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Phenotypic variation in root architecture traits and their relationship with eco-geographical and agronomic features in a core collection of tetraploid wheat landraces (*Triticum turgidum* L.)

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Abstract To obtain varieties with root systems adapted to marginal environments it is necessary to search for new genotypes in genetically diverse materials, such as landraces that are more likely to carry novel alleles for different root features. A core collection of 'durum' wheat, including three subspecies (*dicoccon*, *turgidum* and *durum*) from contrasting eco-geographical zones, was evaluated for root traits and shoot weight at the seminal root stage. Distinctive rooting phenotypes were characterized within each subspecies, mainly in subsp. *durum*. Contrasting rooting types, including large roots with shallow distributions, and others with high root numbers were identified. Correlations with climatic traits showed that root shape is more relevant in adaptation to eco-geographical zones in subsp. *dicoccon*, whereas in subsp. *turgidum* and *durum*, which come from warmer and drier areas, both size and shape of roots could have adaptive roles. Root traits with the largest positive effects on certain yield components under limited water conditions included root diameter in subsp. *dicoccon*, root size in *turgidum*, and root number in *durum*. Additionally, shoot weight at the seedling stage had important effects in subsp. *turgidum* and *durum*. Twenty-eight marker–trait associations (MTAs) previously identified in this collection for agronomic or quality traits were associated with seminal root traits. Some markers were associated with only one root trait, but others were associated with up to six traits. These MTAs and the genetic variability characterized for root traits in this collection can be exploited in further work to improve drought tolerance and resource capture in wheat.

Keywords Germplasm · MTAs · Root characteristics · *Triticum dicoccon* · *Triticum durum*

Abbreviations

D	Mean root diameter
ETP	Evapotranspiration potential
L	Total root length

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MAV	Minimum angle with respect to the vertical
MRA	Mean of all root angles with respect to the vertical
MxAV	Maximum angle with respect to the vertical
NR	Root numbers
PL	Primary root length
RSA	Root system architecture
S	Total root surface area
V	Total root volume
W	Shoot weight

Introduction

One of the largest current problems facing agriculture is declining rainfall in some regions due to climate change, including countries in southern Europe and North Africa, (IPCC 2014). Taking into account the steady increase in world population (Godfray et al. 2010) it is necessary to create new varieties with enhanced adaptation to drought in order to maintain or even increase agricultural production (Manschadi et al. 2008; Ehdaie et al. 2012).

Plant breeding has mostly focused on the aerial parts of plants, namely stems, leaves, flowers, seeds, and fruits, the parts that are normally used as well as the most easily studied. Roots have been less studied and, therefore, hardly ever taken into account in breeding programs. Roots play a key role in plant development; they are the anchor and support of the aerial parts, and are responsible for uptake of water and mineral nutrients (de Dorlodot et al. 2007; Zhu et al. 2011). Therefore, as with other parts of the plant, roots should be taken into account in breeding programs aimed at increasing crop yields because optimization of the root system could lead to varieties that are better adapted to environments prone to water and/or nutrient stress (Manschadi et al. 2008; Comas et al. 2013). The reasons for this lack of interest are the difficulties in root phenotyping because they are underground and highly variable as a result of interaction with the soil environment (e.g. soil type, pH, water availability) (de Dorlodot et al. 2007; Zhu et al. 2011; Comas et al. 2013).

The spatial distribution, age and identity of all roots of a plant is called the root system architecture (RSA) (Lynch 1995), and its configuration regulates the acquisition of water and mineral nutrients. The RSA is very plastic and adapts to the soil conditions during plant growth (de Dorlodot et al. 2007; Ehdaie et al. 2012; Paez-Garcia et al. 2015). To compare RSA between genotypes, studies of root systems must be performed under highly controlled conditions.

Studying RSA in adult plants grown in soil is impracticable when large numbers of individuals have to be studied. Therefore, different "in vitro" methodologies that allow study of roots of seedlings over a few days or weeks have been developed. Many RSA studies have used the gel chamber method to investigate roots at the seedling stage (Bengough et al. 2004; Sanguineti et al. 2007; Liu et al. 2013). Other studies have compared three different root growth systems to study RSA in *Brachypodium* and concluded that the use of Whatman paper, or a similar product, as a support for root development was adequate for analysing large germplasm collections (González et al. 2016).

Analysis of roots at the seedlings stage is of interest for plant breeding only if the results correlate with root development on adult plants (Paez-Garcia et al. 2015). Several studies have described this correlation, e.g. Løes and Gahoonia (2004) found that the RSA of common wheat at the early stages of plant development determined the growth and functionality of mature plant roots; Manschadi et al. (2008) showed that the angle formed by the first pair of seminal roots was correlated with RSA in adult plants; and Wasson et al. (2012) found a relationship between wheat root vigour in the field and in plants developed in a controlled environment. Furthermore, Zhao et al. (2017) reported that the correlation between early-stage root traits and mature root systems can be improved by simulation models.

Durum wheat (*Triticum turgidum* L. subsp. *durum*) is an important food crop grown on approximately 13 million hectares worldwide. This wheat type is primarily grown under rainfed conditions that are characterized by unpredictable rainfall, making drought stress one of the most common challenges for this crop. To obtain varieties with root systems adapted to limited water availability and associated abiotic stress factors, it is necessary to have parental genotypes that have the required traits. There have been several studies of variability of RSA at the seminal root stage in different sets of accessions, representing the most important breeding pools (Sanguineti et al. 2007; Canè et al. 2014). However, a more exhaustive search for new genes governing RSA traits should include genetically diverse materials, such as landraces, and other tetraploid subspecies that might possess novel combinations of RSA features conferring adaptation to marginal environments (Palta et al. 2011). Core collections in which genetic diversity is maximized with minimum repetition is an efficient approach for exploring novel variation and enhancing the use of germplasm. In previous research (Ruiz et al. 2013) we created a core collection of Spanish durum comprising landraces of three tetraploid subspecies from contrasting eco-geographical zones. Study of this collection allowed analysis of the contribution of each subspecies to the overall variability of RSA traits and their potential as sources of genetic variation for wheat improvement.

The main objectives of the study were to: analyse the root architectural complexity of this durum collection at the seedling stage; study associations with field performance; and study genetic associations of RSA and agronomic traits.

Materials and methods

Plant materials

In this study 'durum' refers to cultivated forms of tetraploid wheat. The Spanish core collection of durum wheat (*Triticum turgidum* L.) used in this research comprised 94 genotypes of three subspecies: 10 *dicoccon* (Schrank) Thell., 32 *turgidum*, and 52 *durum* (Desf.) Husn, which were representative of the entire collection of 555 Spanish durum landraces and old cultivars preserved at the National Plant Genetic Resources Centre. In a previous study (Ruiz et al. 2012) the geographic origin of each landrace was recorded and nine agroecological zones of origin were identified. Climatic data covering at least 20 years were extracted for each collection site to characterize each agroecological zone (Ruiz et al. 2012).

Root morphology evaluation

A modification of the filter paper method described by Hund et al. (2009) and González et al. (2016) was used. Twelve grains of similar size from each of the 94 wheat genotypes were surface-sterilized for 15 min in 10% calcium hypochlorite, then rinsed four times with sterile water. Seeds of each accession were spread over two Petri dishes containing blotting paper and 7 ml of sterile water, and stored at 4 °C for 3 days and at 22 °C for 1 day. Three replicates of four seeds of each genotype were created.

