Original scientific paper
DOI: /10.5513/JCEA01/25.3.4220
Journal of Central European Agriculture, 2024, 25(3), p.661-674

Determination of early-stage shoot and root traits of cultivated and wild barley genotypes

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Received: January 3, 2024; accepted: April 6, 2024

ABSTRACT

Rapid shoot and root growth at the early developmental stage of barley is critical for maximising yield and access to water and plant nutrients, particularly in dryland environments. Determining genotypic variation in shoot and root traits at the early seedling stage is essential for germplasm identification. In this study, widely grown old and new barley cultivars and wild barley genotypes were grown in sand media under greenhouse conditions for four weeks. The differences in shoot and root traits between the genotypes, the broad sense heritability and the genetic parameters of these traits were determined. The genotypes showed statistically significant differences for all the traits studied except number of leaves. Old cultivars had higher shoot length, while new cultivars had higher shoot fresh and dry weight. The old cultivars, new cultivars and wild barley genotypes were characterised by root length, root fresh weight and specific root length, respectively. A high correlation was found between root weight and root volume, and it was determined that root volume was more influenced by root thickness than root length. Shoot fresh and dry weight, root fresh weight, root volume and specific root length had the highest broad sense heritability. Among the genotypes, cv. Tokak 157/37 showed potential as a donor for root length and cv. Durusu for root weight and root volume in breeding studies.

Keywords: barley, heritability, root characters, seedling characters, wild barley

INTRODUCTION

The primary goal of agricultural production is to maximize the yield and quality of the cultivated plants. To accomplish this, the plants should be able to overcome the biotic and abiotic stresses of their growing conditions. Establishing healthy and well-developed plants at early growth stages is typically critical to maximizing yield and dealing with environmental challenges at various stages of development. A higher rate of early-season seedling development is also known as "early vigour" (Blum, 2009). Early vigour is a complex trait regulated by multiple genes (Botwright et al., 2002) and caused by various growing characteristics at each organizational level of the plant. Growth performance and cell properties of individual leaves, expansion of whole shoot leaf area and even whole plant traits are examples of growth traits (Parker et al., 2020). This rapid plant and leaf development can provide more light interception and reduce direct water loss through soil evaporation. Reduced evaporative water loss could increase available soil moisture for photosynthesis and transpiration, thus improving plant water use efficiency (WUE) (Blum, 2009). In arid environments, a high rate of WUE due to the early seedling vigour and good stand establishment typically leads to increased dry matter accumulation and enhanced grain yield (Tyagi et al., 2014). Additionally, crops can compete more successfully with weeds thanks to the early shoot growth rate, which results in the conservation of soil moisture (Parker et al., 2020).

A genotype with high vigour also has greater root development potential under drought, resulting in higher WUE and grain yield than one with low vigour (Boudiar et al., 2020a). Deeper root systems provide access to water in deeper soil profiles. This difference becomes more apparent during the seedling stage when seminal and lateral roots absorb most water and nutrients (Ahmed et al., 2018). The architecture of the root system is an important component of plants that differs between and within species because of genotypic and/or environmental influences (Adeleke et al., 2020). Early and vigorous plant growth depends on climatic and soil conditions, cultivation practices, and plant genetic variation. This variation in vigour among cool-season cereals is mainly due to differences in seedling traits between germination and the two leaf stages (Lopez-Castaneda et al., 1996). Identifying genotypes with faster-growing roots, a feature with much genetic variation, could be one way to improve root depth (Adeleke et al., 2020).

Various abiotic and biotic stressors have a detrimental impact on the sustainable production of many cereals, and barley (*Hordeum vulgare* L.) is one of the cereals whose sustainable production is impacted (Boudiar et al., 2020b; Wang et al., 2021). Another reason for the fluctuation in barley production could be genotypic differences in root characteristics, particularly under unfavourable growing conditions (Schwarz et al., 1991). Evaluating the variability in root morphological characteristics between different barley genotypes is crucial for selecting those with the most suitable root characteristics for breeding new varieties more tolerant to stresses (Wang et al., 2021). A focused integration of root traits into plant breeding efforts necessitates understanding existing root diversity and access to simple and low-cost techniques (Nakhforoosh et al., 2014).

Genetic variations in root traits, particularly under harsh growth environments, may also contribute to variations in barley productivity (Schwarz et al., 1991). It is essential to assess the variation in root morphological traits among genotypes to select the barley genotypes with greater root features for developing new, stress-tolerant varieties (Wang et al., 2021). Understanding current root diversity and having access to simple, affordable methods are essential for integrating root characteristics into plant breeding programs with emphasis (Nakhforoosh et al., 2014). On the other hand, repeated use of elite material with similar traits in barley breeding narrowed genetic variation (Tanksley and McCouch, 1997; Newton et al., 2011). Primary gene pools consisting of old varieties, landraces, and wild relatives of barley are valuable resources that can be used for expanding genetic resources in breeding programs to develop new barley varieties with improved traits (Ellis et al., 2000; von Bothmer et al., 2003; Muñoz-Amatriaín et al., 2014; Karagoz et al., 2017).

