Root System Architecture and Its Association with Yield under Different Water Regimes in Durum Wheat

K. El Hassouni, S. Alahmad, B. Belkadi, A. Filali-Maltouf, L. T. Hickey, F. M. Bassi*

ABSTRACT
Durum wheat (*Triticum durum* Desf.) is a major cereal crop grown globally, but its production is often hindered by droughts. Breeding for adapted root system architecture should provide a strategic solution for better capturing moisture. The aim of this research was to adapt low-cost and high-throughput methods for phenotyping root architecture and exploring the genetic variability among 25 durum genotypes. Two protocols were used: the “clear pot” for seminal root and the “pasta strainer” to evaluate mature roots. Analysis of variance revealed significant segregation for all measured traits with strong genetic control. Shallow and deep root classes were determined with different methods and then tested in yield trials at five locations with different water regimes. Simple trait measurements did not correlate to any of the traits consistently across field sites. Multi-trait classification instead identified significant superiority of deep-rooted genotypes with 16 to 35% larger grains in environments with limited moisture, but 9 to 24% inferior in the drip irrigated site. Combined multitrait classification identified a 28 to 42% advantage in grain yield for the class with deeper roots at two environments where moisture was limited. Further discrimination revealed that yield advantage of 37 to 38% under low moisture could be achieved by the deepest root types, but that it also caused a 20 to 40% yield penalty in moisture-rich environments compared with the shallowest root types. In conclusion, the proposed methodologies enable low-cost and quick characterization of root behavior in durum wheat with significant distinction of agronomic performance.

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Abbreviations: BLUE, best linear unbiased estimate; GW, grain weight; GY, grain yield; IG, ICARDA Genebank; PCA, principal component analysis; PLH, plant height; QTL, quantitative trait locus; RA, root angle; RB, root biomass; RR, root ratio; SB, shoot biomass; SN, spike number; SPN, average spikelet number per plant; SRA, seminal root angle; TN, tiller number; TRN, total root number; TWK, 1000-kernel weight.

DURUM WHEAT (*Triticum durum* Desf.) is one of the main sources of daily caloric intake and a major staple crop in the Mediterranean region. It is known for its unique quality characteristics, in particular high protein content and hard kernels that make it ideal for pasta, couscous, and bourghul manufacturing (Able and Atienza, 2014; Habash et al., 2014; Kezih et al., 2014; Stuknytė et al., 2014). Unlike common wheat (*Triticum aestivum* L.), durum wheat is primarily grown in marginal environments of the Mediterranean and semiarid regions of the world, where moisture is mostly provided through rain (Able and Atienza, 2014; Habash et al., 2014). Annual variation in rainfall is common in the Mediterranean environment, with late-season droughts happening frequently. When droughts coincide with the flowering or grain-filling phase, it can dramatically affect yield and grain quality (Loss and Siddique, 1994; Belaid, 2000; Mohammadi et al., 2011; Bassi and Sanchez-Garcia, 2017). Furthermore, the Mediterranean region is predicted to lose 30% of its in-season...
rainfall due to the warming climate (Christensen et al., 2007). However, winter rainfall is typically abundant in Mediterranean climates, which leads to percolation of moisture into the deeper layers of the cultivated soils. According to modeling studies, wheat yield would increase by 55 kg ha⁻¹ on average for each millimeter of water extracted from the soil after anthesis (Manschadi et al., 2006; Christopher et al., 2013). This highlights the importance of identifying root systems that provide better exploration of soil layers to capture rainfall early in the season or with the capacity to reach the residual moisture deep in the soil profile toward the end of the season.

