Morphological Variability and Yield Traits in Softneck Garlics

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ABSTRACT

To improve garlic breeding, it is important to determine the morphological differences between garlic genotypes of local origin. This study was conducted to determine the phenotypic diversity of Turkish softneck garlic (Allium sativum L. sub. var. sativum) genotypes using morphological traits determined based on International Union for the Protection of New Varieties of Plants (UPOV) descriptors. Twenty-six garlic genotypes were characterized using 15 quantitative morphological characteristics. Principal Component Analysis (PCA) revealed that the first four principal components explained 84.58% of the total variation among the 26 garlic genotypes. The characters with the greatest contribution to variability were identified as Plant Height (PH), Pseudostem Diameter (PSD), Leaf Length (LL), Leaf Width (LW), Bulb Weight (BW), Yield (Y), Bulb Height (BH), Bulb Diameter (BD), Clove Height (CH), Bulb Height/Bulb Diameter ratio (BH/BD), Vumber of Cloves (NC), Clove Weight (CW), Clove Width (CWi), and Clove Thickness (CT). Significant differences were observed in the quantitative traits of garlic genotypes. As a result of the study, AS14 stood out for its clove weight, length, width, and thickness, while AS13 had the highest bulb weight and yield. The present findings could be reliably used in the development of new garlic varieties.

Keywords: Allium sativum L., Genetic resources, Phenotypic diversity, Principal component analysis, Quantitative characters.

INTRODUCTION

Garlic belongs to the genus Allium of the family *Alliaceae*. It is widely grown in temperate climate zones and mountainous sections of tropical climate zones (Manjunathagowda et al., 2017; Ayed et al., 2019; Erbaş, 2019). Garlic plays a significant role in human nutrition and health; thus, its production and consumption are continuously increasing worldwide. Garlic constitutes approximately 5% of the total vegetable production in Turkiye. Four percent of the total production (116,840 tons) is used as dry garlic, and 1% (28 552 tons) is used as fresh garlic. "Taşköprü" is the most popular garlic species in Turkiye. Gaziantep province ranks first in production, with an annual production of 33,973 tons. This is followed by the Kastamonu (22,995 tons) and Kahramanmaraş (7,259 tons) provinces (TurkStat, 2022). Garlic (Allium

sativum L.) has also been used as a spice and medicinal plant since ancient times (Etoh, 1985; Gehani and Kanbar, 2013; Petropoulos et al., 2018; El-Fiki and Adly, 2020; Beşirli et al., 2022). It is an important functional food because of its organosulfur and phenolic components. It is used in both traditional and clinical medicine to prevent and treat various diseases (Kim et al., 2013; Koca et al., 2015; Chhouk et al., 2017; Akan, 2022).

The region extending from the Mediterranean Basin to the Caucasus is defined as the second gene center of garlic. Turkiye is located within this region; thus, it has excellent population richness (Etoh and Simon, 2002). Since most of the garlic varieties grown today are sterile, they are propagated vegetatively using cloves (Brewster, 1994; Yulianingsih et al., 2019). Garlic populations are generally divided into two sub-groups: soft neck (unbolting plants, creamy cloves) and hard neck (bolting

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plants, pink-red cloves) (Volk and Stern, 2009; Portela et al., 2012). Soft-necked garlics are strong-smelling and resistant to storage for 6-8 months. Cloves arranged radially or non-radially on the head are also prone to form external cloves. Hard-necked garlics have fewer protective shells, shorter storage life, and lighter scent. The cloves of this group of garlics, which form larger cloves and ostentatious heads than softnecked garlics, are arranged circularly around the flower stalk. They form bulbils on the flower stalk and flower base (Koch et al., 1996). Although garlic is propagated vegetatively, the frequent replacement of clones between producers causes differences in morphological characteristics (Khar et al., 2006; Kılıç, 2021). These variations among garlic genotypes constitute an important source for development of new varieties (Yarali Karakan, 2022). Morphological traits are widely used in breeding programs to select lines with the maximum variation (Liu et al., 2007; Hartings et al., 2008; Zhang et al., 2008; Smykal et al., 2008; Wang et al., 2014; Polyzos et al., 2019). Morphophysiological characteristics are commonly used to elucidate genetic variation within and between populations. Such traits are also used to determine the genetic similarities and dissimilarities between populations (Hunter, 1993). Therefore, they are widely used by the International Association for the Conservation of New Plant Varieties (UPOV). In genetic diversity studies, these traits are generally expressed as numerical values (Sneath and Sokal, 1973). In other words, the similarities or differences between cultivars were expressed by the coefficients. However, in recent years, some techniques have emerged in which more than one variable can be analyzed together (Özdamar, 2004). Multivariate analyses, such as clustering and principal component analysis (PCA), are widely employed to reveal genetic variation (Hair et al., 1995). PCA facilitates selection of traits that can explain the greatest portion of variation. It also facilitates the improvement of low-