Hordeum vulgare subsp. *spontaneum*, the wild progenitor of cultivated barley, is one of the most important genetic resources for barley breeding programs (Harlan and Zohary, 1966; Ellis et al., 2000). Since wild (*H. vulgare* subsp. *spontaneum*) and cultivated forms of barley have the same number of chromosomes (2n=14) and there is no incompatibility barrier between them, the progeny obtained when they are crossed are almost fully fertile (Harlan and Zohary, 1966; Waugh et al., 2017). This allows the transfer of beneficial traits from wild barley to cultivated barley (Ellis et al., 2000; Kreszies et al., 2020). The genetic diversity of wild barley is greater than that of cultivated varieties (Nevo et al., 1979; Kreszies et al., 2020), such that only 40% of the alleles found in wild barley are thought to be present in modern barley varieties (Ellis et al., 2000). This high level of variation in wild barley compared to cultivated barley is also seen in root traits. It is reported that wild barley has a high variation in maximum root length while having a lower number of seminal roots than cultivated barley varieties (Grando and Ceccarelli, 1995; Tyagi et al., 2014). This remarkable genetic variation in root system traits in wild barley is critical for adaptation to abiotic stresses such as drought (Naz et al., 2014).

Breeding programs frequently use heritability to predict how desired traits will be passed from parents to offspring (Falconer, 1981; Shukla et al., 2006). Heritability,

genotypic and phenotypic coefficients of variation, and other key indicators help breeders understand how much the environment influences the trait they are trying to improve. They provide information on how much of the plant's phenotype is caused by the genotype of the trait under consideration (Dey et al., 2019).

This research aims to evaluate the genetic variation and heritability of the seedling and root characteristics of barley cultivars and wild barley genotypes grown under greenhouse conditions and in sand media. Old and new cultivars were used to illustrate the tolerance to drought conditions and high yield capability, respectively. In addition, the potential of wild barley genotypes as a gene source for early seedling stage attributes was investigated.

MATERIALS AND METHODS

Nine registered barley cultivars and six wild barley genotypes were used as plant material in the study. Barley cultivars Tokak 157/37, Bülbül 89, and Tarm 92 were included in the study as old cultivars, and Aydanhanım, Avcı 2002, Durusu, Burakbey, Yalın, and Tosunpaşa were considered as new cultivars. Wild barley (*Hordeum vulgare* L. subsp. *spontaneum* K. Koch) genotypes, which were originally collected from the Şanlıurfa province in southeastern Türkiye, were utilized as the primary material in the study. These genotypes were carefully purified through a process known as single spike selection in the CRIFC's barley breeding program. Table 1 shows release years and some descriptive characteristics of the barley material evaluated in the study.

The seeds of cultivars and wild barley genotypes were sown in a randomized complete block design with three replications in the greenhouse to grow barley seedlings. Similar-sized seeds were used in the study to avoid possible variances in seed size. The seeds were sown by hand in plastic pods (14 cm deep and 7.5 cm in diameter) containing 320 g of washed sand, with one seed per pod. Ten seeds per replication of each genotype were sown. To prevent seedlings from nutrient deficiency, a nutrient solution containing 6% N, 4% P_2O_5, 5% K_2O, 0.013% B, 0.003% Cu, 0.021% Fe (EDTA), 0.011% Mn, 0.0011% Mo, and 0.0058% Zn was given to the pods once a week. The moisture of the pods was maintained with irrigation at two days intervals. During the four-week growing period, the temperature of the greenhouse was around 20-22 °C, and the seedlings were grown under daylight conditions.

Measurements on barley cultivars and wild barley seedlings were conducted as described by Sahnoune et al. (2004), Nakhforoosh et al. (2014) and, Akman (2021) as follows. Four-week-old seedlings were utilized to measure the number of tillers (NOT), number of leaves (NOL), shoot length (SL), shoot fresh weight (SFW), and shoot dry weight (SDW). After washing the roots of the same plant, whose shoot measurements were recorded, the root length (RL), the root fresh weight (RFW), and the root dry weight (RDW) were determined. The root/shoot dry weight ratio (RSR) was calculated by dividing the root dry weight by the shoot dry weight. Specific root length (SRL) was calculated by dividing the root length by root dry weight (m/g). Root volume (RV) was calculated by immersing the roots in a scaled test tube and measuring the volume of water displaced. Shoot and root length was recorded in centimetres (cm), root volume in cubic centimetres (cm^3), root and shoot fresh weight, and root and shoot dry weight in grams (g).

According to the randomized complete blocks design, analysis of variance (ANOVA) was performed on the data obtained from four-week-old barley seedlings. The significance levels of the differences between genotypes in all traits analysed were determined according to the F test, and the means of the genotypes were grouped according to the Student's t-test (Montgomery, 2013). In addition, Pearson correlation analysis was performed to demonstrate the relationship between shoot and root traits examined (Clewer and Scarisbrick, 2013). Estimation of broad-sense heritability and genetic parameters was performed using the methods outlined by Johnson et al. (1955), Wricke and Weber (1986), and Schmidt et al. (2019). The specific formulas used for these calculations are as follows:

