	Sensibility of the epidermis membrane	the Consumption preference
0	Absent	Absent	Absent	Absent	None	None
1	Very low	Very poor	Very low	A little	Very little	Very little
2	Low	Poor	Low	Little	Little	Little
3	Medium	Medium	Medium	Medium	Medium	Medium
4	Strong	Strong	Strong	Strong	Strong	Strong
5	Very strong	Very strong	Very strong	Very strong	Very strong	Very strong

Table 6. Distribution of shallot genotypes according to morphological parameters.

Traits	Level	Value	Number of genotypes	Frequency (%)
Leaf attitude (LA)	Erect	1	8	100.00
	Medium	5	1	12.50
Leaf waxiness (LW)	Strong	7	4	50.00
	Very strong	9	3	37.50
Green color intensity (GCI)	Dark	7	7	87.50
	Medium	5	1	12.50
Leaf cranking (LC)	Weak	1	8	100.00
	Broad elliptic	3	2	25.00
Bulb shape (BS)	Circular	4	5	62.50
	Rhombic	7	1	12.50
Shape of bulb top (SBT)	Slightly raised	3	1	12.50
	Rounded	4	7	87.50
	Flat	2	2	25.00
Shape of bulb base (SBB)	Round	3	5	62.50
	Weakly tapered	4	1	12.50
Position of maximum diameter (PMD)	At middle	2	8	100.00
	Absent or very weak	1	1	12.50
	Weak	3	3	37.50
Tendency to split into bulblets (TSB)	Medium	5	2	25.50
	Strong	7	1	12.50
	Very strong	9	1	12.50
	White	1	1	12.50
Main color of dry skin (MCDS)	Yellow	4	2	25.50
	Pink	6	4	50.50
	Purple	8	1	12.50
Adherence of dry skin	Medium	5	1	12.50
	Strong	7	7	87.50
Flesh color (FC)	White	1	2	25.00
	Purplish	3	6	75.00
Firmness of flesh (FF)	Medium	5	1	12.50
	Firm	7	7	87.50
Fleshy scales thickness (FST)	Thin	3	3	37.50
	Medium	5	5	62.50
Coloration of epidermis of fleshy scales (CEFS)	Absent	1	3	37.50
	Reddish	3	5	62.50
	Early	3	2	25.00
Time of harvest maturity (THM)	Medium	5	5	62.50
	Late	7	1	12.50

(GMSY-3), and round in 62.50% (GMSY-4, GMSY-5, GMSY-6, GMSY-7, and GMSY-8) of the genotypes. The results for number of heads per plant were ranked as very strong (12.50%) (GMSY-1), strong (12.50%) (GMSY-7), medium (25.00%) (GMSY-4 and GMSY-6), weak (37.50%) (GMSY-2, GMSY-5, and GMSY-8), and absent or very weak (12.50%) (GMSY-3).

The main color of the dry skin was yellow in 25.00% (GMSY-3 and GMSY-7), purple in 12.50% (GMSY-6),

white in 12.50% (GMSY-8), and pink in 50.00% (GMSY-1, GMSY-2, GMSY-4, and GMSY-5) of the genotypes. While the adherence of dry skin was moderate in one genotype (GMSY-7), it was determined to be strong in all other genotypes (87.50%).

Flesh color was detected as purplish in 75.00% of the genotypes (GMSY-1, GMSY-2, GMSY-3, GMSY-4, GMSY-5, and GMSY-6) and white in the others (GMSY-7 and GMSY-8). The firmness of the flesh was medium in

one (12.50%) (GMSY-8) and firm in all other genotypes (87.50%).

Fleshy scale thickness was determined as thin in 37.50% of the genotypes (GMSY-1, GMSY-2, and GMSY-3) and medium in the others (62.50%) (GMSY-4, GMSY-5, GMSY-6, GMSY-7, and GMSY-8). While coloration of the epidermis of fleshy scales was not seen in 37.50% of the genotypes (GMSY-3, GMSY-7, and GMSY-8), it was noticeable as reddish in 62.50% of the genotypes (GMSY-1, GMSY-2, GMSY-4, GMSY-5, and GMSY-6).