For each seed, a sheet of sterilized A4-size black cardboard (180 g/m^2, www.liderpapel.com) was placed on the surface of a glass plate of the same dimensions. One 10 mm-deep and 5 mm-wide nick was made at the top of the cardboard to create a place to position the grain, and the cardboard sheet was covered with a piece of filter paper and two black plastic sheets (front and back) to keep the roots in darkness. The entire system was secured using clips at the corners, placed vertically in a plastic container and irrigated with an Aniol solution of 500 µl/l of PPM^{TM} (Plant Cell Technology) and pH 5.8. The seeds were placed in a growth chamber at 22–18 °C with a 12 h light/darkness photoperiod. After 7 days, the plastic and filter paper pieces were removed, the roots were scanned at 300 ppi using a Canon 'LiDE210' scanner and the first image was obtained. The roots were then manually separated and a second scan was carried out to capture the second image. The images were saved in jpg format at maximum quality.

Root analysis

The root images were analysed using the *SmartRoot* software v.3.32 (Lobet et al. 2011) and ImageJ1.46R software (http://imagej.nih.gov/ij/download.html). The first image was used to measure the angles of the roots with respect to the vertical, and the second was used to take other measurements of the roots using manual and semi-automated SmartRoot procedures. For each seedling, the number of seminal roots, as well as the length, mean diameter, surface area and volume of each root, were recorded in a Microsoft Access file.

All of the data were exported to a Microsoft Excel file, and the following variables were annotated or calculated: total root length in cm (L), primary root length in cm (PL), total surface area of the roots in cm^2 (S), total root volume in cm^3 (V), mean root diameter in cm (D), number of seminal roots (NR), mean of all root angles with respect to the vertical in ° (MRA), most vertical root angle in ° (MAV), and least vertical root angle in ° (MxAV) (see Fig. 1a). We also measured the shoot dry weight of each seedling in mg (W).

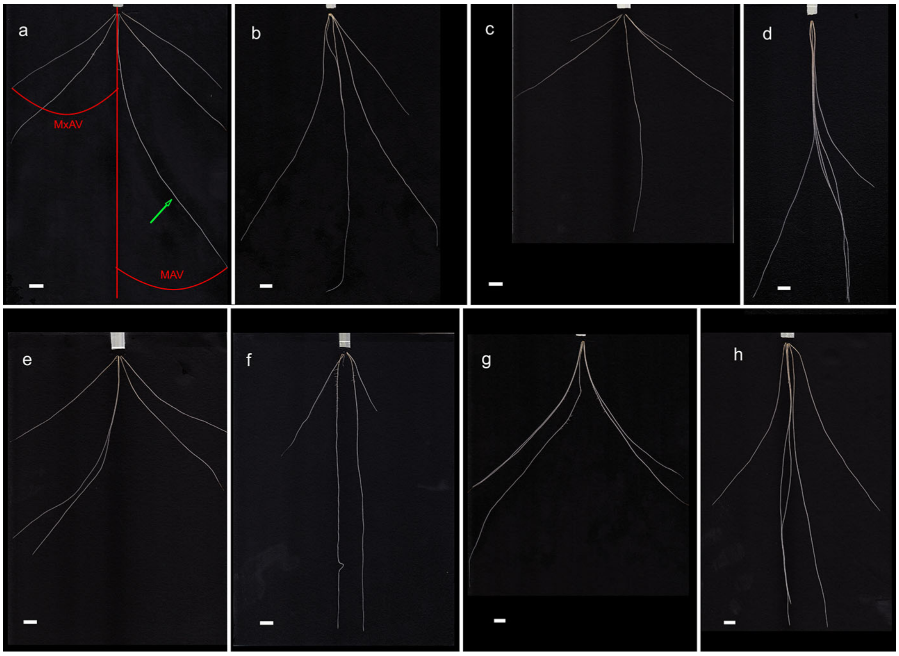

Fig. 1 Examples of root architecture in 8-day-old seedlings in accessions of the three subspecies of *Triticum turgidum*: subsp. *dicoccon* **a** BGE047499; **b** BGE047503; subsp. *turgidum* **c** BGE048497; **d** BGE047505; and subsp. *durum* **e** BGE045658; **f** BGE045651; **g** BGE045631; **h** BGE045655. Scale bar, 1 cm. In **a**, we show how MVA and MxAV were measured. The arrow points to the primary root

Agronomic evaluation

All of the accessions were tested at two diverse Spanish locations: Lleida in the north (N) and Alcala de Henares in the centre (C) of the country. The experiments were carried out at both locations during the season from November 2006 to June 2007 (N and C07), and in the centre during 2013–2014 (C14). A description of the three test environments (combinations of location and year) is provided in Online Resource 1. The locations were separated into dry (N and C14) and wetter (C07) environments according to annual rainfall (200 vs. 400 mm).

To analyse the relationship of root traits and agronomic performance the accessions were evaluated for five agronomic traits (days to heading, days to maturity, plant height, number of spikelets per spike and spike length) in the dry environment (C14), and for eight agronomic traits (days to heading, plant height, number of spikelets per spike, days to maturity, carbon isotope discrimination, spike length, thousand-kernel weight, and test weight (kilograms per hectolitre)) in the dry (N) and wetter environments (C07). Four more agronomic traits (mean tiller weight per plant, spike weight per plant, grain weight per plant and harvest index) were included in the dry environment (N). Details of the agronomic evaluation and field trials are reported in Giraldo et al. (2016). Tiller weight per plant, spike weight per plant, and grain weight per plant were evaluated on 10 plants per plot. Harvest index was calculated as the ratio of grain and above-soil plant weight on a whole sample basis.

Statistical analyses and association mapping

Each root variable showed a near-normal distribution, except for MAV, for which a square root transformation was used for analysis. Only NR deviated from equality of variances tested by Levene's test. For this variable, the nonparametric Kruskal and Wallis (1952) test and the Spearman correlation test were used. Multivariate analysis with principal component analyses (PCA), as proposed by Bodner et al. (2013), was applied to seminal root data to determine the root system distinctiveness among genotypes within each subspecies. The first three principal components, as distinctive composite variables of different rooting types, were used in cluster analysis. Distinctive groups were identified by their separation in cluster analyses and PCA biplots in order to group the genotypes with similar rooting types. Differences in trait means were assessed by two-sided *t test*.

In previous research (Giraldo et al. 2016), 85 stable MTAs were identified for 18 agromorphological and grain quality traits. In the present work, 39 MTAs, detected for traits related to yield (number of spikelets per spike, spike length, tiller weight per plant, spike weight per plant, grain weight per plant, harvest index, test weight, thousand-kernel weight, and carbon isotope discrimination), vegetative development (plant height), or phenology (days to heading, days to maturity), were tested for root variables using a linear regression analysis ($P < 0.05$) for the subspecies *turgidum* and *durum*. The grain quality trait gluten strength was included because other studies had reported RSA-QTLs close to genes controlling this quality trait (Sharma et al. 2011; Botwright Acuña et al. 2014).

Results

A summary of the within subspecies genetic variation of RSA traits and W is presented in Table 1. The analysed landraces exhibited a wide range of variation in RSA traits, especially for root angle in all three subspecies, and for V and S in the subsp. *dicoccon*. The variables L, S, V, and PL were related to root size, whereas the angles were related to geometric shape.