Roots also play an essential role in plant health, as they enable not only access to water, but also nutrients vital for high productivity (Sharma et al., 2009). Hence, targeted breeding for specific root system architecture should ultimately result in more resilient durum wheat cultivars under water-limited environments (Sanguineti et al., 2007; Manschadi et al., 2008). For example, in rainfed cropping systems, it was shown that a narrow and deep root system architecture with more branching at depth provided greater access to soil nutrient and moisture in environments experiencing terminal drought (Manschadi et al., 2006; Kirkegaard et al., 2007; Christopher et al., 2008, 2013). In addition, deeper and more efficient root systems were demonstrated to be significantly correlated to yield increases in wheat (Kirkegaard et al., 2007; Fang et al., 2017), rice (Oryza sativa L.; Ari-Sanoh et al., 2014), and sorghum [Sorghum bicolor (L.) Moench; Mace et al., 2012]. Roots length density (also known as the length of roots per unit of soil volume) and root depth are key components for enhanced deep soil water extraction (Asif and Kamran, 2011; Borrell et al., 2014). Seminal root angle (SRA), also called gravitropic set-point angle (Digby and Firn, 1995), has been shown to be a good proxy to determine the depth of roots in the field across different cereal crops such as wheat (Manschadi et al., 2008), rice (Kato et al., 2006) and sorghum (Mace et al., 2012). This variation in root traits is mostly regulated by multiple adaptive genes with minor additive effects often combined with epistasis, resulting in a degree of genotype × environment interaction (Price et al., 2002; Tuberosa et al., 2002; Giuliani et al., 2005; MacMillan et al., 2006; de Dorlodot et al., 2007; Cooper et al., 2009; Ren et al., 2012; Christopher et al., 2013).

Despite its complexity, breeding for beneficial root system architecture holds great potential to enhance drought adaptation and offers a great opportunity for rapid genetic gain for grain yield (GY) in marginal land (Hammer and Jordan, 2007). The significance of root traits contributing to yield under water-limited environments has long been recognized (Richards, 1991), and roots have likely been subjected to indirect selection in breeding programs as a result of selection for high yield in the target environment (Wasson et al., 2012). However, incorporating selection for root traits directly in a breeding program has been met with many challenges, foremost the difficulty of phenotyping large numbers of genotypes in a cost- and time-efficient manner (Mace et al., 2012).

Several wheat studies have evaluated roots using different phenotyping methods including rhizotrons (Nagel et al., 2012; Lobet and Draye, 2013; Clarke et al., 2017), soil coring (Trachsel et al., 2011; Wasson et al., 2012; Wasson et al., 2014), lysimeters (Ehdaie et al., 2014; Elazab et al., 2016), hydroponics (Liu et al., 2015), paper roll culture and Petri dishes for seedling (Tomar et al., 2016), rhizoboxes (Fang et al., 2017), and X-ray-computed tomography (Gregory et al., 2003; Mairhofer et al., 2013; Colombi and Walter, 2017; Flavel et al., 2017). However, most of these techniques are either expensive or not precise enough and reproducible. This has encouraged researchers to develop high-throughput strategies that focus on key proxy traits linked to root system architecture displayed in the field (Petrarulo et al., 2015; Richard et al., 2015). One such example is the “clear pot” method first developed by Richard et al. (2015) in hexaploid wheat, and later adapted to barley (Hordeum vulgare L.; Robinson et al., 2016). It involves growing genotypes in plastic transparent pots under semicontrolled conditions in the glasshouse, whereby the SRA can be measured without removing the seedlings from the soil. Some degree of infrastructure is still required to perform the assay, but some success in breeding has already been shown (Hickey et al., 2017).

Another method for characterization of mature roots is the “basket” or “pasta strainer” method. It involves sowing isolated genotypes directly in the field inside a plastic container with holes on all its sides. The baskets are then removed from the soil to assess the behavior of the roots in proximity of the crown. This inexpensive and fast phenotyping technique was originally developed for wheat (Oyanagi et al., 1993) to assess the growth angle of seminal roots in greenhouse pots. It was then adapted to field conditions to study rice roots 6 wk after sowing (Uga et al., 2009; Uga, 2012).