The genotypes differed in terms of time of harvest maturity: 25.00% (GMSY-1 and GMSY-8) matured early, 62.50% (GMSY-2, GMSY-4, GMSY-5, GMSY-6, and GMSY-7) matured in the middle of the season, and 12.50% (GMSY-3) matured at a later date (Table 6). Morphological features that differ between genotypes are decisive characteristics that can be used to identify shallot genotypes.

3.2. Biometric parameters

The biometric parameters used to identify the shallot genotypes included plant height, pseudostem length, pseudostem diameter, number of leaves, leaf length, leaf diameter, bulb weight, bulb height, bulb diameter, neck width of bulb, dry skin thickness, root disc diameter, bulblet number per plant, water-soluble solid contents, and yield properties. The findings are presented in Tables 7–9.

Statistical analyses showed that there were significant differences in the biometric parameters of the eight shallot genotypes. There was a difference in terms of plant length, which varied between 35.10 and 58.27 cm. While the shortest plants were seen in the GMSY-8 (35.10 cm) and GMSY-8 (36.67 cm) genotypes, the tallest plants were seen in the GMSY-4 (56.17 cm), GMSY-5 (58.03 cm), and GMSY-6 (58.27 cm) genotypes.

Table 7. Plant and leaf biometric parameters of shallot genotypes.

Genotypes	Plant height (cm)	Pseudostem length (cm)	Pseudostem diameter (mm)	Number of leaves	Leaf length (cm)	Leaf diameter (mm)
GMSY-1	36.67±0.82d	7.60±0.27d	7.98±0.31e	8.07±0.38c	27.13±0.62d	5.89±0.14f
GMSY-2	49.23±0.69b	9.23±0.34b	12.40±0.41c	7.50±0.29c	41.00±0.65b	9.71±0.23c
GMSY-3	42.53±0.61c	8.30±0.21cd	11.14±0.34d	7.27±0.24c	34.90±0.55c	8.75±0.20d
GMSY-4	56.17±0.94a	9.72±0.36b	17.40±0.41a	9.56±0.40b	47.39±0.65a	12.84±0.27a
GMSY-5	58.03±0.97a	9.43±0.40b	17.02±0.39a	9.90±0.30ab	49.03±0.84a	12.44±0.37a
GMSY-6	58.27±0.84a	10.77±0.38a	16.32±0.50a	10.63±0.42a	48.80±0.86a	10.90±0.28b
GMSY-7	49.97±0.70b	9.10±0.18bc	14.65±0.37b	8.13±0.27c	41.17±0.62b	11.57±0.30b
GMSY-8	35.10±0.74d	8.30±0.38cd	7.04±0.28e	6.27±0.36d	24.77±0.47e	7.52±0.19e
F	138.74***	9.57***	107.61***	19.82***	198.27***	86.53***

Different letters in the same column indicates statistical differences at $p \leq 0.05$. ns: not significant. *, **, *** indicates $p \leq 0.05, 0.01, \text{ and } 0.001$, respectively.

Table 8. Biometric parameters of bulbs of shallot genotypes.

Genotypes	Bulb weight (g)	Bulb height (mm)	Bulb diameter (mm)	Neck width of bulb (mm)	Bulblet number per plant
GMSY-1	21.67±1.27e	29.79±0.44f	40.56±1.34c	4.94±0.28d	2.33±0.09c
GMSY-2	90.74±4.31ab	53.66±1.45cd	56.40±1.33b	9.61±0.36a	2.00±0.00d
GMSY-3	50.78±2.52d	62.89±1.21a	40.64±0.88c	8.36±0.31b	2.00±0.00d
GMSY-4	73.58±4.09c	50.05±2.41de	54.08±1.29b	9.88±0.57a	2.89±0.24b
GMSY-5	81.98±4.00bc	57.99±1.50b	55.25±1.91b	10.08±0.39a	2.67±0.09b
GMSY-6	76.15±2.98c	58.77±1.85b	54.32±1.19b	9.82±0.51a	2.00±0.00d
GMSY-7	93.82±4.85a	55.15±1.49bc	63.88±1.73a	6.83±0.33c	4.00±0.15a
GMSY-8	44.95±3.28d	49.05±1.12e	43.14±1.21c	4.95±0.23d	2.00±0.00d
F	52.28***	51.90***	38.23***	35.46***	62.48***

Different letters in the same column indicates statistical differences at $p \leq 0.05$. ns: not significant. *, **, *** indicates $p \leq 0.05, 0.01, \text{ and } 0.001$, respectively.