The relationships between RSA traits were analysed by PCA. The PCA (Fig. 2) showed that the three subspecies were located along PC1, differing mainly in the root size-related attributes and NR (positive PC1), and root shape (negative PC1). Overall, these parameters captured 56% of the variance and were independent of D (positive PC2), which increased the explained variance to 74%. Landraces in subsp. *dicoccon* possessed lower PC1 values than *durum* landraces. Subspecies *turgidum* exhibited an intermediate position with respect to PC1 but higher PC2 values. ANOVA (Online Resource 2) revealed significant differences among subspecies for all seminal root traits. Subsp. *dicoccon* had the lowest values of L, S, V and NR and the widest angles. The opposite was found in subsp. *durum*, which also showed the highest PL values. Landraces of *turgidum* were intermediate stage between *dicoccon* and *durum*, and had the highest D values. No significant differences among subspecies were detected for W.

The four root traits related to root size were correlated in the three subspecies, and with NR also in subsp. *durum* (Table 2). The three seminal angles showed significant correlations, although the correlations between MAV and the other two angles were weaker in *dicoccon*. Significant correlations were detected between root size and shape-related variables in *dicoccon* and *turgidum*, but not in *durum*. Shoot weight was correlated with D in *dicoccon* and *turgidum*, and with NR and PL in *durum*.

Variation of RSA features and W for each subspecies

To identify RSA similarities among landraces, a PCA was conducted with the root variables of the genotypes within each subspecies. For subsp. *dicoccon*, the PCA (Fig. 3a) indicated that PC1 was positively correlated with L, PL, S, V, and D and negatively correlated with MRA and MxAV. This component explained the 69% of the variance. PC2 was correlated with MAV, and PC3 was correlated with NR (not shown). In total, the RSA variables explained 91% of the variance in this subspecies. The dendrogram performed with the first three PCs showed three functional groups (G1, G2 and G3) that contained all landraces with similar seminal rooting types (Fig. 3b). These groups were clearly identified in the PCA biplot (Fig. 3a). Taking into account the root traits contained in the principal component-based rooting types, landraces in G1 shared the longest root pattern, with large D and narrow angles (high PC1), whereas those in G2, on the opposite side, had a small horizontal pattern (negative PC1). G3 occupied an intermediate rooting type with larger sized and steeper roots than those in G2, but with a more horizontal primary roots than G1 and G2 (high PC2). There were no clear differences among groups in terms of NR, which was correlated to PC3. Figure 1 shows an example of rooting types corresponding to G2 (a) and G1 (b) groups.

For subspecies *turgidum*, PC1 captured size (positive scores) and shape (negative scores) related attributes, whereas PC2 was correlated with D and NR (Fig. 3c), and PC3 was correlated with NR and MAV (not shown). The first two and three principal components explained 75 and 83%, respectively, of the variation in this subspecies. The resulting

Table 1 Statistical estimation of the RSA traits and W for each subspecies of the 94 genotypes analysed

Subspecies	Variable	n	Mean	SD	CV	Min	Max
dicoccon	L (cm)	10	74.58	11.86	15.9	59.30	91.31
	PL (cm)	10	20.59	2.24	10.9	17.73	23.22
	S (cm^2)	10	12.68	2.57	20.2	10.12	16.57
	V (cm^3)	10	0.18	0.05	25.3	0.13	0.25
	D (cm)	10	0.05	0.00	7.0	0.05	0.06
	NR (no.)	10	4.88	0.15	3.1	4.58	5.17
	MRA (°)	10	33	8	23.5	20	43
	MAV (°)	10	19	7	33.9	11	33
	MxAV (°)	10	48	9	19.5	32	59
	W (mg)	10	12.39	2.05	16.6	8.96	15.11
turgidum	L (cm)	32	86.28	9.65	11.2	63.71	104.32
	PL (cm)	32	21.81	2.00	9.2	17.45	25.29
	S (cm^2)	32	14.87	1.80	12.1	10.43	18.20
	V (cm^3)	32	0.21	0.03	14.1	0.14	0.27
	D (cm)	32	0.06	0.00	8.0	0.05	0.07
	NR (no.)	32	5.08	0.17	3.4	4.75	5.50
	MRA (°)	32	26	6	24.4	14	43
	MAV (°)	32	13	4	33.9	4	21
	MxAV (°)	32	42	8	18.6	29	64
	W (mg)	32	11.37	1.49	13.1	8.93	14.23
durum	L (cm)	52	96.33	9.87	10.3	78.64	115.62
	PL (cm)	52	23.17	2.06	8.9	19.39	28.05
	S (cm^2)	52	16.25	1.89	11.6	12.57	20.43
	V (cm^3)	52	0.23	0.03	14.5	0.17	0.30
	D (cm)	52	0.05	0.00	5.0	0.05	0.06
	NR (no.)	52	5.30	0.37	6.9	4.50	6.58
	MRA (°)	52	21	6	26.2	12	33
	MAV (°)	52	11	4	37.0	4	25
	MxAV (°)	52	34	8	22.1	19	47
	W (mg)	52	12.21	1.66	13.6	8.12	16.10

L total root length, *PL* primary root length, *S* total root surface area, *V* total root volume, *D* mean root diameter, *NR* roots number, *MRA* mean of all roots angles with respect to the vertical, *MAV* minimum angle with respect to the vertical, *MxAV* maximum angle with respect to the vertical, *W* shoot weight

dendrogram identified five main clusters (G1–G5 in Fig. 3d). An analysis of these groups in the PCA biplot (Fig. 3c) indicated that G1, G3 and G2 included large roots with narrow angles (high PC1), but root types in G3 and G1 had the highest and lowest D and NR values, respectively (high and low PC2, respectively). G4 occupied an intermediate position with respect to PC1, whereas G5 represented the smallest root type with the shallowest angles (negative PC1). Examples of rooting types of G5 (c) and G3 (d) are shown in Fig. 1.

Durum landraces exhibited more continuous variation in root variables. Root size and NR were related to PC1, root shape to PC2, and D to PC3 (Fig. 4a, b). The first two PCs explained 70% of the variance, and the first three PCs explained 83%. A cluster analysis of *durum* genotypes based on similarity in their seminal root characteristics resulted in seven main groups (G1–G7 in Fig. 4c). Analysis of these groups in the PCA biplots showed that G1, G2 and G6 had the largest root size (positive PC1), G3 and G5 had an intermediate size, and G4 and G7 had the smallest roots. G1 had the highest NR values, whereas G7 had the lowest (5.72 vs. 5.02). For root shape, G2, G3 and G4 included accessions with wide angles (high PC2) and G1, G5, G6 and G7 had narrower angles (low PC2). G4, G5 and G6 had thicker roots (positive PC3), whereas G1, G3 and G7 had thinner roots (negative