heritability traits, especially in early generations, for use in hybridization and selection programs (Doumbia et al., 2013; Pal et al., 2018). PCA reveals similarities and dissimilarities between the populations. It also reveals genotypes with superior traits (Escribano et al., 1991; Cartea et al., 2002).

For garlic, the characteristics with the greatest contribution to genetic variability were identified as bulb weight, diameter, yield, number of cloves per bulb, plant height, number of leaves per plant, and bulb binding (Bradley et al., 1996; Gad El-Hak and Abd El-Mageed, 2000; Beşirli, 2005; Petropoulos et al., 2018; Kıraç, 2019). Various studies have been conducted on some subjects, such as molecular and morphological characterization and chemotaxonomic classification, to reveal the variations between garlic genotypes. In these studies, positive correlations were reported between clove and bulb weight, and negative correlations between clove weight and number of cloves (Akan, 2022). It has also been reported that changes in yield are directly proportional to the number of leaves and bulb weight, and morphological variations could be used in garlic selection studies (Baghalian et al., 2006; Panthee et al., 2006; Mohammadi et al., 2014; Portela et al., 2015; Akbarpour et al., 2021). Although many studies have examined Turkiye's local garlic genotypes, no studies have examined a large number (25 genotypes+1 control) of soft-necked garlic genotypes. Therefore, this study aimed to elucidate the morphological variability of soft-neck garlic genotypes obtained from the "Garlic Gene Bank." Multivariate analyses were used to present the nature of variability and group traits with the greatest variability.

MATERIALS AND METHODS

Twenty-six soft-neck garlic genotypes were used as plant materials in the present study. Garlic genotypes collected from local or commercial garlic production areas in Turkiye were obtained from the "Garlic Gene Bank" generated within the scope of the projects. These projects have been entitled "Research on the Breeding of Turkish Garlics by Selection Method," "Selection of Kastamonu Garlic (Allium sativum L.), and Creation by Irradiation in a Selected Clone, " and The Research Project of The Conservation and Evaluation of Edible Allium spp. Genetic Resources at the Atatürk Horticultural Central Research Institute between 1975-2020. The registered "Taşköprü 56" garlic variety (AS26) was used as the control variety. The collection sites of garlic accessions and the GPS coordinates of the sampling locations are provided in Table 1.

Garlic cloves were planted in the experimental fields of the Atatürk Horticultural Central Research Institute, located in the Yalova Province (Latitude 40º 28′ N Longitude 28º 45′E), on December 12, 2020. Experiments were conducted in a Randomized Complete Block Design (RCBD) with three replications. Sowing was performed at 20 cm row spacing and 10 cm on-row plant spacing. The soil physicochemical properties were determined by Chapman and Pratt (1961), and the results are given in Table 2.

From sowing to harvest, the same cultural practices were performed for each genotype. Approximately 100 kg ha⁻¹ N, 70 kg ha⁻¹ K, and 40 kg ha^{-1} S fertilizer were applied during the vegetation period. During the initiation of bulbing, Zn-based microelements were applied three times through drip irrigation at an interval of one week. However, Phosphorus (P) was not applied to plants because the soils were sufficient for phosphorus (Table 2). Irrigation was performed twice per week from the beginning of May to the middle of