Table 1. Release years and some descriptors of barley genotypes

Genotype	Source	Year of release	Number of rows	Grain type	Usage
Tokak 157/37	CRIFC[1]	1963	Two rows	Hulled	Feed
Bülbül-89	CRIFC	1989	Two rows	Hulled	Feed
Tarm-92	CRIFC	1992	Two rows	Hulled	Feed
Avcı-2002	CRIFC	2002	Six rows	Hulled	Feed
Aydanhanım	CRIFC	2002	Two rows	Hulled	Malting
Durusu	AEBC[2]	2007	Two rows	Hulled	Malting
Burakbey	CRIFC	2013	Two rows	Hulled	Feed
Yalın	CRIFC	2014	Two rows	Hulless	Food
Tosunpaşa	CRIFC	2016	Two rows	Hulled	Feed
HSPON(NE)-1	CRIFC	Not registered	Two rows	Hulled	Wild type
HSPON(NE)-2	CRIFC	Not registered	Two rows	Hulled	Wild type
HSPON(NE)-3	CRIFC	Not registered	Two rows	Hulled	Wild type
HSPON(NE)-4	CRIFC	Not registered	Two rows	Hulled	Wild type
HSPON(NE)-5	CRIFC	Not registered	Two rows	Hulled	Wild type
HSPON(NE)-6	CRIFC	Not registered	Two rows	Hulled	Wild type

[1] CRIFC: Central Research Institute for Field Crops-Ankara/Türkiye; [2] AEBC: Anadolu Efes Brewery Company

Genotypic variance: $\sigma^2 g = (MSg - MSe) / r$

where *MSg* represents the genotype mean square, *MSe* indicates the error mean square and *r* represents the number of replications.

Phenotypic variance: $\sigma^2 p = \sigma^2 g + \sigma^2 e$

In this formula, $\sigma^2 p$ refers to the phenotypic variance, $\sigma^2 g$ represents the genotypic variance and $\sigma^2 e$ indicates error variance.

Genotypic coefficient of variation: $GCV\ (\%) = \frac{\sqrt{\sigma^2 g}}{\bar{X}} \times 100$

where *GCV* represents the genotypic coefficient of variation, $\sigma^2 g$ represents the genotypic variance and $\bar{X}$ represents the mean value of the related character.

Phenotypic coefficient of variation: $PCV\ (\%) = \frac{\sqrt{\sigma^2 p}}{\bar{X}} \times 100$

In this formula *PCV* represents the phenotypic coefficient of variation, $\sigma^2 p$ represents the phenotypic variance and $\bar{X}$ represents the mean value of the related character.

Broad sense heritability: $h^2 B\ (\%) = (\sigma^2 g / \sigma^2 p) \times 100$

In this formula, $h^2 B$ represents the broad sense heritability, $\sigma^2 g$ is the genotypic variance, $\sigma^2 p$ is the phenotypic variance. Broad sense heritability was categorised as high (>60%), moderate (30–60%) and low (<30%) (Johnson et al. 1955; Regmi et al 2021).

Genetic advance: $GA\ (\%) = K \times \sqrt{("\sigma 2 p")} \times h^2 B$

In this formula, *GA* represents the genetic advance, *K* refers to the intensity of selection for the trait, $\sigma^2 p$ is the phenotypic variance for the trait, and $h^2 B$ is the broad sense heritability of the trait.

The *K* value was used as 2.06 at 5% selection intensity (Johnson et al. 1955). Genetic advance was categorized as high (>20%), moderate (10–20%) and low (<10%) (Johnson et al. 1955; Iannucci et al. 2021).

RESULTS AND DISCUSSION

The differences among barley genotypes were found statistically significant for all measured seedling traits except the number of leaves (Table 2). Similar results have been reported in previous studies (Zhao et al., 2010; Tyagi et al., 2014; Abdel-Ghani et al., 2015). This variation is a positive indicator of the diversity needed in breeding programmes to improve early root and seedling development in barley. When the number of tillers (NOT) in all genotypes was examined, the highest NOT was in HSPON(NE)-5 wild barley genotype with 3.26 tillers per plant and the lowest NOT was in cv. Tokak 157/37 with 2.42 tillers per plant (Table 3).

The highest NOT among the cultivars was found in cvs. Yalın and Durusu. Similar to the NOT, the highest NOL was found in HSPON(NE)-5 wild barley genotype with 8.38 leaves per plant. It was followed by cvs. Yalın and Durusu with 8.30 and 8.08, respectively. The lowest value in this trait was recorded in Tokak 157/37 with 6.67 leaves per plant. In barley shoot architecture and final productivity are related to tillers (Haaning et al., 2020). In addition, NOT is highly correlated with leaf number, which is directly related to photosynthetic activity. Leaf development characters are an essential element of plant breeding in cereals (Alqudah and Schnurbusch, 2015). In our study, differences were found between genotypes in the NOT. The presence of genotypes with high tillering capacity in wild barley highlights their potential for use in breeding programmes to improve this trait.

The highest shoot length (SL) was measured in cv. Bülbül-89 with 38.71 cm, while the lowest SL was observed in cv. Aydanhanım with 32.06 cm. The shoot lengths of the cultivars were higher than those in wild barley genotypes. The average SL of the cultivars was 34.34 cm, while the average SL of the wild barley genotypes was 24.04 cm. Among the wild barley genotypes, HSPON(NE)-1 had the lowest SL with 22.50 cm and HSPON(NE)-3 had the highest with 25.13 cm (Table 3). The shoot fresh weight (SFW) in the plants' above-ground sections was found to be highest in cv. Durusu (2.94 g). The wild barley genotype HSPON(NE)-1 had the lowest SFW, which was 1.24 g. Tokak 157/37, the cultivar with the lowest NOT and SFW value among the cultivars, had an SFW value of 2.00 g.