Table 9. Bulb and yield characteristics of shallot genotypes.

Genotypes	Dry skin thickness (mm)	Root disc diameter (mm)	Water-soluble solid content (%)	Yield kg/m ²
GMSY-1	0.03±0.00cd	9.42±0.30d	8.83±0.16ab	2.17±0.13e
GMSY-2	0.05±0.00b	11.28±0.40c	9.50±0.27a	9.07±0.43ab
GMSY-3	0.06±0.00a	11.75±0.33c	9.00±0.55ab	5.08±0.25d
GMSY-4	0.04±0.00c	12.20±0.48bc	8.78±0.06ab	7.36±0.41c
GMSY-5	0.04±0.00cd	12.87±0.28b	8.67±0.18b	8.20±0.40bc
GMSY-6	0.04±0.00cd	14.99±0.32a	8.67±0.23b	7.62±0.30c
GMSY-7	0.05±0.00b	15.73±0.38a	7.00±0.15c	9.38±0.49a
GMSY-8	0.03±0.00d	12.24±0.41bc	2.33±0.09d	4.49±0.33d
F	16.00***	33.39***	80.01***	52.28***

Different letters in the same column indicates statistical differences at $p \leq 0.05$. ns: not significant. *, **, *** indicates $p \leq 0.05$, 0.01, and 0.001, respectively.

The differences between the pseudostem lengths of the genotypes were found to be significant as these lengths varied from 7.60 mm to 10.77 mm. The shortest pseudostem was seen in the GMSY-1 (7.60 mm) genotype and the longest pseudostem was seen in the GMSY-6 (10.77 mm) genotype. The differences between the pseudostem diameters of the plants were found to be significant depending on the genotypes; the thinnest pseudostem was seen in GMSY-8 (7.04 mm) and GMSY-1 (7.98 mm), while the thickest were seen in GMSY-6 (16.32 mm), GMSY-5 (17.02 mm), and GMSY-4 (17.40 mm) in increasing order, respectively.

The differences between genotypes regarding leaf number were found to be significant; the fewest leaves were found in the GMSY-8 genotype (6.27 per plant), while the highest leaf number was found in GMSY-6 (10.63 per plant).

Leaf length varied between 24.77 and 49.03 cm and the differences between the genotypes were found to be significant. Plant length and leaf length were found to parallel each other and the longest leaves were obtained from the same genotypes [GMSY-4 (47.39 cm), GMSY-6 (48.80 cm), and GMSY-5 (49.03 cm)].

The shortest leaves were seen in the GMSY-8 genotype (24.77 cm). The differences between genotypes in terms of leaf diameter were also significant. The narrowest leaves were seen in GMSY-1 (5.89 mm), while the widest leaves were detected in GMSY-5 (12.44 mm) and GMSY-4 (12.84 mm), respectively (Table 7). Head weight differed among the genotypes and varied between 21.67 and 93.82 g. While the smallest heads were obtained from GMSY-1 (21.67 g), the largest heads were obtained from GMSY-2 (90.74 g) and GMSY-7 (93.82 g). The longest heads were seen in the GMSY-3 genotype (62.89 mm) and the shortest heads were seen in GMSY-1 (29.79 mm), similarly to the findings for head weight.