In this study, the clear pot method was deployed in combination with an adapted space-planted field pasta strainer (basket) method to explore the seminal and mature root traits for a set of durum wheat genotypes under two different water regimes. The objective was to assess the suitability and high throughput of these methods, and to use them to investigate the available genetic diversity for rooting pattern. Further, we examined whether root systems respond differently to water availability. Finally, yield trials were conducted in different environments with a range of water regimes to evaluate the potential value of shallow or deep rooting systems, and the possibility of incorporating selection for root traits in breeding programs.
MATERIAL AND METHODS

Plant Material
A subset of 25 durum wheat ($2n = 4x = 28$, AABB) genotypes derived from a collection of 384 accessions originating from different countries were evaluated for root growth pattern using the clear pot and pasta strainer methods. The panel comprised four landraces, six cultivars, and 15 ICARDA accessions. Details for the different genotypes are provided in Table 1.

Evaluating Seminal Root Traits Using the Clear Pot Method
Clear plastic pots (ANOVApot, 200-mm diam., 190-mm height) were filled with peat moss soil known for its high water- and nutrient-holding capacity. Seed sowing was performed according to Richard et al. (2015). A randomized complete block design was used, where each pot containing 24 seeds was considered a block, and a total of 20 blocks were used. Genotype Maci115 was omitted from this experiment to facilitate a balanced experimental design, since only 24 entries could be accommodated in each clear pot. Using forceps, seeds were positioned vertically between the pot wall and soil with the embryo facing downward at $\sim 3$-cm depth, ensuring the easy visualization of the seed embryo through the transparent pot. Plants were grown in the glasshouse under diurnal natural light conditions. Images were captured 5 d after sowing (seminal roots 3–5 cm in length) using a digital camera. The images were analyzed for SRA, where the angle between the first pair of seminal roots emerging from the seed was measured using online free software ImageJ (http://imagej.nih.gov/ij/).

Evaluating Mature Root System Traits Using the Pasta Strainer Method
The method of Uga et al. (2009), initially developed for assessing rice roots in plastic baskets, was adapted to durum wheat as follows. The space-planting field conditions experiment was performed in Guich Station (33°59’ N, 6°50’ W; Rabat, Morocco) in the field, in sandy soil (silt loam 0–20 cm, sandy loam 20–40 cm) under the protection of a net house. Plastic pasta strainers (height = 11 cm, diameter = 20 cm) were filled with sandy soil and buried at 11-cm depth (Fig. 1A). Strainers were placed with 20 cm distance between each other to ensure 40–cm spacing between plants, to avoid barriers to the growth of the root system in all three dimensions. Three seeds were placed in the middle of each strainer representing one genotype. At the four-leaf stage (growth stage 14, according to the Zadoks decimal growth scale; Zadoks et al., 1974), the three seedlings were thinned, retaining the most vigorous plant.

During the growing season, standard cultural practices were used, with 150 kg ha$^{-1}$ of N, P, and K incorporated in the soil before planting, followed by 50 kg ha$^{-1}$ of NH$_4$NO$_3$. Cherokee fungicide (1.5 L ha$^{-1}$, Syngenta, chlorothalonil–cyproconazole–propiconazole) was applied to prevent development of fungal diseases, and Pirimor (500 g ha$^{-1}$, Syngenta, pyrimicarb) was applied to control aphid infestation. Weeds were controlled by two applications of tank mixture of Mustang (0.6 L ha$^{-1}$, DOW Agrosciences, 2,4-D-florasulame) and Pallas (0.5 L ha$^{-1}$, DOW Agrosciences, pyroxulame), with additional mechanic weeding to ensure pristine plot.