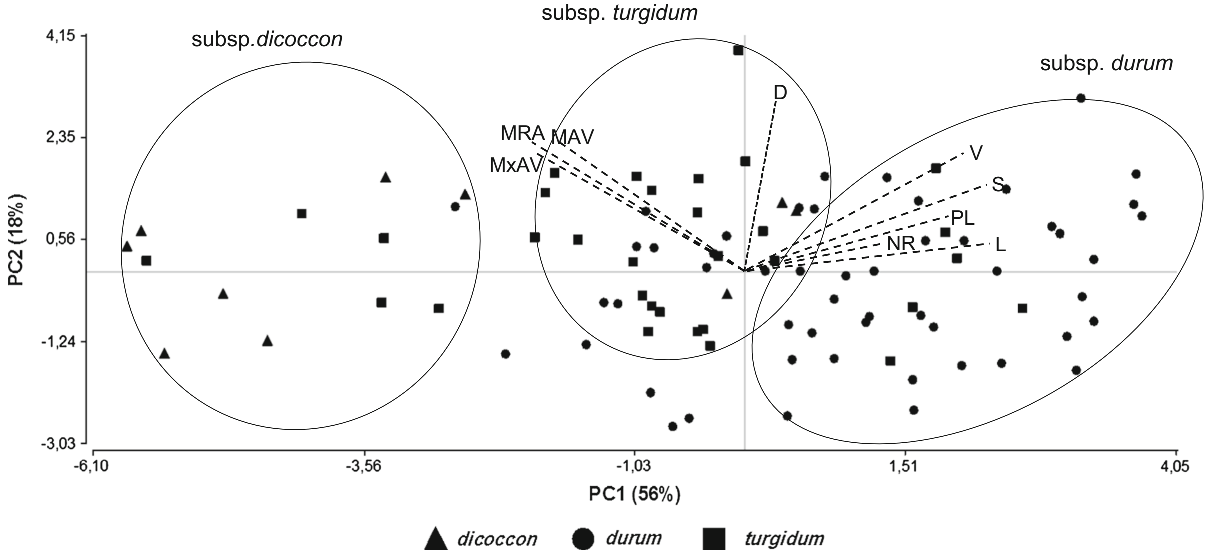

Fig. 2 Biplots showing trait vectors and the location of genotypes of subsp. *dicoccon*, *turgidum* and *durum*

Table 2 Correlations (*r* values) between the RSA traits and W for each subspecies

	L			**PL**			**S**			**D**			**NR**			**MRA**			**MAV**			**MAxV**		
	dicoccon	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*
PL	0.96	0.89	0.77																					
S	0.96	0.91	0.90	0.95	0.75	0.72																		
D	0.55	0.05	0.05	0.60	0.06	0.09	0.75	0.25	0.47															
NR	0.42	0.22	0.48	0.49	0.07	0.01	0.42	0.18	0.44	0.29	0.41	0.01												
MRA	-0.81	-0.65	-0.22	-0.74	-0.59	0.02	-0.81	-0.53	-0.15	-0.51	0.13	0.05	-0.21	0.12	-0.15									
MAV	-0.30	-0.49	-0.13	-0.16	-0.50	-0.07	-0.34	-0.43	-0.06	-0.29	0.01	0.08	-0.21	0.14	-0.09	0.66	0.84	0.77						
MAxV	-0.81	-0.72	-0.24	-0.80	-0.54	0.04	-0.84	-0.65	-0.14	-0.60	0.08	0.10	-0.19	0.00	-0.17	0.96	0.89	0.92	0.52	0.63	0.60			
W	0.20	0.21	0.40	0.39	0.15	0.37	0.37	0.37	0.38	0.67	0.40	0.02	0.11	0.11	0.37	-0.10	0.19	0.20	0.36	0.19	0.07	-0.28	0.04	0.15

Correlation values for V (total volume of the roots) were similar to those for S

L total root length, *PL* primary root length, *S* total root surface area, *D* mean root diameter, *NR* roots number, *MRA* mean of all roots angles with respect to the vertical, *MAV* minimum angle with respect to the vertical, *MxAV* maximum angle with respect to the vertical, *W* shoot weight

$P < 0.05$, $P < 0.01$

PC3). Figure 1 gives examples of the rooting patterns of G4 (e), G7 (f), G2 (g) and G6 (h).

Correlation among RSA features and climatic parameters in the zone of origin

The three subspecies were derived from zones with significant ($P < 0.05$) differences among all climatic parameters, except for the minimum temperature of the coldest month, and for the hot period for *turgidum* and *dicoccon*. These differences indicated that *dicoccon* came from colder and wetter zones than *durum*, with *turgidum* showing a transitional position (i.e., annual rainfall = 898, 717 and 562 mm, annual temperature = 11.6, 13.5 and 15.1 °C and annual ETP = 681, 752 and 809 mm, for subsp. *dicoccon*, *turgidum* and *durum,* respectively). Table 3 shows the significant correlations between the RSA traits and climatic variables for collection sites. In subsp. *dicoccon*, only root shape showed significant

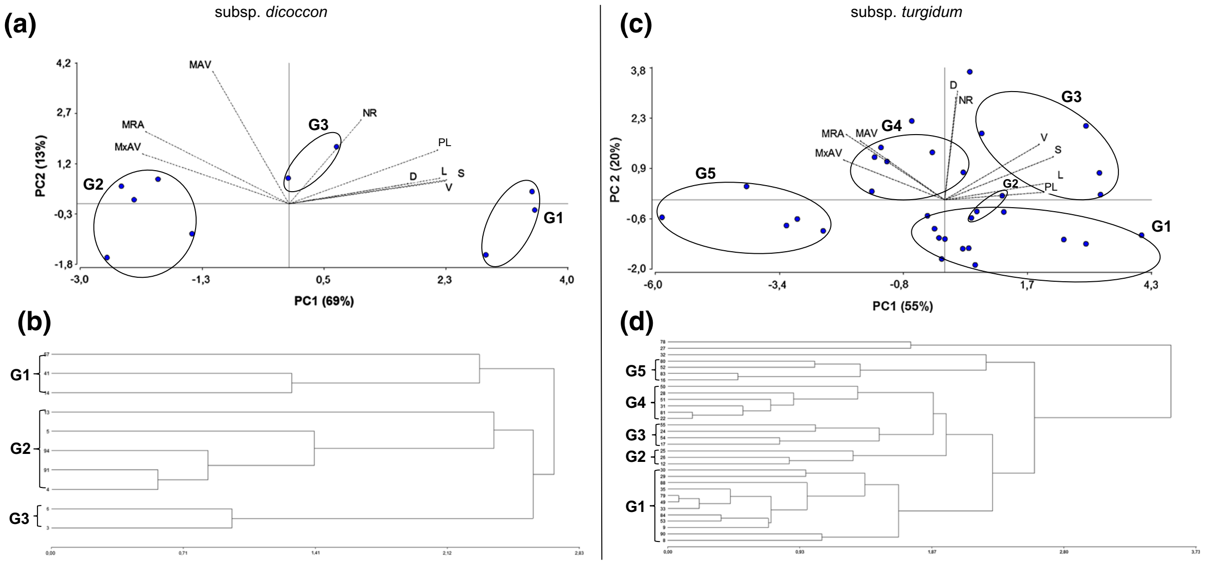

Fig. 3 Biplots showing trait vectors and the location of genotypes of subsp. *dicoccon* (**a**) and *turgidum* (**c**). Dendrograms showing the classification result from principal component-based seminal root traits used as classifiers of the subsp. *dicoccon* (**b**) and *turgidum* (**d**). The main clusters identified in the dendrograms are indicated in the biplots