Differences in bulb diameter were found to be significant and these values varied between 40.56 and 63.88 mm. The widest head diameter was seen in GMS-

7 (63.88 mm), while the shortest bulb diameters were seen in GMSY-1, GMSY-3, and GMSY-8 with average values of 40.56 mm, 40.64 mm, and 43.14 mm, respectively. The differences between the width of the necks of the heads were significant among the genotypes, and the thinnest head necks were obtained from GMSY-1 (4.94 mm) and GMSY-8 (4.95 mm). The widest heads were seen in the GMSY-2, GMSY-6, GMSY-4, and GMSY-5 genotypes with average values of 9.62 mm, 9.82 mm, 9.88 mm, and 10.08 mm, respectively. Bulblet numbers per plant varied between 2.00 and 4.00, and the highest number was seen in the GMSY-7 genotype. Numbers varied between 2.00 and 2.89 per bulb in the other genotypes (Table 8).

The differences between genotypes in terms of dry skin firmness, an important feature for the preservation of edible onions and shallots, were found to be significant, with values varying between 0.03 and 0.06 mm. The differences between genotypes in terms of root disc width were found to be highly significant. The heads with the smallest root disc were detected in the GMSY-1 genotype (9.42 mm), while the two genotypes with the widest root discs were GMSY-6 (14.99 mm) and GMSY-7 (15.73 mm), respectively (Table 9).

The differences between genotypes in terms of water-soluble solid contents were found to be significant, with values varying between 2.33% and 9.50%. While the lowest water-soluble solid content was observed for GMSY-8 (2.33%), average values of 8.78%, 9.00%, and 9.50% were obtained for GMSY-4, GMSY-3, and GMSY-2, respectively (Table 9). Statistical groupings of genotypes regarding yield paralleled the results for head weight. Yield values varied between 2.17 and 9.38 kg/m² (Table 9).

3.3. Clustering of biochemical parameters

The determined biochemical parameters were pyruvic acid, total antioxidant capacity, total anthocyanins, and total phenolic and total flavonoid contents. The data obtained were evaluated with the HCA and PCA methods and the findings are presented in Figures 2 and 3.

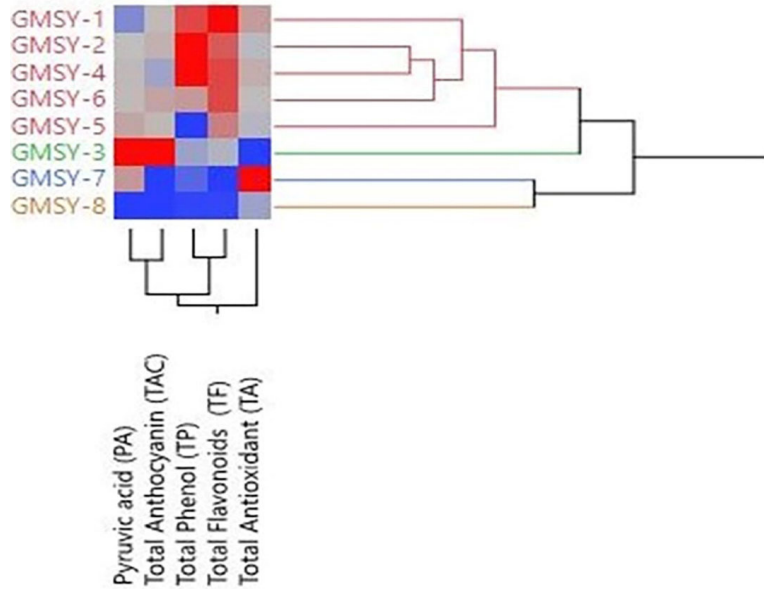


Figure 2. Grouping of shallot genotypes by biochemical contents. The color scale from blue to red represents lower (blue) to higher (red) values.