The experimental design was an $\alpha$ lattice with two replications and five incomplete blocks of size five. Two independent

### Table 1. Name, origin, and genetic background of the 25 durum wheat genotypes evaluated in this study.

<table>
<thead>
<tr>
<th>Accession name</th>
<th>Origin</th>
<th>Pedigree</th>
</tr>
</thead>
<tbody>
<tr>
<td>IG:86075</td>
<td>India</td>
<td>Landrace</td>
</tr>
<tr>
<td>IG:79509</td>
<td>Ethiopia</td>
<td>Landrace</td>
</tr>
<tr>
<td>IG:85026</td>
<td>Spain</td>
<td>Landrace</td>
</tr>
<tr>
<td>IG:85620</td>
<td>Afghanistan</td>
<td>Landrace</td>
</tr>
<tr>
<td>Jabal2</td>
<td>ICARDA</td>
<td>Korifta/AegSpeltoidesSyr/Mrb5</td>
</tr>
<tr>
<td>Amina</td>
<td>ICARDA</td>
<td>Korifta/AegSpeltoidesSyr/Loukos</td>
</tr>
<tr>
<td>Heirum</td>
<td>ICARDA</td>
<td>Heider/TAraticumMA/Mrb5</td>
</tr>
<tr>
<td>Icamator</td>
<td>ICARDA</td>
<td>IcamorTA041/4/Aghrass1/3/HFN94N8/Mrb5/2/3/Azn1/4/BEZAIZSHF/SD19539/</td>
</tr>
<tr>
<td>Ouassarar1</td>
<td>ICARDA</td>
<td>Ouasloukos1/5/Anen1/A/BEZAI/SZH//SD19539/Wahsa/3/Gdr2</td>
</tr>
<tr>
<td>Margherita2</td>
<td>ICARDA</td>
<td>Terboi975/Gerufterl2</td>
</tr>
<tr>
<td>Icadeful</td>
<td>ICARDA</td>
<td>Geromtel1/IRANYTO53/Mgnl3/Anzen1</td>
</tr>
<tr>
<td>Icarasha2</td>
<td>ICARDA</td>
<td>Stj3/Bcr/Lks4/3/Ter3</td>
</tr>
<tr>
<td>Icamoram7</td>
<td>ICARDA</td>
<td>ICAMORTA0472/Ammer7</td>
</tr>
<tr>
<td>Maci115†</td>
<td>ICARDA</td>
<td>Maamouri2/Citrus5/3/Fit/3/Arthurr7/Lahna/Bilk/Lahn/4/Quar</td>
</tr>
<tr>
<td>Miki3</td>
<td>ICARDA</td>
<td>Stj3/Bcr/Lks4</td>
</tr>
<tr>
<td>Secondroue</td>
<td>ICARDA</td>
<td>Stj3/Bcr/Lks4/3/Ter3/3/Bcr/Gro1/Mgnl1</td>
</tr>
<tr>
<td>Omrabi17</td>
<td>ICARDA</td>
<td>Jori c69/Hau</td>
</tr>
<tr>
<td>Bellaroi</td>
<td>Australia</td>
<td>920405/920274</td>
</tr>
<tr>
<td>Jupare C2003</td>
<td>CIMMYT</td>
<td>STOT//ALTAR84/ALD</td>
</tr>
<tr>
<td>Yavaro879</td>
<td>CIMMYT</td>
<td>JORI69/ANHINGA/FALMINGO</td>
</tr>
<tr>
<td>Creso</td>
<td>Italy</td>
<td>Yaktana-54/Norin 10-B/22/Cappelli-63/3/Teuucan-60/4/Cappeli-B144</td>
</tr>
<tr>
<td>Marzak</td>
<td>Morocco</td>
<td>BD113</td>
</tr>
<tr>
<td>Kofa</td>
<td>USA</td>
<td>dicoccum alpha poly-85 S-1</td>
</tr>
</tbody>
</table>

† Genotype was not evaluated using the clear pot method.
trials were conducted over two seasons, each with independent randomization based only on the genotype factor. Each trial received a different amount of moisture as follows: after flowering (growth stage 55 on the Zadoks scale), a plastic tarp was placed over the roof and sides of the net house to prevent rainfall from reaching the experimental setup. The well-watered trial then received four irrigation events, one every 10 d (total amount of 40 mm), whereas the deficit trial did not receive any additional moisture for 40 d. At this point, one final irrigation of 20 mm was provided to both. These experiments were conducted during the seasons 2014–2015 and 2015–2016 from December to May.