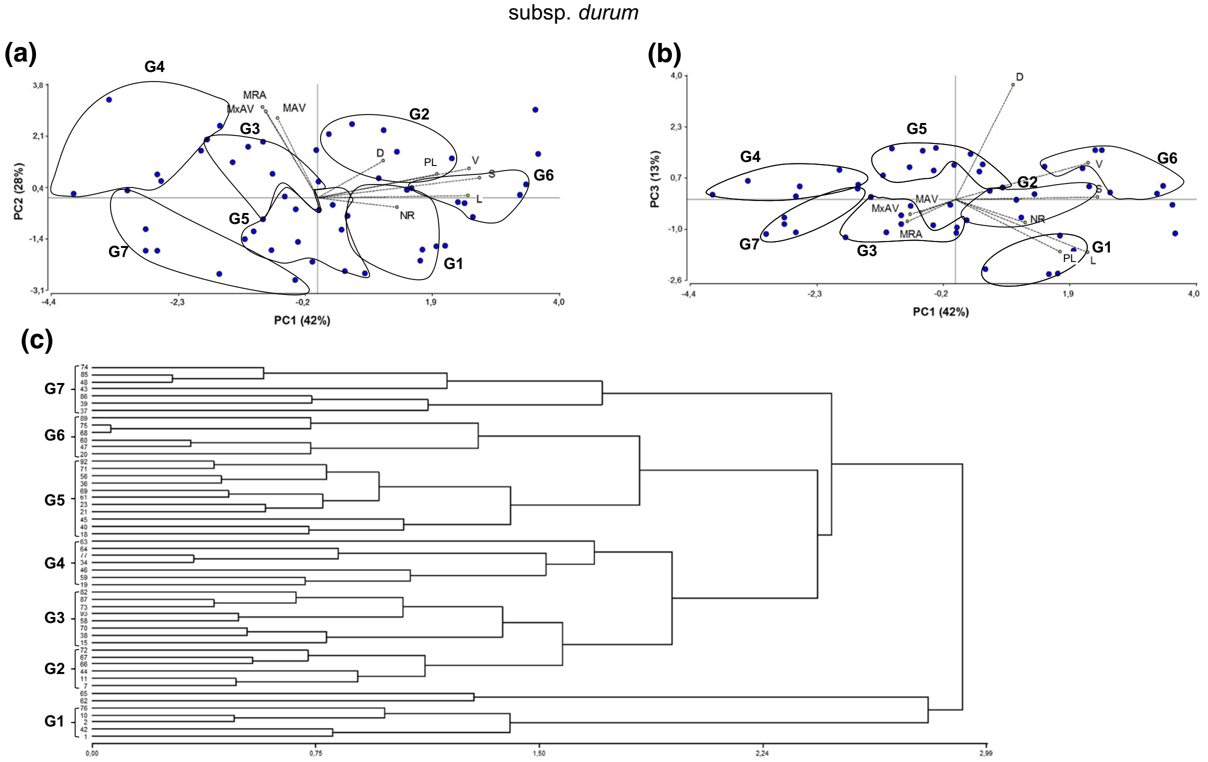

Fig. 4 Biplots showing trait vectors and the location of genotypes of subsp. *durum* (**a** and **b**). Dendrogram showing the classification result from principal component-based seminal root traits used as classifiers (**c**). The main clusters identified in the dendrogram are indicated in the biplots

Table 3 Correlations (r values) between RSA traits and the climatic variables of the collecting sites for each subspecies

	L	PL	S	V	D	NR	MRA	MAV	MAxV	W
dicoccon										
ETP (mm)	0.49	0.27	0.45	0.42	0.20	− 0.06	− 0.64	− 0.69	− 0.46	− 0.38
Hot period (month)	0.17	− 0.04	0.10	0.07	− 0.07	0.00	− 0.20	− 0.39	0.00	− 0.44
Cold period (month)	− 0.59	− 0.40	− 0.58	− 0.55	− 0.32	0.07	0.79	0.75	0.65	0.29
Dry period (month)	0.51	0.30	0.48	0.45	0.22	− 0.06	− 0.67	− 0.71	− 0.50	− 0.36
Annual rainfall (mm)	− 0.56	− 0.36	− 0.54	− 0.51	− 0.28	0.06	0.75	0.74	0.59	0.32
Annual temperature (°C)	0.45	0.23	0.40	0.37	0.16	− 0.06	− 0.58	− 0.65	− 0.39	− 0.40
Max. temp. of the hottest month (°C)	0.51	0.30	0.48	0.45	0.22	− 0.06	− 0.67	− 0.71	− 0.50	− 0.36
Min. temp. of the coldest month (°C)	− 0.59	− 0.40	− 0.58	− 0.55	− 0.32	0.07	0.79	0.75	0.65	0.29
turgidum										
ETP (mm)	0.39	0.30	0.37	0.33	− 0.10	− 0.20	− 0.45	− 0.49	− 0.42	0.09
Hot period (month)	0.43	0.23	0.46	0.45	− 0.06	− 0.21	− 0.44	− 0.48	− 0.48	0.23
Cold period (month)	− 0.37	− 0.33	− 0.34	− 0.29	0.11	0.19	0.45	0.49	0.39	− 0.05
Dry period (month)	0.28	0.17	0.30	0.29	− 0.04	− 0.12	− 0.33	− 0.34	− 0.36	0.03
Annual rainfall (mm)	− 0.17	− 0.08	− 0.24	− 0.27	− 0.06	− 0.02	0.22	0.24	0.27	− 0.05
Annual temperature (°C)	0.39	0.31	0.38	0.34	− 0.11	− 0.18	− 0.48	− 0.52	− 0.43	0.09
Max. temp. of the hottest month (°C)	0.30	0.18	0.37	0.35	0.04	− 0.19	− 0.32	− 0.36	− 0.37	0.18
Min. temp. of the coldest month (°C)	0.36	0.35	0.24	0.13	− 0.26	− 0.27	− 0.47	− 0.50	− 0.32	− 0.04
durum										
ETP (mm)	0.11	0.15	0.17	0.20	0.12	0.18	0.11	− 0.05	0.16	0.07
Hot period (month)	0.04	0.01	0.07	0.08	0.04	0.22	0.05	− 0.09	0.05	0.18
Cold period (month)	− 0.06	− 0.11	− 0.14	− 0.18	− 0.14	− 0.14	− 0.12	0.02	− 0.18	0.02
Dry period (month)	0.32	0.29	0.31	0.28	0.05	0.33	− 0.12	− 0.09	− 0.10	0.24
Annual rainfall (mm)	− 0.31	− 0.20	− 0.29	− 0.25	− 0.08	− 0.24	0.31	0.10	0.31	− 0.10
Annual temperature (°C)	0.12	0.15	0.20	0.23	0.17	0.21	0.07	− 0.08	0.14	0.05
Max. temp. of the hottest month (°C)	0.07	0.07	0.07	0.07	0.00	0.17	0.15	0.02	0.12	0.19
Min. temp. of the coldest month (°C)	0.10	0.15	0.19	0.24	0.19	0.17	0.09	− 0.06	0.18	0.00

L total root length, *PL* primary root length, *S* total root surface area, *V* total root volume, *D* mean root diameter, *NR* roots number, *MRA* mean of all roots angles with respect to the vertical, *MAV* minimum angle with respect to the vertical, *MxAV* maximum angle with respect to the vertical, *W* shoot weight

$P < 0.05$, $P<0.01$

correlations with climatic variables. In general, those climatic variables related to higher water availability or lower temperatures were positively correlated with angle value, suggesting that *dicoccon* landraces coming from colder and wetter zones had shallower seminal root systems than those from warmer and drier areas. In contrast, correlations between the minimum temperature of the coldest month and angle were positive. Subsp. *turgidum* showed the highest number of correlations between root traits and climatic variables, mainly for seminal angles and temperature related variables. Root size and shape-related traits showed opposite correlations with the climatic variables. Therefore, landraces derived from warmer and drier areas tended to have larger and more vertical root patterns than landraces coming from colder and wetter zones. Subsp. *durum* only showed significant correlations with the climatic variables annual rainfall and dry period. Very few correlations with angle variables and none with MAV were found in this subspecies. The results indicated that *durum* landraces coming from drier zones had larger roots with a narrower distribution than those coming from wetter zones. D and W showed no significant correlations with the climatic variables in any subspecies.