Table 2. ANOVA table of data obtained from barley cultivars and wild barley genotypes

Traits	Sum of Squares	Mean Square	F	CV (%)
NOT	1.95	0.14	2.60*	7.98
NOL	1292.72	92.34	14.41	8.18
SL (cm)	8.84	0.63	1.69**	8.40
RL (cm)	671.17	47.94	3.49**	9.11
SFW (g)	12.07	0.86	9.27**	14.93
RFW (g)	12.16	0.87	8.26**	19.11
SDW (g)	0.14	0.01	10.01**	13.42
RDW (g)	0.11	0.01	4.48**	23.46
RSR	0.28	0.02	0.95*	20.99
SRL (m/g)	31.45	2.25	7.59**	19.98
RV (cm^3)	0.13	0.01	7.99**	18.88

* Statistically significant at $P < 0.05$ level; ** Statistically significant at $P < 0.01$ level; NOT Number of tillers; NOL Number of leaves; SL Shoot length; RL Root length; SFW Shoot fresh weight; RFW Root fresh weight; SDW Shoot dry weight; RDW Root dry weight; RSR Root/shoot dry weight ratio; SRL Specific root length; RV Root volume; CV (%) Coefficient of variation (%)

The situation observed in SL was also observed in this trait, and the average SFW of the cultivars was higher than the average SFW of the wild barley genotypes. While the average SFW was 2.43 g in barley cultivars, the average SFW was 1.47 g in wild barley genotypes. Shoot dry weight (SDW) values also showed a parallel trend with the SFW values. The highest SDW value was observed in cv. Durusu with 0.35 g, followed by cv. Tosunpaşa with 0.29 g. Among the cultivars, the lowest SDW values were measured in Burakbey and Tokak 157/37 varieties with 0.25 g. The SDW values in wild barley genotypes ranged between 0.22 g and 0.14 g in HSPON(NE)-5 and HSPON(NE)-1, respectively (Table 3). In the results of a similar study, it was reported that SDWs measured in cultivated barley were higher than those in wild barley (Barati et al., 2015).

Shoot dry weight was more correlated (0.82) with SL in new cultivars, whereas it was correlated (0.99) with NOT in old cultivars. In wild genotypes, NOL was found positive (0.85) and statistically effective on shoot fresh weight (Table 4). Earlier and improved establishment of shoot biomass, known as early vigour, can result in increased interception of light and reduced direct water loss through evaporation from the soil. Reduced water loss through evaporation can increase available soil moisture for transpiration and photosynthesis, improving the WUE of plants (Blum, 2009). The average root length (RL) measured in all genotypes was 40.66 cm. The highest RL was found in cv. Tokak 157/37 with 49.67 cm. Cvs. Tarm 92 and Bülbül 89, which are known as drought tolerant, were the other cultivars with high RL values.

Table 3. Seedling characteristics of barley cultivars and wild barley genotypes

Genotype	NOT	NOL	SL (cm)	SFW (g)	SDW (g)
Tokak 157/37	2.42^{d}	6.67^{d}	32.91^{c}	2.00^{c-e}	0.25^{b-d}
Bülbül-89	2.87^{bc}	7.38^{a-d}	38.71^{a}	2.47^{a-c}	0.28^{b}
Tarm-92	2.93^{a-c}	7.67^{a-d}	34.74^{a-c}	2.36^{bc}	0.28^{b}
Avcı-2002	2.80^{cd}	7.47^{a-d}	33.32^{c}	2.48^{a-c}	0.27^{b}
Aydanhanım	2.73^{cd}	7.00^{d}	32.06^{c}	2.36^{bc}	0.26^{bc}
Durusu	3.22^{ab}	8.08^{a-c}	37.98^{ab}	2.94^{a}	0.35^{a}
Burakbey	2.89^{a-c}	7.09^{cd}	34.02^{bc}	2.30^{b-d}	0.25^{b-d}
Yalın	3.22^{ab}	8.30^{ab}	32.99^{c}	2.33^{b-d}	0.26^{bc}
Tosunpaşa	2.77^{de}	7.53^{a-d}	32.30^{c}	2.67^{ab}	0.29^{b}
HSPON(NE)-1	2.80^{cd}	7.27^{b-d}	22.50^{d}	1.24^{g}	0.14^{g}
HSPON(NE)-2	3.00^{a-c}	7.53^{a-d}	24.35^{d}	1.46^{fg}	0.17^{e-g}
HSPON(NE)-3	2.80^{cd}	7.27^{b-d}	25.13^{d}	1.50^{e-g}	0.18^{e-g}
HSPON(NE)-4	3.00^{a-c}	7.38^{a-d}	22.65^{d}	1.35^{fg}	0.16^{fg}
HSPON(NE)-5	3.26^{a}	8.38^{a}	24.57^{d}	1.82^{d-f}	0.22^{c-e}
HSPON(NE)-6	2.85^{bc}	7.17^{cd}	25.05^{d}	1.45^{fg}	0.20^{d-f}
Mean	2.90	7.45	30.11	2.04	0.24
Standard Deviation	0.30	0.75	6.24	0.59	0.06
CV (%)	10.43	10.09	20.72	29.08	26.86