Before maturity, the number of fertile spikes (SN) and tillers (TN), as well as the number of spikelets on each spike (SPN), was recorded for each plant, together with the plant height (PLH) excluding the awns. In addition, a relative surrogate for the chlorophyll content was measured using a chlorophyll meter (Konica Minolta SPAD 502) during the grain-filling stage to confirm the treatment effects. At maturity, the shoot was cut 3 mm above the soil and weighed to determine the dry shoot biomass (SB). The spikes were then threshed to determine the grain weight (GW), and the weight of 1000 kernels (TKW) was determined using a precision balance. The stay-green trait was calculated as the days elapsing between the heading and maturity dates.

The belowground traits were recorded by first removing the strainer from the soil using a shovel. The strainer was then divided into three sections (Fig. 1B): an upper Layer 1 (2–8 cm), a middle Layer 2 (8–10 cm), and a lower Layer 3 (10–13 cm). The three layers marked on the strainer corresponded to the angles from the horizontal ground level of 0 to 30, 30 to 60, and 60 to 90°, respectively. The number of roots protruding from the holes in the sides of the plastic container were counted for each level, and the number of roots for each layer was then expressed as a ratio of the total root number (TRN), resulting in three root ratios (RR 2–8 cm, RR 8–10 cm, and RR 10–13 cm). The root sections protruding from the plastic container were cut off to leave an exact volume of soil and roots corresponding to the volume contained inside the pasta strainer, equivalent to 2100 cm$^3$. The sandy soil was then gently removed from the container, avoiding damaging the roots in the process. The type of soil used was ideal for this task, as its loam content prevented loss through the holes of the strainer until a small pressure was imposed, whereas its sandy nature facilitated the task of removing the soil without damaging the roots. Roots were then rinsed in water to remove any remaining sand and allowed to dry for 10 d. The dry roots were first weighed to obtain root biomass (RB) and then scanned using an Epson Perfection V700 scanner. This image was analyzed with ImageJ to measure the root angle (RA), setting the center of the angle in the middle of the crown and the two extreme sides of roots as the final point of the angle (Fig. 1C). All roots angles were visually controlled to prevent errors. Image scanning and ImageJ was performed only in the second season.

**Field Trials**

The 25 entries used in this study were included in yield trials containing a larger set of 384 genotypes. Each trial was conducted using an augmented design of 19 blocks of 24 plots, each block with four commercial checks. Sown plot size was 3.6 m$^2$ (six rows), and 2.4 m$^2$ (four rows) were harvested for assessing yield performances. In addition, 1000 randomly selected kernels were weighted to determine TKW. The five environments listed in Table 2 included two rainfed environments with strong terminal droughts (Marchouch in Morocco [MCH16] and Kfardan in Lebanon [KFD16]) and three irrigated environments. The...
irrigated environments were Terbol (TER16), where supplemental moisture was provided via three sprinkler irrigations; Melk Zehr (MKZ16), where drip irrigation was used to provide the majority of the moisture; and Tessaut (TES16), where nearly all in-season water was provided via gravity-fed irrigation. Optimal agronomic management practices were applied in all environments. The total rainfall and the amount of irrigation water applied are presented in Table 2.

### Data Analysis

All statistical analyses for root trials were computed in R (R Development Core Team, 2016). A mixed linear model was formulated in accordance with the experimental design to obtain best linear unbiased estimates (BLUEs) for all traits, where genotype, treatment, and year were considered as fixed effects and replication and block as random effects nested in treatment and year. The model was fit using the `lmer` function of the lme4 package (Bates et al., 2015). Broad-sense heritability was estimated based on a random model as the ratio between the genotype variance ($\sigma^2_g$) and phenotypic ($\sigma^2_p$) variance (Falconer and Mackay, 1996):

$$H^2 = \frac{\sigma^2_g}{\sigma^2_p}$$

Phenotypic variance was calculated using the method suggested by DeLacy et al. (1996):

$$\sigma^2_p = \sigma^2_{G\times T} + \frac{\sigma^2_{G\times Y}}{t} + \frac{\sigma^2_{G\times T\times Y}}{ty} + \frac{\sigma^2_e}{tyr}$$

where $\sigma^2_{G\times T}$ is the genotype × treatment variance, $\sigma^2_{G\times Y}$ is the genotype × year variance, $\sigma^2_{G\times T\times Y}$ is the genotype × treatment × year variance, $\sigma^2_e$ is the residual variance, $r$ is the number of replications per treatment, $t$ is the number of sites, and $y$ is the number of years.