Correlation among RSA features and agronomic traits

Agronomic traits of the landraces were evaluated in field trials in three environments (Online Resource 1): one with higher water availability during the crop cycle (C07) and two with high temperatures and water-limited conditions (N and C14). Effects of water stress were detected for some agronomic traits, indicating that landraces grown in dry environments (N, C14) had significantly ($P < 0.05$) lower values for spike length (84 vs. 103 mm), spikelets per spike (21 vs. 24), plant height (106 vs. 129 cm), and days to maturity (202 vs. 215 days) than landraces grown in wetter environments (C07). Analysis of correlations between RSA traits and agronomic performance in different environments (Online Resource 3) revealed significant correlations for each subspecies in the wet or dry environments (summarized in Table 4). In subsp. *dicoccon*, independent of water requirements, larger size and steeper root growth was associated with a lower number of spikelets per spike, whereas deeper primary roots were associated with earlier maturity. Larger root systems in dry environments were associated with fewer days to heading, whereas root diameter was negatively correlated with days to heading and was positively correlated with harvest index and thousand-kernel weight. W showed positive correlations with traits related to the plant and spike size (plant height, spike length and spikelets per spike number). In the wetter environment, a wider MAV was associated with higher test weight. In subsp. *turgidum*, a larger and more vertical root system was negatively and positively correlated, respectively, with days to heading in both water conditions, whereas it was correlated with a lower number of spikelets per spike and a higher spike weight per plant, and grain weight per plant under water stress. A higher W was positively correlated with spike weight per plant, grain weight per plant, and thousand-kernel weight evaluated in the dry environment.

In subsp. *durum*, fewer significant correlations between agronomic and root traits were found, none with angle variables. The most significant correlation was positive between NR and thousand-kernel weight under water stress conditions. Negative correlations were detected between the root traits D and V with the test weight and harvest index, respectively, in the dry environment, and between PL and thousand-kernel weight in the wetter environment. Positive associations of W with tiller weight per plant, spike weight per plant, grain weight per plant and thousand-kernel weight, and negative correlations with harvest index, were found in the dry environment.

MTAs for agronomic or quality traits significantly associated with RSA traits

We studied associations among root traits and QTLs previously detected for agronomic or quality traits in this collection (Giraldo et al. 2016) due to the strong correlation between agronomic and root traits.

Results of the AM analysis are provided in Online Resource 4. In total, 28 markers significantly associated with RSA traits were mapped to all linkage groups except chromosome 4A or B. These markers were associated with several agronomic/quality traits, mainly with spikelets per spike number and gluten strength. Seminal root traits NR and MRA were the most frequently associated in subsp. *durum*, and L and PL were the most frequently correlated in subsp. *turgidum*. Some markers were associated with only one RSA trait, but others, such as wPt-2858 (2A) and wPt-7426 (6B), were associated with up to six RSA traits in subsp. *turgidum*.

Discussion

Phenotypic variability

The durum wheat germplasm analysed in the present work contained a wide diversity in a number of important RSA traits. CV ranged from 6.3 for NR to 41.8 for MAV, and were higher than those reported for MRA and NR in a panel of 183 elite accessions of durum wheat (Canè et al. 2014) and for L and NR in 57 elite durum wheat accessions representative of the

Table 4 Significant correlations (P < 0.05) between the RSA and the agronomic traits for each subspecies in wetter (in blue) or dry (in yellow) environments

	L			PL			S			V			D			NR			MRA			MAV			MAxV			W		
	dicoccon	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*	*dicoccon*	*turgidum*	*durum*
DH (day)	🟨 -	🟦 - 🟨 -			🟦 - 🟨 -		🟨 -	🟦 - 🟨 -		🟨 -	🟦 - 🟨 -		🟨 -							🟦 + 🟨 +			🟦 + 🟨 +			🟦 + 🟨 +				🟦 -
DM (day)		🟦 -																	🟨 +	🟨 +		🟦 + 🟨 +	🟨 +							
PH (cm)															🟦 +					🟨 -						🟨 -		🟨 +		
SS (no.)	🟦 - 🟨 -	🟨 -		🟨 -	🟨 -		🟦 - 🟨 -			🟦 - 🟨 -									🟦 + 🟨 +	🟨 +		🟦 + 🟨 +	🟨 +		🟦 + 🟨 +	🟨 +		🟨 +		
SL (cm)		🟦 +			🟦 +			🟦 +			🟦 +											🟦 +				🟦 -		🟨 +		
TWP (g)					🟨 +								🟨 -																	🟨 +
SWP (g)		🟨 +						🟨 +			🟨 +																		🟨 +	🟨 +
GWP (g)		🟨 +						🟨 +			🟨 +																		🟨 +	🟨 +
HI (%)												🟨 -	🟨 +																	🟨 -
TKW (g)						🟦 -							🟨 +					🟨 +											🟨 +	🟨 +
TW (kg/hl)															🟨 -							🟦 +								

DH days to heading, *DM* days to maturity, *PH* plant height, *SS* spikelets number per spike, *SL* spike length, *TWP* tiller weight per plant, *SWP* spike weight per plant, *GWP* grain weight per plant, *HI* harvest index, *TW* test weight, *TKW* thousand-kernel weight

\+ positive *r* values, − negative *r* values

most relevant germplasm cultivated in the Mediterranean region and North America (Sanguineti et al. 2007). Wheat usually has five seminal roots, although NR values ranging from 3.6 to 6 were found in an analysis of six wheat species (Nakhforoosh et al. 2014). Remarkably, some genotypes in our collection displayed seven roots, which might have a particular functional relevance for exploration of deeper soils (Nakhforoosh et al. 2014). There were also outstanding landraces characterized by extraordinary horizontality in *dicoccon* or large diameter in *turgidum*. Although some overlap was detected in root traits among subspecies, mainly between *durum* and *turgidum*, the subspecies grouping had a large effect on seminal root variation. In general, *dicoccon* and *turgidum* landraces had roots with wider angles and restricted penetration, whereas *durum* genotypes had larger and higher numbers of roots with more vertical growth. Gioia et al. (2015) also found that *durum* varieties had deeper roots than *dicoccon* varieties. The size and shape of the root system were closely linked in subsp. *dicoccon* (except for MAV) and *turgidum*, but not in *durum*. Overall, the genotypes in the present study exhibited considerable variability in RSA, consistent with the high genetic diversity reported for this collection by Ruiz et al. (2013).