Accessions	City/Province/Village	Longitude	Latitude	Altitude (m)
AS1	Kastamonu/Taşköprü	34°29'	$41^{\circ} 30'$	553±5
AS ₂	Sakarya	30° 40'	40° 78'	31 ± 5
AS3	Yalova	28° 45'	$40^{\circ} 28'$	30±5
AS4	Edirne/Uzunköpü/Yeniköy	26°69'	41° 27'	$10+5$
AS5	Tekirdağ	$26^{\circ} 43'$	$40^{\circ} 36'$	$37 + 5$
AS6	Kırklareli	26°53'	$41^{\degree}44'$	203 ± 5
AS7	Edirne	26°55'	41° 67'	42 ± 5
AS8	Afyonkarahisar	29° 40'	$37^\circ\,45^\prime$	1021 ± 5
AS9	Batman	37° 50^\prime	41° 10^{\prime}	540±5
AS10	Kastamonu/Taşköprü	$34^\circ\,29^\prime$	$41^{\circ} 30'$	553±5
AS11	Gaziantep	36°28'	$37^{\circ} 32'$	850±5
AS ₁₂	Aydın	27°84'	37°83'	67 ± 5
AS13	Eskişehir	$30^{\circ} 32'$	39°40'	788±5
AS14	Muğla/Fethiye	29°12'	36°65'	660 ± 5
AS15	Kayseri	36°59'	$37^{\circ} 45'$	1050 ± 5
AS16	Cyprus/Nicosia ^a	$33^{\circ} 36'$	33° 21'	220 ± 5
AS17	Kilis	$37^{\circ} 11'$	36°71'	660 ± 5
AS18	Muğla	28°21'	37°12'	660±5
AS19	Kütahya	29°59'	39°25'	970 ± 5
AS20	Muğla	28°21'	$37^{\circ} 12'$	660±5
AS21	Balıkesir	$27^{\circ} 87'$	$40^{\circ} 23'$	$70 + 5$
AS22	Samsun/Çarşamba	36° 43'	$41^\circ 11'$	$15 + 5$
AS23	Kayseri/Pinarbaşı	36°39'	38°72'	1050 ± 5
AS24	Yalova	28° 45'	$40^{\circ} 28'$	30±5
AS25	Yalova	$28^{\circ} 45'$	$40^{\circ} 28'$	30 ± 5
AS26	Kastamonu/Taşköprü	34°29'	$41^{\circ} 30'$	553 ± 5

Table 1. Accession numbers, collecting sites and geographical coordinates.

^a This genotype, collected from Cyprus/Nicosia in 1975, has been grown for 48 years in Yalova, Turkey.

Table 2. Some physical and chemical properties of the experimental soil (0-30 cm).

July. Manual weed control was practiced. Climate data of Yalova Province for the years 2020 and 2021 and long-term averages (1991/2020) are given in Table 3 (Turkish State Meteorological Service, 2023).

Morphological measurements were carried out at two different stages: pre-harvest and post-harvest. Measurements of aboveground parts (plant height, leaf length and width, and pseudostem diameter) were made in the field at the bulb formation stage of the plants 150 days after planting. Harvesting was carried out at the beginning of August when 80% of the plants started to turn yellow and dry. For a positive effect on the protection of the bulbs during storage, harvested bulbs were left over the field in sunny and openair conditions for the drying process for two days (Kaynas et al., 1997). The dried green parts and roots were cleaned, and the bulbs placed in the nets were brought to the Vegetables Department laboratories to

determine their morphological characteristics. Morphological measurements (Table 4) were performed on ten randomly selected plants from each genotype. Measurements were performed using the criteria described by the UPOV for Allium sativum L. (UPOV, 2001).

The data on morphological traits were analyzed using XLSTAT 2016 software. Principal Component Analysis (PCA) was performed using Pearson's correlation matrix to compute the association that accounted for diversity in 15 quantitative traits. For the principal components, the squared cosines $(cos²)$ were used to determine the most important variables for each component. The squared cosine values generated were used to indicate the traits that contributed significantly to each component, where high values of squared cosine for traits in individual principal components revealed traits with greater

Table 3. Monthly climate data of Yalova Province (1991-2021).

Morphological traits	Parameters
	Plant height (cm)
	Leaf length (cm)
Plant	Leaf width (cm)
	Pseudostem diameter (mm)
	Bulb weight (g)
	Yield (ton ha^{-1})
Bulb	Bulb height (mm)
	Bulb diameter (mm)
	Bulb height/Bulb diameter
	Dry external thickness (mm)
	Number of cloves
	Clove weight (g)
Clove	Clove height (mm)
	Clove width (mm)
	Clove thickness (mm)

Table 4. Determined morphological traits used in the morphological characterization of softneck garlic genotypes.

weight in defining those principal components (Nyabera et al., 2019).