The adjusted means of the field trials were calculated using ACBD-R. The adjusted means of the tested genotypes were extracted from the full field experiment and then used for a simple two-way ANOVA to test the main effects of root type (class), environment, and class × environment interaction using car (Fox and Weisberg, 2011) and FSA (Ogle, 2016) packages. To assess the differences between the average performances of the different root classes, a Tukey test was performed for all pairwise comparisons.

Pearson correlation coefficients were estimated for single plant studies for all traits that displayed a significant genotype effect, rather than using the BLUEs across treatments or the single treatment mean, when the treatment effect was significant. The critical value of the correlation significance was determined at 0.505 for $p < 0.01$ and 0.617 for $p < 0.001$ (df = 23) using the corrplot package (Wei and Simko, 2017).

### Multitrait Analyses to Define Root Architecture Behavior

The first multitrait root classification method was performed using multivariate statistical analysis with principal component analysis (PCA) for RR per level. The packages MASS (Venables and Ripley, 2002) and ggplot2 (Wickham, 2009) were used for this scope. The graphical biplot was then divided into three root classes by splitting in two each angle of the main vectors. The second method for multitrait classification was generated by calculating the Fisher LSD for each relative root number for the three layers at a significance level of 0.05 using the agricolae package. Each class was then graphically represented with a different color for values nonsignificantly different than twice the LSD from the maximum value. Through graphical representation, four classes of genotypes could be determined. The third multitrait method compared the SRA against the mature RA, where averages for each axis on the biplot were used to separate the genotypes into four root classes.

### Combined Multitrait Analyses to Define Root Architecture Behavior

The results obtained from the three multitrait methods were used for hierarchical clustering of genotypes using the average method (ggdendro and ggplot2 packages) as was used for functional classification based on rooting types in the study of Bodner et al. (2013). “Main” root classes were determined by separating hierarchical dendrogram once at 90% of the total variation, and then at 50% of the total variation to determine “extreme” classes. Two extreme classes were identified, and three genotypes from each were selected: Jabal2, 79509, and Bellaroi representing the shallowest, and Icamator, Margherita2, and Omrabi17 representing the deepest rooted. The yield performance of these extreme classes was graphically represented using box-and-whisker plots (ggplot2 package).

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**Table 2. Site characteristics and irrigation practices at five experimental stations used to determine field performances of different root types.**

<table>
<thead>
<tr>
<th>Code</th>
<th>Site</th>
<th>Country</th>
<th>Year</th>
<th>Climate</th>
<th>Irrigation method</th>
<th>Moisture†</th>
<th>Coordinates</th>
<th>Soil type</th>
</tr>
</thead>
<tbody>
<tr>
<td>MKZ15</td>
<td>Melk Zhar</td>
<td>Morocco</td>
<td>2014–2015</td>
<td>Mediterranean, hot and temperate</td>
<td>Drip irrigation</td>
<td>297 + 324</td>
<td>30°2′33″N, 9°33′4″W</td>
<td>Sandy limestone</td>
</tr>
<tr>
<td>TER16</td>
<td>Terbol</td>
<td>Lebanon</td>
<td>2015–2016</td>
<td>Mediterranean, temperate</td>
<td>Sprinkler</td>
<td>356 + 80</td>
<td>33°48′29″N, 35°59′22″W</td>
<td>Chromic vertisols</td>
</tr>
<tr>
<td>TES16</td>
<td>Tessaut</td>
<td>Morocco</td>
<td>2015–2016</td>
<td>Hot steppe</td>
<td>Gravity irrigation</td>
<td>132 + 360</td>
<td>29°49′48″N, 8°34′48″W</td>
<td>Calcic vertisols</td>
</tr>
<tr>
<td>MCH16</td>
<td>Marchouch</td>
<td>Morocco</td>
<td>2015–2016</td>
<td>Mediterranean, warm temperate</td>
<td>–</td>
<td>183</td>
<td>33°34′31″N, 6°38′01″W</td>
<td>Clay vertisol</td>
</tr>
<tr>
<td>KFD16</td>
<td>Kfaridan</td>
<td>Lebanon</td>
<td>2015–2016</td>
<td>Mediterranean, temperate</td>
<td>–</td>
<td>236</td>
<td>33°32′54″N, 35°51′18″W</td>
<td>Sandy clay</td>
</tr>
</tbody>
</table>