Root characteristics commonly exhibit a high degree of phenotypic plasticity in response to temporal and spatial variation in the rooting environment (Poorter and Nagel 2000; Fitter 2002), and this may complicate identification of genotypic variation in root traits (Price et al. 2002). According to Bodner et al. (2013), multivariate statistics provides efficient classifiers to capture root system diversity and can identify distinct groups of rooting types related to phylogenetic relationships, regional origin, and functional plant adaptation to different environments. In this study, PCA indicated that seminal root traits captured almost all of the variability (above 80%) within each subspecies, showing that the root parameters studied here were efficient in identifying distinctive rooting types among the analysed genotypes. This analysis also revealed characteristic groups of genotypes and traits within each subspecies. In the three subspecies, there was a high influence of root system size (described by L, S, V and PL traits) on the first principal component. Root patterns in *dicoccon* and *turgidum* could be grouped into a larger vertical type or a smaller horizontal type, although more variation in size and shape existed among *turgidum* accessions than in *dicoccon* genotypes. This result was not unexpected, as higher genetic variability in *turgidum* than *dicoccon* landraces was previously reported (Ruiz et al. 2012) probably due to the latter subspecies being less widespread, with its current cultivation area restricted to few Spanish regions. In subsp. *dicoccon*, the two groups within the category of a small superficial root type differed in verticality of the primary roots (described by MAV values), in agreement with the independence of this angle variable from the remainder of the root angles in this subspecies. In *turgidum*, the large and narrow patterns differed in D, NR or PL values, whereas small and shallow rooting types could have either thin or thick roots. A higher number of distinctive rooting patterns were identified in subsp. *durum*, probably due to the independence of the root size from root shape and diameter. Therefore, similar-sized patterns could indicate thin or thick roots, and vertical or horizontal growth, although a narrow distribution was more common, probably due to the selection pressure for drought tolerance on durum wheat since its domestication. The lack of correlation between root shape and other RSA traits, such as L, PL, D, or NR, concurred with other studies (O'Brien 1979; Sanguineti et al. 2007; Manschadi et al. 2008). Our results also agreed with the absence of correlation between D and W, and the significant correlation between L and S, V, PL and W, reported by Narayanan et al. (2014), and correlation between NR and L, as reported by Sanguineti et al. (2007). Significant correlations between D and PL (Sanguineti et al. 2007) and L and PL (Narayanan et al. 2014) were not found in the present study.

Analysis of correlations with eco-geographical origin

The Spanish Core Collection represents all of the agro-ecological zones of durum wheat cultivation in the country. Therefore, the variability found for seminal root traits may, to a certain extent, reflect the adaptive value of such features for the environmental conditions prevailing in each region. The characterization of the three subspecies derived from contrasting eco-geographical areas (*dicoccon* and *turgidum* from the North, and *durum* from the South) also allowed assessment of the roles of geographic origin on environmental adaptation profiles of the three subspecies. In subsp. *dicoccon*, climatic parameters were correlated with root shape phenotype, but no correlation was found with root size. This could indicate that root shape has a more relevant function in adaptation of this subspecies to the environment. *Dicoccon* zones of origin are the coldest and wettest, with a high spring rainfall, thus resulting in a lower requirement for water uptake from deeper soil horizons. Accordingly, root shape could make a significant contribution to root distribution in soil layers to capture spring rainfall during heading. In subsp. *turgidum* and *durum*, coming from warmer and drier zones than *dicoccon*, both size (except the verticality of the primary root in *durum*) and shape varied with climatic conditions, showing that both morphological root characteristics have adaptive roles in these subspecies. This morphological adaptation was mainly affected by temperature-related variables in *turgidum*, and by humidity in *durum*. These differences between both subspecies could be because *durum* came from the warmest southern areas with fewer differences in temperature, in contrast to *turgidum,* which came from relatively wetter zones. Nevertheless, few correlations between climatic conditions and RSA traits were found in subsp. *durum*.

In both *dicoccon* and *turgidum*, small and horizontal root systems were frequent in accessions from wetter and colder zones. Accordingly, the shallow small root patterns (G2 and G3 groups in *dicoccon*, and G5 in *turgidum*) were present in zones with higher precipitation (from 863 to 1135 mm) and lower annual temperature (11 °C) compared to zones with large vertical root types, G1 in *dicoccon*, and G1 and G3 in *turgidum* (precipitations from 559 to 668 mm and temperatures from 12 to 15 °C). This result is consistent with low root temperatures restricting root growth in some species (Nagel et al. 2009).

It may be significant that in both subspecies the largest and most vertical root pattern involved thick roots (G1 in *dicoccon* and G3 in *turgidum*). In the subsp. *turgidum*, accessions with this pattern originated from warmer and drier zones than the large pattern with thinner roots in group G1 (precipitation 591 vs. 668 mm, maximum temperature of the hottest month 33 vs. 30 °C and minimum temperature of the coldest month 1.8 vs. 2.8 °C). Thick roots seem to have a greater mechanical resistance and improved penetration ability in dry soils (Clark et al. 2008; Nakhforoosh et al. 2014). Furthermore, high xylem conductance (correlated with large xylem diameter) facilitates the flow of water and nutrients in zones with water in the upper soil layers (Nakhforoosh et al. 2014). Thus, a higher D may be an adaptive trait for drier and hotter conditions in zones where wheat is mostly grown on stored soil water. In this respect lower minimum temperatures in the coldest month were correlated with smaller angles in subsp. *dicoccon*. This result agrees with the relationship found between deep root systems and cold tolerance as reported by Worzella (1932) in common wheat. Therefore, selection for a narrower root distribution may lead to better adaptation in areas with low minimum temperatures.

In subsp. *durum*, the largest and vertical types (G1 and G6 groups) were present in zones with significantly higher values of annual ETP (874 mm) or dry periods (5 months), annual temperature (17 °C), and minimum temperature in the coldest month (4 °C). Those deep rooting types suited to arid zones with high temperatures throughout the crop season could be useful for adaptation to steadily increasing temperatures associated with climate change. The better adaptation of genotypes with small and horizontal root systems to wetter conditions, and large and vertical root type adapted to drier environments, were in agreement with other studies on wheat (Oyanagi 1994; Ehdaie et al. 2012). The two largest and vertical types in *durum* displayed different RSA strategies to achieve large size; G1 increased NR (5.72 roots) but G6 enhanced D (0.06 mm). According to the significant correlation between D and S in subsp. *durum*, thick roots could provide a greater root surface and better mechanical resistance to penetrate deep soil layers. On the other hand, a higher number of seminal root axes may result in more intensive root branching and root length density at depth, substantially enhancing the capacity of root systems to more effectively explore deeper soil layers (Maccaferri et al. 2008). Genotypes expressing a high number of seminal roots combined with narrow growth, such as those in G1, may be ideally suited to environments where plants rely largely on subsoil water. In this case, a smaller xylem diameter as that in the G1 genotypes would imply conservative water use during vegetative growth and sufficient stored soil water remaining for the reproductive period (Richards and Passioura 1989). Genetic analysis of this collection (Ruiz et al. 2012) indicated that genotypes in G1 were more related to *durum* landraces from North Africa, whereas those in G6 seemed to be closer to subsp. *turgidum*, which also produced thick roots in dry environments. Such results could provide useful information for further functional analyses to identify loci underlying the phenotypic differences found in both rooting types. Remarkably, the other large rooting type present in subsp. *durum* had a shallow distribution (group G2). This type came from colder and wetter zones than the G1 and G6 groups, supporting the importance of horizontal roots in the warm but more humid areas of origin in this subspecies. Independence between shape and size is key to improving root plasticity in response to changes in environmental conditions. According to Bektas et al. (2016), a preferred root system should be large enough and well distributed throughout the soil profile in order to capture nutrients and water effectively throughout the season (Hurd 1974; Blum 1996). This kind of root system may also be important to capture precipitation from light rains at the end of the growing season, which may also help them reach non-mobile nutrients. This pattern may be ideal but may not be easy to achieve since it is difficult to breed a plant with the above root characteristics. It should be noted that the root type in G2 was a large shallow root system with high values for L, PL, MxAV and MRA (102 mm, 25 mm, 38.6° and 25°, respectively), which could capture water and nutrients in shallow soil layers and water from deep soil zones. The plasticity of this phenotype could be valuable for breeding programs targeting marginal environments.