RESULTS

Squared cosine values of the generated characters were used as a measure of their contribution to the explanation of the variation in Principal Components. Significant differences were observed for all the traits of the genotypes (Table 5). A scree plot of several components and eigenvalues is shown in Figure 1. PCA results revealed that the first ten principal components explained 99.15% of the total variation. A number of "useful" dimensions were automatically detected, and the first four principal components with eigenvalues greater than 1, representing a cumulative variance of 84.58%, were considered as principal components. The first principal component (F1) had an eigenvalue of 6.785 and contributed 45.235% of the total variability, whereas F2, F3, and F4, with eigenvalues of 3.519, 1.324, and 1.059, accounted for 23.462, 8.824, and 7.061% of the total variation, respectively.

It was considered that the greater the squared cosines, the greater the link with the corresponding axis. Based on the squared

cosine values, the first principal component (F1) includes Plant Height (PH), Pseudostem Diameter (PSD), Leaf Length (LL), Leaf Width (LW), Bulb Weight (BW), Yield (Y), Bulb Height (BH), Bulb Diameter (BD), Clove Height (CH). F2 includes the Bulb Height/Bulb Diameter (BH/BD) ratio, Number of Eloves (NC), Clove Weight (CW), Clove Width (CWi), and Clove Thickness (CT). The third component (F3) did not include any important contributing traits, whereas the fourth component (F4) included only Dry External Thickness (DET) (Table 6). As a result, traits that show a high contribution towards genetic variability may be used for positive selection by breeders.

The correlation circle generated below F1 and F2 was used to interpret the axes (Figure 2). Variables had significant positive correlations if they were far from the center, but close to each other (with r values close to 1); they had significant negative correlations if they were on the opposite side of the center (with r values close to -1), and they were not correlated if they were orthogonal to each other (with r values close to 0). In this case, the horizontal axis was linked with the Plant Height (PH), Pseudostem Diameter (PSD), Leaf Length and Width (LL and LW), Bulb Weight and

Principal Component	Eigenvalue	Variability (%)	Cumulative $(\%)$
PC ₁	6.785	45.23	45.23
PC2	3.519	23.46	68.70
PC ₃	1.324	8.82	77.52
PC ₄	1.059	7.06	84.58
PC ₅	0.800	5.34	89.92
PC ₆	0.449	3.00	92.91
PC7	0.338	2.25	95.16
PC8	0.245	1.63	96.79
PC ₉	0.202	1.35	98.14
PC10	0.152	1.01	99.15
PC11	0.059	0.39	99.55
PC12	0.036	0.24	99.79
PC13	0.020	0.14	99.92
PC14	0.007	0.05	99.97
PC15	0.004	0.03	100.00

Table 5. Eigenvalues and contribution of the principal component axes towards total genetic variation among garlic genotypes.

Figure 1. Principal scree plot between component number and corresponding eigenvalue.

Figure 2. The correlation circle among quantitative traits associated with PC1 and PC2.

Height (BW and BH), and Yield (Y). The vertical axis was linked with Clove Height, Weight, Width, and Thickness (CH, CW, CWi, TC).

The distribution of genotypes based on the contribution of some quantitative traits from