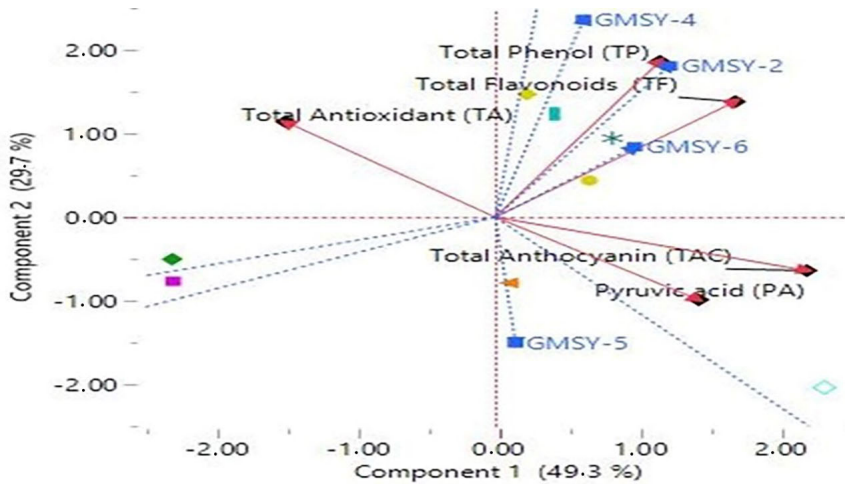


Figure 3. Determination of the relationships between biochemical contents of the bulbs of the shallot genotypes by PCA.

Based on HCA, the genotypes were divided into four different clusters. In the heatmap analysis of the biochemical parameters of the shallot genotypes, GMSY-1, GMSY-2, GMSY-4, GMSY-6, and GMSY-5 formed a separate cluster with high total phenol and total flavonoid contents. However, the GMSY-5 genotype was partially distinguished by its lower total phenolic contents. GMSY-3 formed a separate cluster with high pyruvic acid and total anthocyanin contents and low total antioxidant capacity. GMSY-7 formed a different cluster with low total anthocyanins, total phenolics, and total flavonoids and high total antioxidant capacity. Finally, GMSY-8 formed a

different cluster, separated from the other groups with its low pyruvic acid, total anthocyanins, total phenolics, and total flavonoids (Figure 2).

The distributions of the correlations between the biochemical parameters of the shallot genotypes in the basic coordinate plane defined by PCA are given in Figure 3. As a result of PCA, it was seen that the total variation consisted of the first two principal component axes, and the variation between biochemical parameters and genotypes was found to be 79.00%.

The first principal component axis accounted for 49.30% of the total variation and the second accounted

for 29.70% of the total variation. Therefore, these axes were important in evaluating the analysis results. It was observed that the total phenolic and total flavonoid content values defined by PCA paralleled each other. Similarly, pyruvic acid and total anthocyanin values were determined to be parallel. However, total antioxidant values displayed negative correlations with pyruvic acid and total anthocyanin values (Figure 3).

3.4. Sensory properties

In HCA, the genotypes were divided into four different clusters. In heat mapping analysis, the Akgün 12/1 and GMSY-8 genotypes formed a separate cluster with high aroma, priority in consumption preference, and crunchiness in terms of sensory characteristics. Akgün 12/2 and GMSY-6 formed a separate cluster with high flavor and consumption preference, low pungency, and perceptibility of the membrane. GMSY-1, Kantartopu 3, GMSY-2, GMSY-3, GMSY-4, and GMSY-5 formed a separate cluster with high pungency and perceptibility of the membrane, as well as low smell, aroma, consumption preference, and crunchiness. GMSY-7 was distinguished from the other clusters with its strong smell and low aroma properties (Figure 4; Table 10).

4. Discussion

4.1. Morphological characterization

Eight shallot genotypes including one vegetatively propagated and seven generatively propagated were

evaluated regarding a total of 16 morphological parameters including leaf attitude, leaf waxiness, green color intensity, leaf cranking, bulb shape, shape of the bulb top, shape of the bulb base, position of maximum diameter, tendency to split into bulblets, main color of dry skin, adherence of dry skin, flesh color, firmness of flesh, fleshy scales thickness, coloration of the epidermis of fleshy scales, and time of harvest maturity. Brewster (1994), Tendaj (2005), Khosa et al. (2014), and Perkovic et al. (2020) indicated the importance of these 16 parameters in distinguishing shallot genotypes. In the present study, most of the morphological parameters differed among the genotypes, excluding leaf attitude, green color intensity, leaf cranking, and position of maximum bulb diameter.