Although some similarities can be found between root patterns among subspecies (e.g. G1 in *dicoccon* and *turgidum* with G5 in *durum*), the rooting patterns identified in this study were subspecies-specific. Such findings demonstrated that root type is the joint result of phylogenetic relationships and environmental, as well as human, selection pressure, although common environmental adaptation seems to be relevant for root system formation (Bodner et al. 2013).

Analysis of correlations with agronomic traits

The relationships between RSA and different agronomic features were analysed across water regimes. Landraces of subsp. *durum* had the best yields under drought when evaluated by harvest index and grain weight per plant respectively, 30% and 5.4 g, 30% and 5.6 g, and 35% and 5.7 g for *dicoccon*, *turgidum* and *durum*). Several correlations between seminal root morphology and agronomic traits were detected in the three subspecies. The relationship was less evident in *durum,* as also shown by Canè et al. (2014). In dry environments, a larger vertical root was associated with earlier heading and a lower number of spikelets per spike in the subsp. *dicoccon* and *turgidum*. The lower number of spikelets per spike resulted from the positive relationship between days to heading and spikelet number per spike detected in dry environments, in agreement with previous reports (Worland et al. 1998; Ruiz et al. 2008). This reduction in spikelets per spike seemed to be associated with higher yield under drought conditions due to the larger size and weight of grain (Lafitte et al. 2004; Khan et al. 2005). Larger root size in the subsp. *turgidum* was associated with higher spike weight and grain weight per plant, indicating a positive effect of large roots on yield components under limited water conditions. In the more humid environments, shallow or shorter primary roots were related to better agronomic performance (higher test weight in *dicoccon* and thousand-kernel weight in *durum*), indicating the influence of morphology of the primary root under limited water. No significant correlations between agronomic parameters and seminal root shape were detected in subsp. *durum*. In contrast, Canè et al. (2014) found significant correlations between root angle and number of kernels per square metre (positive) and thousand-kernel weight (negative) in high and medium yield environments. In our study, thicker roots were associated with higher test weight and harvest index in subsp. *dicoccon*, and with lower test weight in subsp. *durum*, as evaluated under drought conditions. In contrast, a positive effect of NR on thousand-kernel weight was detected in *durum* in the dry environment. These results agreed with the beneficial adaptive role of a large root diameter in subsp. *dicoccon*, and of NR in subsp. *durum* in drier zones. In the latter subspecies, NR was positively correlated with root size and showed great variation among landraces, supporting that NR as a crucial parameter for environmental adaptation. This result was consistent with other studies finding positive influences of NR on yield under limited water conditions (Liu et al. 2013; Canè et al. 2014).

Shoot weight was associated with higher vegetative development in dry environments in all three subspecies, and with better values of some yield components in subsp. *turgidum* and *durum*. The negative effect of W on harvest index in subsp. *durum* seemed to be due to a greater influence of W on tiller weight than on grain weight per plant, which negatively affected harvest index values. Higher W values reflected faster leaf area development, which seemed to be related to higher tiller number and greater shoot biomass (Rebetzke and Richards 1999). Other studies have reported positive associations between shoot biomass and dry weight and size of roots (Palta et al. 2011; Jain et al. 2014; Bektas et al. 2016), consistent with the positive correlation found in our study between shoot weight and root volume and surface area in *durum* and *turgidum* genotypes. In areas where terminal drought often occurs, a vigorous root system could facilitate crop establishment and growth and could improve the capture of water and nitrogen (Palta et al. 2007, 2011). Consequently, measuring shoot weight at an early stage could provide meaningful indications of root system vigour and vegetative development, key traits for enhancing yield under stress conditions.

MTAs for agronomic and quality traits significantly associated with RSA traits

Dissecting the genetic control of RSA traits is very important in tetraploid wheat due to their cultivation under rainfed conditions often associated with low water availability. We searched for QTLs that were expressed at the seedling stage under non-stress conditions, aiming to identify constitutively expressed QTLs whose effects were eventually maintained in field-grown plants. Only RSA–QTLs co-located with agronomic traits are reported.

The meta-QTL analysis approach made by Petrarulo et al. (2015) allowed us to identify genomic regions involved in the control of root traits. Some of the genomic regions matched the locations of markers identified in the present work according to previous genetic map information (Marone et al. 2012). Markers *wPt-0196* and *wPt-8770* (1A), associated with MRA and NR, respectively, were located in the interval *Xgwm136-wPt-4177* where a strong QTL for root length, surface area, number of tips, and volume was described. Marker *wPt-1890* (6B), associated with D, V and W, was located in the interval *wPt-9256-wPt-219*, where a QTL for the number of tips was identified. Marker *wPt-6012* (1B), associated with V and MAV, was also coincident with a QTL for root length identified in the same work. Marker *wPt-2202* (3A), associated with V, D and NR, was relatively close to marker *Xwmc428* associated with root length, surface and volume. The markers identified on chromosomes 1A and 1B were coincident with the QTLs for root length identified in recent work in common wheat (Ayalew et al. 2017), and marker *wPt-2858* (2A) was identified as being associated with a root angle QTL that also occurred in common wheat (Christopher et al. 2013).

The most comprehensive dissection of the QTLome for RSA in durum wheat was recently published (Maccaferri et al. 2016). Some of the genomic locations identified in the present work are coincident or close to QTLs identified in that study (Marone et al. 2012; Bordes et al. 2014). Marker *wPt-4120* (7B), associated with L, co-localized with *wPt-6865*, which is also associated with root length. Marker *wPt-0196* (1A), associated with MRA, is very close to *wPt-4676*, which is linked to QTLs for root volume, and *wPt-6376* (3A), associated with W, which is relatively close to *Xbarc51* that is associated with shoot dried weight. Finally, *wPt-1163* (7A), associated with L and PL, is located in map interval *Xcfd2049-Xcfd2028*, where Kabir et al. (2015) reported a QTL for number of root tips.

Conclusions

Both regional origin and genetic background were responsible for the high root system diversity observed in this wheat collection. Distinctive phenotypes were identified within each subspecies. Some of them were remarkable, such as large roots with shallow distribution or high root numbers. Root shape was more relevant in adaptation to wetter conditions, whereas both root size and shape could have had an adaptive role in warmer and drier zones. RSA traits with the largest positive effects on yield components under water limited conditions were root diameter in subsp. *dicoccon*, root size in *turgidum*, and root number in *durum*. Shoot weight had an important effect in subsp. *turgidum* and *durum*. The genetic variability identified in root traits in this research could be exploited in breeding for drought tolerance and resource capture in wheat.

Some of the molecular markers identified in the present work were associated with root traits co-mapped with MTAs for previously identified agronomic or quality traits. These regions are of particular interest and should be targets for future studies. A forthcoming association study with a high number of markers will confirm these results and should provide new knowledge about the genetic basis of root traits and their potential use in marker-assisted selection programs in durum wheat.

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