† Determined by rainfall + irrigation.
RESULTS

Mixed-Model ANOVA for Individual Traits

An ANOVA for seminal root traits measured using the clear pot method revealed highly significant differences among the tested genotypes (Table 3). Average SRA was 48.6°, and it ranged from 8.5 (Jupare C2003) to 115.7° (Miki3). Space-planted field testing for adult plant root behavior revealed significant (p < 0.001) genotype differences for all root and shoot traits (Table 3). The treatment effect was significant for RB at p < 0.01 and for RA at p < 0.001. There was no genotype × treatment interaction for RR per level, but all other root traits did show this interaction. The genotypes presented a large diversity in terms of rooting pattern. The RR ranged from 0.05 to 0.51 in the upper section, followed by 0.13 to 0.54 in the middle section and from 0.18 to 0.77 in the deepest section. The broad-sense heritability was relatively high for all the morphological and architectural root traits, and the highest values were for RB (broad-sense heritability = 0.90) and RR 2 to 8 cm, RR 8 to 10 cm, and RR 10 to 13 cm (0.82, 0.52, and 0.87, respectively). The SRA and adult plant RA also showed high heritability at 0.64 and 0.82, respectively (Table 3).

Correlations among All Traits

The matrix in Fig. 2 shows only the significant (p < 0.001) positive and negative correlations among traits. Traits for which the water treatment had no significant effect were presented as combined values, whereas when the treatment was significant, a letter D for simulated drought or a letter W for well-watered were added to separate the two values. The adult RA under water deficit was strongly associated (r = 0.68) with RA under well-watered conditions, and both were positively correlated with RR 2 to 8 cm and RR 8 to 10 cm and negatively associated with RR 10 to 13 cm, whereas RR 2 to 8 cm and RR 10 to 13 cm were in repulsion under both water scarce (r = −0.85) and well-watered (r = −0.81) treatments. Total root number exhibited a significant correlation with RB and RA in both treatments. Adult plant RA was not directly correlated to SRA. Strong correlations also existed between above- and belowground traits. Root angle in the well-watered treatment was positively associated with GW and its components TN, SN, and SB under the same treatment. The opposite trend was observed for well-watered RR 10 to 13 cm that was negatively correlated to GW (well-watered), TN, SN, SB, and PLH as well. Deficit RR 10 to 13 cm was negatively associated with only TN. Total root number and RB were positively correlated to almost all shoot traits, with the exception of chlorophyll content, TKW, and harvest index, which showed a negative correlation. The estimated stay-green was negatively correlated to SB, SPN, PLH, and TRN and positively correlated to chlorophyll content and SRA.