Using the morphological characters discussed in this study, Poulsen and Henriksen (2001) collected 161 types of shallots from northern European countries; Khosa et al. (2014) collected 35 different *Allium* genotypes, including one shallot genotype, grown in India; and Perkovic et al. (2020) reported the variations among 35 different shallot genotypes.

The most important feature that distinguishes shallots from onions morphologically is that more than one head with its own peel is formed from one shallot plant. Brewster (1994) defined shallots as having 3–20 heads for one plant, while Puizina (2013), Tendaj and Mysiak (2013), and Beşirli et al. (2014) reported that 1–22 heads can be formed. Jones and Mann (1963), Brewster (1994),

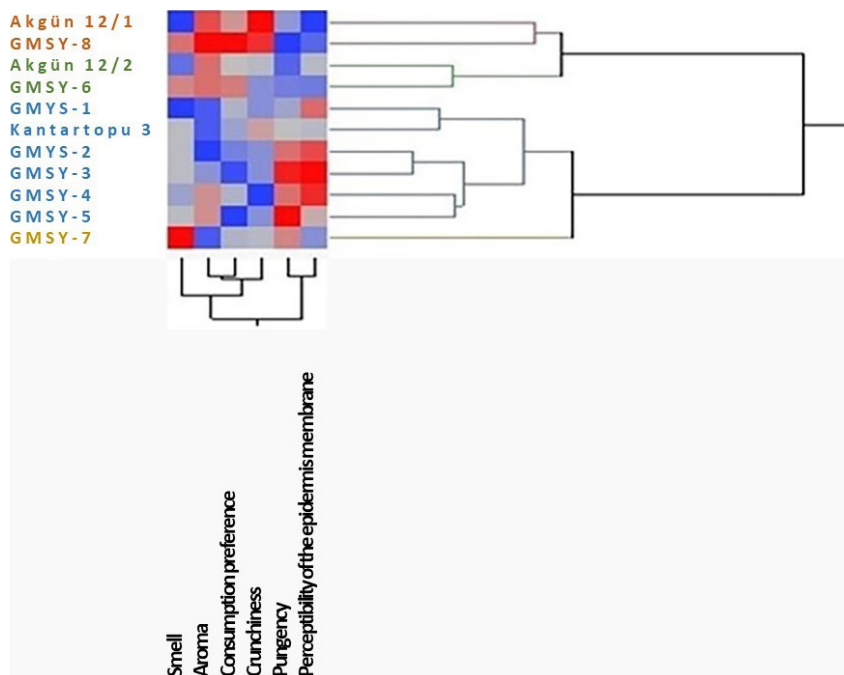


Figure 4. Grouping of genotypes according to sensory analysis. The color scale from blue to red represents lower (blue) to higher (red) values.

Table 10. Sensory evaluation of shallot genotypes.

Genotypes	Pungency	Aroma	Crunchy	Smell	Consumption preference	Sensibility of the epidermis membrane
Akgün 12/1	2.83±0.39bcd	3.33±0.28 ^{NS}	3.75±0.28 ^{NS}	2.00±0.41c	3.42±0.36abc	1.50±0.31b
Akgün 12/2	2.50±0.44cd	3.25±0.22	3.33±0.28	2.17±0.24bc	3.17±0.41bc	2.00±0.35ab
GMSY-3	3.75±0.28ab	2.92±0.34	3.25±0.25	2.42±0.29bc	2.58±0.42c	2.50±0.36a
GMSY-4	3.42±0.3abc	3.17±0.24	3.08±0.23	2.33±0.19bc	3.08±0.29bc	2.42±0.23a
GMSY-5	3.83±0.21a	3.17±0.34	3.25±0.28	2.42±0.26bc	2.50±0.42c	2.08±0.31ab
GMSY-6	2.67±0.22cd	3.25±0.22	3.25±0.22	2.67±0.14abc	3.58±0.29ab	1.75±0.39ab
GMSY-7	3.33±0.41abc	2.75±0.30	3.33±0.28	3.17±0.30a	3.08±0.40bc	1.83±0.32ab
GMSY-8	2.25±0.30d	3.50±0.34	3.67±0.26	2.75±0.22ab	4.33±0.19a	1.67±0.28ab
GMYS-1	2.92±0.36abcd	2.75±0.33	3.25±0.25	2.00±0.17c	3.08±0.36bc	2.25±0.28ab
GMYS-2	3.42±0.40abc	2.67±0.33	3.25±0.22	2.42±0.29bc	2.83±0.34bc	2.33±0.36ab
Kantartopu 3	3.08±0.31abcd	2.75±0.30	3.42±0.15	2.42±0.23bc	3.00±0.37bc	2.00±0.33ab
F	2.27*	0.92 ^{ns}	0.63 ^{ns}	1.73*	2.02 [†]	1.00 [†]