* The same letters indicate that the means are statistically in the same group; NOT Number of tillers; NOL Number of leaves; SL Shoot length; SFW Shoot fresh weight; SDW Shoot dry weight; CV (%) Coefficient of variation (%)

Table 4. Correlation coefficients between seedling characters of barley cultivars and wild barley genotypes

	NOT			NOL			SL			SFW		
	N	O	W	N	O	W	N	O	W	N	O	W
NOL	0.86*	0.99	0.94**									
SL	0.55	0.66	0.07	0.37	0.53	0.15						
SFW	0.24	0.94	0.77	0.39	0.88	0.85*	0.75	0.87	0.63			
SDW	0.46	0.99*	0.56	0.54	0.99	0.59	0.82*	0.64	0.76	0.96**	0.94	0.90*

* Statistically significant at $P < 0.05$ level; ** Statistically significant at $P < 0.01$ level; NOT Number of tillers; NOL Number of leaves; SL Shoot length; SFW Shoot fresh weight; SDW Shoot dry weight; N New cultivars; O Old cultivars; W Wild barley genotypes

These two cultivars, which inherited the genes from the cv. Tokak 157/37, were found to have longer roots than modern cultivars (Table 5). Previous studies also reported that landraces have a deeper root system than modern cultivars (Boudiar et al., 2020a). On the other hand, the lowest RL was measured in the wild barley genotype HSPON(NE)-2 with 34.48 cm. The average RL of the cultivars was 41.90 cm, while the average RL of the wild barley genotypes was 38.80 cm. Among the cultivars, the six-row cv. Avcı 2002 had the lowest RL value (Table 5). The results of previous studies also reported that the roots of six-row barley cultivars were shorter and shallower than those of two-row cultivars (Jia et al., 2019). Many studies have shown significant differences in the angle of root spread between wild, landrace and modern cultivated barley. Landraces and wild barley genotypes have the narrowest root angle, which may be related to their environment, as they often grow in water-limited conditions where obtaining water from depth is essential for survival (Bengough et al., 2004). An important selection criterion for drought-tolerant genotypes is the ability of deep, thick and extensive root systems to extract water from a deeper soil profile under water deficit conditions (Hasanuzzaman et al., 2017). Conversely, modern cultivars are grown on well-fertilised agricultural soils where nutrients are abundant near the surface (Grando and Ceccarelli, 1995; Bengough et al., 2004; Hargreaves et al., 2009). In well-watered soils, shallow roots have significantly higher water uptake per unit root length than deeper roots (Müllers et al., 2022)

There was a large variation between the RFW and RDW values of the genotypes. The mean values of RFW and RDW of the cultivars were found to be higher than those of the wild barleys. Root length had a greater effect on RFW and RDW in old cultivars (Tokak 157/37, Tarm-92 and Bülbül-89). In wheat and barley, root weight was reported to be highly correlated with root length in homogeneous samples and explained most of the variation in root length (Løes and Gahoonia, 2004). The highest RFW was measured in cv. Durusu. In cv. Durusu, one of the cultivars with the highest NOT value, RFW was found as 2.46 g. The lowest value in terms of this characteristic was found in HSPON(NE)-1 wild barley genotype with 0.67 g. The lowest RFW (1.78 g) among the cultivars was observed in cv. Burakbey. The mean RFW of the cultivars (2.08 g) at the seedling stage was higher when compared to the mean RFW of the wild barley genotypes (1.13 g). The highest root dry weight (RDW) values were found in cvs. Tokak 157/37 and Durusu. Abdel-Ghani et al. (2015) reported that improved varieties and breeding lines exhibited greater RDW than landraces, which may indicate that selection for higher yield and greater above-ground biomass indirectly leads to more productive root systems at the seedling stage. While RDW value varied between 0.17 g and 0.25 g in cultivars, RDW in wild barley genotypes was in the range of 0.08-0.16 g. A wide variation in RDW was found in wild barley genotypes. Similar results were reported in previous studies (Zhao et al., 2010; Tyagi et al., 2014). The highest root/shoot dry weight ratio (RSR) among the cultivars was Tokak 157/37

with 0.96, while HSPON(NE)-6 had the highest value with 0.85 among the wild barley genotypes. The cultivar with the lowest value in terms of this trait was cv. Durusu with 0.69. Although RFW and RDW values were high in cv. Durusu, SFW and SDW values were also high, which caused the RSR ratio to be relatively low (Table 5). In this respect, both measurements used in the calculation should be given importance in order to make a correct evaluation of the RSR. The root/shoot dry weight ratio (RSR) displays the distribution of assimilates between above- and below-ground organs (Nakhforoosh et al., 2014). RSR variation is influenced by genotype, water in the rhizosphere, and their interactions. Due to reduced shoot rather than root growth, this ratio was greater under drought conditions than well-watered conditions (Sahnoune et al., 2004; Tavakol and Pakniyat, 2007; Li et al., 2020). Under drought stress, RSR increases the accessibility of the root system to deeper soil profiles, which helps plants to better absorb water.