Eigenvalues	PC axis				
	PC1	PC ₂	PC ₃	PC4	PC5
Eigenvalue	6.785°	3.519	1.324	1.059	0.800
Variability (%)	45.235	23.462	8.824	7.061	5.335
Cumulative $(\%)$	45.235	68.697	77.521	84.582	89.918
Squared cosines of the variables					
Plant Height (PH)	0.602	0.066	0.231	0.002	0.002
Pseudostem Diameter (PSD)	0.493	0.029	0.189	0.001	0.032
Leaf Length (LL)	0.633	0.001	0.206	0.038	0.009
Leaf Width (LW)	0.605	0.045	0.122	0.004	0.007
Bulb Weight (BW)	0.797	0.041	0.107	0.007	0.002
Yield (Y)	0.773	0.049	0.120	0.007	0.003
Bulb Height (BH)	0.471	0.029	0.000	0.263	0.200
Bulb Diameter (BD)	0.640	0.207	0.070	0.001	0.015
Height/Bulb Bulb Diameter					
(BH/BD)	0.184	0.294	0.167	0.211	0.077
Dry External Thickness (TDE)	0.119	0.039	0.020	0.418	0.386
Plant Height (PH)	0.011	0.731	0.033	0.003	0.000
Pseudostem Diameter (PSD)	0.336	0.582	0.034	0.002	0.016
Leaf Length (LL)	0.493	0.298	0.006	0.037	0.027
Leaf Width (LW)	0.433	0.450	0.011	0.052	0.001
Bulb Weight (BW)	0.194	0.657	0.007	0.014	0.023

Table 6. Principal components for fifteen selected quantitative traits of garlic.

 a Values in bold correspond for each variable to the factor for which the squared cosine is the largest.

Figure 3. Distribution of garlic genotypes among accessions in F1 and F2 for quantitative traits.

F1 to F2 showed phenotypic variation among accessions (Figure 3). According to axis 1 and 2, the most active genotypes AS7, AS15, AS8, AS1 and AS19 were placed into the first quarter (+ve F1, +ve F2); AS17, AS3, AS20, AS21, AS5, AS11 and AS26 were in the second quarter (-ve F1, +ve F2); AS16, AS9, AS2 and AS14 were in the third quarter (-ve F1, -ve F2); AS10, AS12, AS4, AS25 and AS13 were in the fourth quarter (+ve F1, -ve F2) on the discriminant axis

and also these genotypes had the highest contribution to variation (%). AS14 had the highest clove weight, height, width, and thickness, and AS13 ranked high. AS13 exhibited the highest bulb weight and yield.

DISCUSSION

In this study, the principal components for fifteen selected quantitative traits of garlic indicated that the squared cosine values revealed the importance of each component (with a large value of squared cosine) for a given observation (Abdi and Williams, 2010). The first ten principal components (from PC1 to PC10) for qualitative characters explained 99.15% of the total variation and were associated with 15 characters that made some varieties distant from the others. The present findings are similar to those of Sharma et al. (2018), who studied the diversity of 131 garlic accessions from India. In their study, 12 qualitative characteristics were investigated, and the first ten principal components with Eigenvalues≥1 represented a cumulative variance of 99.17%. Wang et al. (2014) assessed 28 morphological traits of garlic from China and reported that the first eight principal components with an Eigenvalue of ≥1 accounted for 71.35% of the total variation. Based on "Guttman lower bound Principle," the components with an eigenvalue of <1 were ignored (Kaiser, 1960; Mohammadi and Prasanna, 2003). For PCA to be used effectively and interpreted correctly, the ratio of the first two or three components of the total variation should be greater than 25% (Mohammadi and Prasanna, 2003; Gözen, 2008). If the cumulative variances of the first three components are $\leq 50\%$, the genetic diversity of the gene pool is high. Although this case is important for breeders, it limits the use of PCA (Gözen, 2008). In this study, the first three axes of the component axes were greater than 25% and defined 77.52% of the total variation. The first two components explained more than 50% of the total

variation; therefore, PCA is a useful statistical method that can be applied effectively. The first and second PC axes represented more than half of the total variation (68.70%) (Table 5). Wang et al. (2014) assessed 29 morphological traits to determine the diversity of 212 Chinese garlic accessions and reported that the first 8 components were able to explain 71.35% of the total variation. Polyzos et al. (2019) assessed the phenotypic variation of 34 Greek garlic genotypes growth in two different locations (Kavasila and Velestino) and indicated that the first seven axes explained 71.49 and 75.86% of the total variation, respectively. Sharma et al. (2018) assessed the genetic diversity of Indian garlic germplasm and indicated that the first three PCs explained 68.03% of the total variation.