*: The difference between the means with the same letter in the same column is significant at the $p < 0.05$, $p < 0.01$ and $p < 0.001$ level. ns: not significant.

and Rabinowitch and Kamenetsky (2002) stated that the head shape and dry shell color are determining features used in the preparation of catalogs of edible *Allium* species and that shallot genotypes have a cluster structure with wide structures rather than long heads and yellow, brown, pink, and purple shell colors.

The head shapes of the shallot genotypes examined in this study could be divided into three groups: broadly elliptic, circular, and rhombic. Shell colors were determined as white, yellow, pink, and purple. Thus, these findings are in line with the aforementioned descriptions in the literature. In light of the findings obtained, it was concluded that all morphological characteristics examined in this study, which were seen to be decisive in distinguishing shallot genotypes, could be used as selection criteria in future shallot breeding studies.

4.2. Biometric parameters

As biometric parameters, plant height, pseudostem length, pseudostem diameter, number of leaves, leaf length, leaf diameter, bulb weight, bulb height, bulb diameter, neck width of bulb, dry skin thickness, root disc diameter, bulblet number per plant, water-soluble solid contents, and yield were evaluated and variations among the genotypes based on those parameters were determined.

In *Allium* species, plant height, pseudostem height, pseudostem diameter, leaf length, and leaf diameter provide preliminary information about plant development and performance as they all determine the photosynthetic capacity of the plant. These characteristics may vary largely depending on the genetic structure, climatic conditions, and growing conditions, such as dry versus irrigated conditions (Brewster, 1994). The plant heights of the genotypes examined in this study, except for GMSY-1

and GMSY-8, were found to be similar to the data obtained by Shimeles (2014) from plants propagated by seeds and grown under irrigated conditions.

While GMSY-1 is a vegetatively propagated genotype, GMSY-8, unlike the others, has white-skinned heads. In this study, significant differences were found between the genotypes in terms of pseudostem height and diameter characteristics, which are among the UPOV criteria. However, these features were not taken into account by other researchers. While the leaf length and diameter of the genotypes examined in this study were similar to the findings of studies conducted by other researchers, the numbers of leaves were found to be low (Major et al., 2018; Perkovic et al., 2020).

Considerable variation in head weight was observed among the genotypes examined in this study and the data obtained were comparable to those of Tendaj (2005), Shimeles (2014), and Perkovic et al. (2020). Head height and head diameter are important features in terms of giving information about the head shapes of *Allium* species. If the height/diameter ratio is close to or equal to 1, the head shape is closer to being round (Brewster, 1994).

There is an inversely proportionate relationship between head size and the number of heads formed in a plant. Furthermore, the number of bulbs formed in a plant is higher in vegetatively produced genotypes than in seed-produced genotypes, and the variation between the shallot genotypes examined in this study in terms of these two features was found to be significant (Rabinowitch and Kamenetsky, 2002). Researchers have generally examined the weight and diameter of shallot heads. While the diameters of the shallot genotypes examined in this study were larger than the diameter sizes of the genotypes

examined by Major et al. (2018), the values were similar to those reported by Shimeles (2014) and Perkovic et al. (2020). Head diameter in *Allium* species is a calibration criterion for products to be marketed and for heads to be used in planting (Rabinowitch and Kamenetsky, 2002).