However, the effect of stress on these traits is highly influenced by genotype due to the strong genotype by environment interaction (Barati et al., 2015). Deficiency of nutrients such as nitrogen is also among the factors that increase the RSR (Ruggiero and Angelino, 2007). High RSR genotypes can maintain root contact with increased root volume to maintain cell turgor, survive the dry period, and meet vegetative growth nutrient requirements (Abdel-Ghani et al., 2015). In specific root length (SRL), the highest value was found in HSPON(NE)-1 wild barley genotype with 5.19 m/g, while the lowest values were measured in cvs. Durusu and Tosunpaşa with 1.77 m/g and 1.79 m/g, respectively (Table 5). While the average SRL value of the cultivars was 2.25 m/g, the average SRL value of the wild barley genotypes was found as 3.43 m/g. Lower SRL in new and high-yielding cultivars is related to higher concentrations of roots in the upper soil layers which are rich in water and nutrients in these genotypes. New cultivars generally have wider roots allowing them to utilize the topsoil nutrients found in fertilized soils (Hargreaves et al., 2009). The SRL values of wild barley genotypes were high because their RL was close to average, but their RDW were quite low (Table 5). This may be explained by the thinner root structure (Corneo et al., 2017) and less seminal root number of wild barley genotypes (Grando and Ceccarelli, 1995; Tyagi et al., 2014). In addition, it is assumed that there is a negative correlation between SRL and root diameter (Kramer-Walter et al., 2016). High SRL is considered to be an indicator of a high proportion of fine roots and therefore a high root surface area in contact with nutrients and water (Gao et al., 2023). Plants with higher SRL develop more root length for a given dry mass input and are commonly assumed to have higher water and nutrient uptake and shorter root life than plants with lower SRL (Pérez-Harguindeguy et al., 2013).

Cultivars with the highest root volume (RV) were Durusu and Tarm-92 with 0.25 cm^3. They were followed by cv. Tokak 157/37 and cv. Tosunpaşa which were statistically in the same group (Table 5). The lowest RV value was found in HSPON(NE)-1 and HSPON(NE)-4 wild barley genotypes (0.08 cm^3 and 0.09 cm^3, respectively). High and statistically significant ($P < 0.01$) correlations were found between RV and RDW and RFW (0.95 and 0.94, respectively). This relationship is also clearly visible in Figure 1, which shows the high regression coefficient (R2:0.9063) between RV and RDW. A lower correlation coefficient (0.56) was found between RV and RL than for weight-related characteristics of roots (Table 6). This indicates that the RV of the genotypes in our study is affected by root thickness and quantity rather than root length.

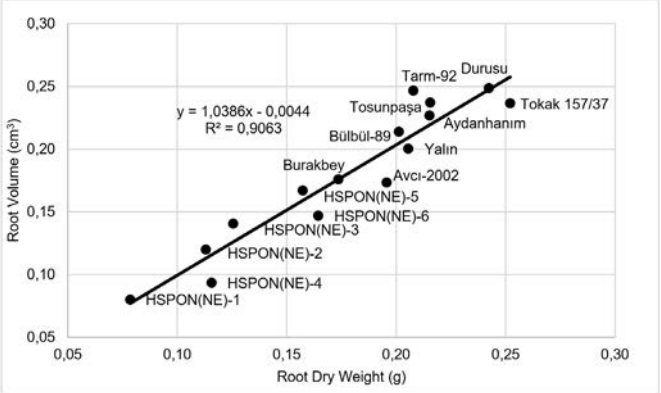

Figure 1. Regression line showing the relationship between root dry weight and root volume

Table 5. Root characteristics of barley cultivars and wild barley genotypes

Genotype	RL (cm)	RFW (g)	RDW (g)	RSR	SRL (m/g)	RV (cm^3)
Tokak 157/37	49.67^{a}	2.23^{ab}	0.25^{a}	0.96^{a}	2.95^{b-d}	0.24^{a}
Bülbül-89	44.07^{a-d}	2.16^{ab}	0.20^{a-c}	0.74^{a-c}	2.23^{de}	0.21^{a-c}
Tarm-92	44.49^{a-c}	1.91^{a-d}	0.21^{a-c}	0.74^{a-c}	2.20^{de}	0.25^{a}
Avcı-2002	36.55^{fg}	2.02^{a-c}	0.20^{a-d}	0.72^{a-c}	2.20^{de}	0.17^{b-e}
Aydanhanım	40.61^{b-g}	1.96^{a-c}	0.22^{a-c}	0.82^{a-c}	2.08^{de}	0.23^{ab}
Durusu	39.95^{b-g}	2.46^{a}	0.24^{ab}	0.69^{bc}	1.77^{e}	0.25^{a}
Burakbey	38.73^{c-g}	1.78^{b-d}	0.17^{b-e}	0.72^{a-c}	2.44^{c-e}	0.18^{b-e}
Yalın	45.14^{ab}	2.03^{a-c}	0.21^{a-c}	0.82^{a-c}	2.50^{c-e}	0.20^{a-d}
Tosunpaşa	37.91^{d-g}	2.20^{ab}	0.22^{a-c}	0.77^{a-c}	1.79^{e}	0.24^{a}
HSPON(NE)-1	38.10^{d-g}	0.67^{f}	0.08^{f}	0.60^{c}	5.19^{a}	0.08^{g}
HSPON(NE)-2	34.48^{g}	1.02^{ef}	0.11^{ef}	0.70^{bc}	3.22^{bc}	0.12^{e-g}
HSPON(NE)-3	41.15^{b-f}	1.16^{ef}	0.13^{d-f}	0.71^{bc}	3.56^{bc}	0.14^{ef}
HSPON(NE)-4	37.07^{e-g}	1.01^{ef}	0.12^{ef}	0.76^{a-c}	3.28^{bc}	0.09^{fg}
HSPON(NE)-5	43.17^{b-e}	1.52^{c-e}	0.16^{c-e}	0.73^{a-c}	2.89^{b-d}	0.17^{c-e}
HSPON(NE)-6	38.83^{c-g}	1.37^{de}	0.16^{c-e}	0.85^{ab}	2.45^{c-e}	0.15^{df}
Mean	40.66	1.69	0.18	0.75	2.72	0.18
Standard Deviation	5.95	0.59	0.06	0.15	1.01	0.05
CV (%)	14.63	35.18	34.77	19.62	37.13	29.78