There were significant variations in the quantitative traits. The first component (F1) included plant height, pseudostem diameter, leaf length and width, bulb weight, height and diameter, yield, and clove height; these traits had the highest contribution to variation. The second component (F2) includes the bulb height/bulb diameter ratio, number of cloves, clove weight, width and thickness, and dry external thickness. Sharma et al. (2018) indicated that PC1 explained 46.04% of total variation and included plant height, leaf length, number of green and dry leaves per pseudostem, pseudostem height and diameter, bulb polar and equatorial diameter, bulb weight per plant, number of cloves per bulb, clove length and weight; PC2 explained 12.68% of total variation and included plant height, pseudostem height, bulb equatorial diameter, number of cloves per bulb; PC3 and PC4 included a number of green and dry leaves per plant, bulb polar and equatorial diameter. Polyzos et al. (2019) assessed morphological traits of garlic genotypes and indicated that PC1 explained 22.79% of total variation and included pseudostem diameter, leaf length, width and chlorophyll content, number of cloves, dry matter, and yield.

There were considerable variations in 15 morphological traits of the 26 garlic genotypes. The genotypes diverged from the others mostly based on yield components (plant height, pseudostem diameter, leaf length and width, bulb weight, height, diameter and thickness, dry external thickness, number of cloves, and clove weight, height, width, and thickness). Garlic quality and calibration are largely determined by the number of cloves, clove weight, and height, width, and bulb weight. Figliuolo et al. (2001) and Fanaei et al. (2014) indicated that the number of cloves was an important yield-contributing trait. These differences are mainly attributed to genetic variations and environmental factors (Benke et al., 2018; Atif et al., 2020; Akan, 2022).

CONCLUSIONS

In this study, 15 quantitative traits of 26 Turkish soft-neck garlic accessions were evaluated using PCA. Significant variations were observed in plant growth and bulb development parameters. The PCA results revealed that plant height, pseudostem diameter, leaf length and width, bulb weight, height and diameter, yield, number of cloves, clove height, width, weight, thickness, and bulb height/bulb diameter ratio were important yield-contributing traits. AS14 stood out for its clove weight (5.98 g), length (31.74 mm), width (23.62 mm), and thickness (18.96 mm), while AS13 had the highest bulb weight (25.68 g) and yield (16.44 ton ha-1). These traits can be reliably used in future garlic selection studies and breeding programs to develop new high-yield and high-quality garlic varieties.

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تغییرات مورفولوژیکی و صفات عملکرد در سیر نرم (سیر نرمگردن)

گ. بشیرلی

چکیده

برای بهبود اصلاح نژاد سیر، تعیین تفاوت های مورفولوژیکی بین ژنوتیپ های سیر با منشاء محلی مهم است. این پژوهش به منظور تعیین تغییر و تنوع فنوتیپی ژنوتیپهای سیر نرم گردن ترک (.var sub .L sativum Allium sativum (با استفاده از صفات مورفولوژیکی تعیینشده بر اساس توصیفگرهای اتحادیه بینالمللی حفاظت از واریتههای جدید گیاهان (UPOV (انجام شد. بیست و شش ژنوتیپ سیر با استفاده از ۱۵ ویژگی کمی مورفولوژیکی مشخص شدند. تجزیه و تحلیل مؤلفه های اصلی (PCA (نشان داد که چهار مؤلفه اصلی اول ۸۴.۵۸ درصد از تغییرات کل را در بین ۲۶ ژنوتیپ سیر توضیح می دهد. صفتهایی که بیشترین سهم را در تغییرپذیری داشتند شامل موارد زیر بود: ارتفاع بوته (PH(، قطر ساقه کاذب (PSD(، طول برگ (LL(، عرض برگ (LW(، وزن پیاز (BW(، عملکرد (Y(، ارتفاع پیاز (BH(، قطر پیاز (BD(، ارتفاع میخک (CH(، نسبت ارتفاع حباب به قطر حباب (BH/BD)، تعداد یا میخک (NC)، وزن حبه یا میخک (CW)، عرض میخک (CWi) و ضخامت میخک (CT(. دراین صفات کمی ژنوتیپ های سیر تفاوت معنی داری مشاهده شد. بر اساس نتایج این پژوهش، 14AS به دلیل وزن، طول، عرض و ضخامت حبه یا میخک (clove(خود متمایز بود، در حالی که 13AS بالاترین وزن و عملکرد پیاز (bulb (را داشت. این یافته ها می تواند به طور قابل اعتمادی در اصلاح ژنتیکی انواع سیر جدید استفاده شود.