The neck width of the bulbs, an important feature in distinguishing genotypes, showed high variation among the genotypes examined in the study. Neck thinness is particularly reported as being important in rainy regions; the flow of water drops along a thinner neck after rainfall prevents the development of diseases and is important in ensuring faster postharvest drying. Thus, varieties with thin-necked heads are preferred in the market (Brewster, 1994; Singh et al., 2021). Dry shell thickness is an important feature for the healthy preservation of heads of *Allium* species during storage and marketing.

Variation in dry shell thickness was found to be significant among the shallot genotypes examined in this study. The shell thickness of early shallot genotypes and white-skinned shallots is thinner, as seen in onions (*Allium cepa* L.). It is desirable to have higher values of dry shell thickness in genotypes that will be sent to distant markets or stored for long periods of time (Jones and Mann, 1963; Kate et al., 2022).

The root disc diameter variation among the genotypes considered in this study was found to be significant. Brewster (1994) reported that root disc width was an important feature in identifying varieties, with smaller values being preferred. The variation between genotypes in terms of bulblet number per plant was also found to be important, as were other head characteristics.

Many researchers have reported that the most important feature that distinguishes shallots from common onions is the number of heads formed on a plant, and the number of heads formed may vary depending on propagation by head, seedling, or seed (Beşirli et al., 2014; Perkovic et al., 2020). Water-soluble solid contents showed high variation across genotypes. Francke and Klasa (2009) reported that soluble solid properties and other nutritional values vary depending on the genetic structure, climate, care, and nutrition conditions in shallots. The soluble solid values obtained in this study were below the values reported by Francke and Klasa (2009) and Shimeles (2014).

The differences between the examined shallot genotypes in terms of yield were found to be significant. Higher yield was obtained from seed-propagated genotypes compared to the vegetatively propagated GMSY-1 genotype. The shallot yield obtained in this study was above the values reported by Brewster (1994), Tendaj (2005), and Shimeles (2014).

4.3. Biochemical parameters

In this study, as biochemical parameters of the genotypes, pyruvic acid, total antioxidants, total anthocyanins, total

phenolics, and total flavonoids were examined and the variations between genotypes were found to be important for these properties. Sun et al. (2019) reported that the most important chemicals in shallots with regards to human health and their use as medicinal plants are phenolic compounds, antioxidants, vitamins, and minerals. The presence and importance of these components in shallots was also emphasized by Fenwick and Hanley (1990). Fattorusso et al. (2002) and Adeyemo et al. (2023) reported that the amount of these phytochemicals in shallots varies depending on the genotype, the conditions in which samples are taken (e.g., from the leaf, head, or whole plant), climatic conditions, and cultural practices. The nutritional value of a food is more important than the amount consumed in human nutrition. In breeding studies, it is important to use genotypes with higher phytochemical values in terms of the chemical properties discussed here and transfer those properties to variety candidates.

4.4. Sensory analysis

The genotypes were examined in terms of pungency, aroma, crunchiness, smell, consumption preference, and perceptibility of the epidermis membrane. Significant variation was found among them in terms of pungency, smell, epidermis membrane perceptibility, and consumption preference. Brewster (1994) reported that pungency in shallots is related to pyruvic acid and water-soluble solid contents, and genotypes with low values for these parameters are sweeter. The results of the present study showed that the GMSY-8 genotype was the best example of those findings.

5. Conclusion

The results of this study have confirmed that the variation among eight Turkish shallot genotypes is important in terms of morphological characterization, biochemical components, and sensory characteristics. Each of the shallot genotypes in question (except GMSY-1) stands out with different characteristics and has the potential to be used as a parent in breeding programs with different purposes. By registering them as standard varieties in their current state, they can be used to fill the gap in shallot varieties needed in Türkiye.

Author contributions

GB was responsible for conceptualization, methodology, software, validation, formal analysis, investigation, data curation, writing the original draft, reviewing and editing, project administration, and funding acquisition. The author has read and agreed to the published version of the manuscript.

Ethics approval and consent to participate

Not applicable.

Availability of data and material

All data generated or analyzed during this study were included.

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