* The same letters indicate that the means are statistically in the same group; RL Root length; RFW Root fresh weight; RDW Root dry weight; RSR Root/shoot dry weight ratio; SRL Specific root length; RV Root volume; CV (%) Coefficient of variation (%)

Table 6. Correlation coefficients between root characters of barley cultivars and wild barley genotypes

	RL	RFW	RDW	RSR	SRL
RFW	0.47	-	-	-	-
RDW	0.56*	0.97**	-	-	-
RSR	0.56*	0.42	0.58*	-	-
SRL	-0.13	-0.83**	-0.81**	-0.39	-
RV	0.56*	0.94**	0.95**	0.44	-0.79**

* Statistically significant at $P < 0.05$ level; ** Statistically significant at $P < 0.01$ level; RL Root length; RFW Root fresh weight; RDW Root dry weight; RSR Root/shoot dry weight ratio; SRL Specific root length; RV Root volume

The root volume is higher in the parts of the roots closer to the soil surface, while significant reductions in root volume occur as they penetrate deeper (Sahnoune et al., 2004). In breeding studies, it is suggested to select genotypes with the highest seminal and adventitious root length as well as higher root volume to improve drought tolerance at the seedling stage (Abdel-Ghani et al., 2015). There was also a high correlation between RL and RDW in our study. This result is similar to that of Løes and Gahoonia (2004), who reported that root length could be accurately predicted by a regression equation derived from root weight.

The genotypic variances (σ^2g) of all shoot and root traits were lower than the phenotypic variances (σ^2p) of the same traits. Their coefficients of variation showed the same pattern (Table 7). For all traits, the genotypic coefficient of variation (GCV) was lower when compared to the phenotypic coefficient of variation (PCV). Shoot length (GCV 17.7% and PCV 19.6%), shoot fresh weight (24.8% and 28.9%), shoot dry weight (23.3% and 26.8%), root fresh weight (29.7% and 35.3%) and root volume (28.8% and 34.5%) had closer GCV and PCV values than other traits. The significant differences between GCV and PCV indicate that environmental factors have a significant influence on this trait. In our study, the traits with the highest differences between GCV and PCV were RSR (11.1% and 22.1%), number of leaves (3.9% and 9.1%), and number of tillers (5.8% and 9.9%). Phenotypic variance describes the variation in a trait caused by variation in the environment as well as genetic variation. Genotypic variance is the variation caused by genetic variation. The genotypic and phenotypic coefficients of variation are frequently utilized to assess the level of variation in breeding material, find appropriate selection strategies, and predict the influence of breeding on enhancing desired traits. The strong relationship between PCV and GCV levels suggests that phenotypic variation is largely determined by genotype. Phenotypic selection on such characters is very useful as it also allows for genotypic progression (Dey et al., 2019; Awad-Allah et al., 2022). The maximum estimated broad sense heritability (h^2B) was found in the shoot length with 81.7% among the traits considered in the study (Table 7). Abdel-Ghani et al. (2015) also reported that they found 75.0% heritability for SL under optimal growing conditions in barley. Significantly high broad sense heritabilities were also found for SDW (75.0%), SFW (73.4%), RFW (70.7%), RV (70.0%), and SRL (68.7%). RDW and RL traits were determined as traits with moderate broad sense heritability at 53.7% and 45.4%, respectively (Table 7). Jia et al. (2019) reported that they found 42.9% broad sense heritability for average seminal root length and 73.8% for shoot dry weight in barley. The results of the researchers support our findings. The traits with the lowest broad sense heritability were NOT (34.8%), RSR (25.1%), and NOL (18.7%). The heritability of a trait is the ratio of phenotypic variance to genotypic variance. Heritability is commonly utilized in breeding programs to predict the transmission of desirable traits from parents to offspring (Falconer, 1981; Shukla et al., 2006). Estimating heritability gives information on the degree of genetic influence in the expression of a specific trait as well as the phenotype's predictability in estimating breeding value (Taneva et al., 2019). The high heritability suggested that the traits were less impacted by environmental factors (Dyulgerova and Valcheva, 2014). Another important criterion for evaluating the predicted outcome of the selection is genetic advance (GA), which is a measure of how much is gained via phenotypic selection for a characteristic (Shukla et al., 2006). Knowledge of genetic advancement when combined with heredity is more effective for selection (Tesfaye, 2021). RFW (51.5%), SRL (50.6%), RV (49.7%), and SFW (43.7%) had the highest genetic advance ratio (Table 7). The heritability of these four traits was about 70%. SL and SDW had the highest heritabilities (81.7% and 75.0%), the GA values for these two traits were also found to be high. The traits with the lowest broad sense heritabilities, RL, RSR, NOT, and NOL also had the lowest GA values. The GA values for these traits were 11.5%, 11.4%, 7.1%, and 3.5% respectively (Table 7).

The biplot graph of the principal component analysis (PCA), which was performed via correlations among the genotypes' root traits, is shown in Figure 2.

Table 7. Estimation of genetic parameters for shoot and root traits of barley cultivars and wild barley genotypes

Traits	σ^2g	σ^2p	GCV (%)	PCV (%)	h^2B (%)	GA (%)
NOT	0.029	0.082	5.8	9.9	34.8	7.1
NOL	0.086	0.460	3.9	9.1	18.7	3.5
SL (cm)	28.643	35.052	17.7	19.6	81.7	33.1
RL (cm)	11.406	25.129	8.3	12.3	45.4	11.5
SFW (g)	0.256	0.350	24.8	28.9	73.4	43.7
RFW (g)	0.254	0.360	29.7	35.3	70.7	51.5
SDW (g)	0.003	0.004	23.3	26.8	75.0	41.5
RDW (g)	0.002	0.004	25.2	34.5	53.7	38.1
RSR	0.007	0.028	11.1	22.1	25.1	11.4
SRL (m/g)	0.650	0.946	29.6	35.7	68.7	50.6
RV (cm^3)	0.003	0.004	28.8	34.5	70.0	49.7

σ^2g: Genotypic variance; σ^2p: Phenotypic variance; GCV: Genotypic coefficient of variation; PCV: Phenotypic coefficient of variation; h^2B: Broad sense heritability; GA: Genetic advance.

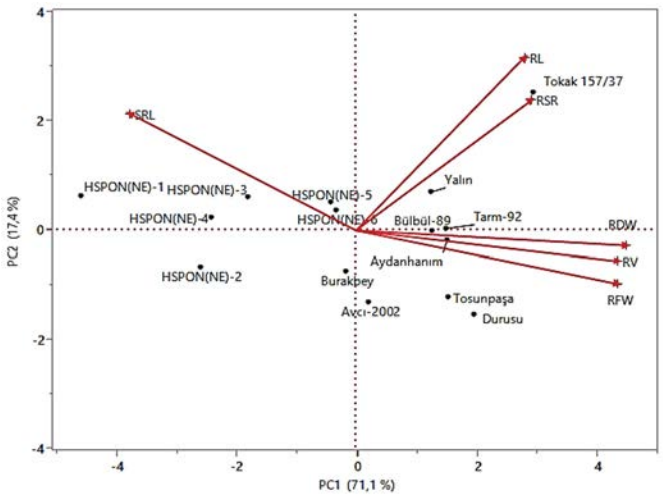

Figure 2. Biplot of the principal component analysis (PCA) displaying the relationship between root traits and barley genotypes

According to the PCA results, the first principal component (PC1) represents 71.1% of the total variation, while the second principal component (PC2) represents 17.4% variation. The two principal components together represent 88.5% of the total variation. The PC1 was mostly characterized by RDW, RFW, and RV, while the second component was mainly associated with RL and RSR. RDW, RFW, and RV are closely correlated to each other (Figure 2). However, there is a significant and negative correlation (r = -0.81, r = -0.83, and r = -0.79 respectively) between these three traits and SRL. It can also be seen that there is a high and positive correlation (r=0.56) between RL and RSR (Figure 2). It can be proposed that RL has the greatest effect on the change in the RSR trait. Analyzing the distribution of the genotypes on the graph, the cv. Tokak 157/37 is closer to the RL axis and stands out in terms of this trait. On the other hand, Durusu, Tosunpaşa, and Aydanhanım are the cultivars defined in terms of RV and RFW traits. It was found that the wild barley genotypes were in a different direction from the cultivars and were more characterized in terms of SRL.

CONCLUSION

In this study, the genetic variation in shoot and root characteristics of old and new barley cultivars, and wild barley genotypes grown under greenhouse conditions were investigated. Old cultivars had higher values for shoot and root length, while new cultivars had higher values for shoot and root fresh weight. Wild barley genotypes had lower values for shoot and root characteristics than the

cultivars, except for specific root length. Root weight was highly correlated with root volume, and root volume was more influenced by root thickness than length. The traits with the highest broad sense heritability are SFW, SDW, RFW, RV, and SRL. This indicates that these traits could be improved by selective breeding. NOT, RSR and NOL have low broad sense heritabilities. This suggests that these traits are strongly influenced by the environment and are more difficult to generate by breeding. The results of this study suggest that old cultivars could be utilized as a gene source for root and shoot length, which are essential traits in breeding programmes focusing on drought tolerance. On the other hand, new cultivars with high root and shoot fresh weight and root mass may serve as genetic resources for breeding cultivars suitable for water and nutrient-rich soils. Alternatively, wild barley genotypes could be included in crossing programs for specific root length, root length and root surface area to develop new cultivars for dry areas.

ACKNOWLEDGEMENTS

The authors are grateful to the Central Research Institute for Field Crops for the provision of the experimental greenhouse and other research facilities.

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