To determine if the above- and belowground traits measured under space-planted conditions had a significant

<table>
<thead>
<tr>
<th>Trait</th>
<th>Abbreviation</th>
<th>Mean</th>
<th>LSD</th>
<th>Min.</th>
<th>Max.</th>
<th>CV†</th>
<th>H²‡</th>
<th>G</th>
<th>T</th>
<th>Y</th>
<th>G × T</th>
<th>T × Y</th>
<th>G × Y</th>
<th>G × T × Y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Root ratio at 2–8 cm</td>
<td>RR 2–8 cm</td>
<td>0.20</td>
<td>0.07</td>
<td>0.05</td>
<td>0.51</td>
<td>23</td>
<td>0.82</td>
<td>***</td>
<td>ns§</td>
<td>*</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
<td>**</td>
</tr>
<tr>
<td>Root ratio at 8–10 cm</td>
<td>RR 8–10 cm</td>
<td>0.34</td>
<td>0.08</td>
<td>0.13</td>
<td>0.54</td>
<td>15</td>
<td>0.52</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
</tr>
<tr>
<td>Root ratio at 10–13 cm</td>
<td>RR 10–13 cm</td>
<td>0.46</td>
<td>0.08</td>
<td>0.18</td>
<td>0.77</td>
<td>12</td>
<td>0.87</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>ns</td>
<td>*</td>
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<td>*</td>
</tr>
<tr>
<td>Total root no.</td>
<td>TRN</td>
<td>40</td>
<td>12</td>
<td>15</td>
<td>83</td>
<td>20</td>
<td>0.56</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Root biomass (g)</td>
<td>RB</td>
<td>3</td>
<td>0.8</td>
<td>0.6</td>
<td>14</td>
<td>27</td>
<td>0.90</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>ns</td>
<td>***</td>
</tr>
<tr>
<td>Root angle</td>
<td>RA</td>
<td>62</td>
<td>8</td>
<td>37</td>
<td>106</td>
<td>10</td>
<td>0.82</td>
<td>***</td>
<td>***</td>
<td>*</td>
<td>***</td>
<td>***</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Days to maturity (d)</td>
<td>DTM</td>
<td>142</td>
<td>10</td>
<td>117</td>
<td>158</td>
<td>4</td>
<td>0.66</td>
<td>***</td>
<td>ns</td>
<td>–</td>
<td>**</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Flag leaf chlorophyll content (SPAD)</td>
<td>CC</td>
<td>55</td>
<td>7</td>
<td>33</td>
<td>66</td>
<td>7</td>
<td>0.10</td>
<td>***</td>
<td>ns</td>
<td>–</td>
<td>***</td>
<td>–</td>
<td>–</td>
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</tr>
<tr>
<td>Stay-green</td>
<td>SG</td>
<td>52</td>
<td>23</td>
<td>10</td>
<td>67</td>
<td>15</td>
<td>0.61</td>
<td>*</td>
<td>ns</td>
<td>–</td>
<td>ns</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Days to heading (d)</td>
<td>DTH</td>
<td>97</td>
<td>4</td>
<td>76</td>
<td>125</td>
<td>4</td>
<td>0.97</td>
<td>***</td>
<td>ns</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
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</tr>
<tr>
<td>Plant height (cm)</td>
<td>PLH</td>
<td>70</td>
<td>4</td>
<td>35</td>
<td>175</td>
<td>6</td>
<td>0.93</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>***</td>
<td>***</td>
</tr>
<tr>
<td>Tiller no. per plant</td>
<td>TN</td>
<td>7</td>
<td>1</td>
<td>2</td>
<td>16</td>
<td>21</td>
<td>0.65</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>ns</td>
<td>ns</td>
<td>**</td>
<td>*</td>
</tr>
<tr>
<td>Spikes no. per plant</td>
<td>SN</td>
<td>6</td>
<td>1</td>
<td>2</td>
<td>14</td>
<td>20</td>
<td>0.63</td>
<td>***</td>
<td>**</td>
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<td>ns</td>
<td>ns</td>
<td>**</td>
<td>ns</td>
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<tr>
<td>Avg. spikelet no. per plant</td>
<td>SPN</td>
<td>17</td>
<td>2</td>
<td>8</td>
<td>29</td>
<td>12</td>
<td>0.83</td>
<td>***</td>
<td>*</td>
<td>*</td>
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<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Shoot biomass (g)</td>
<td>SB</td>
<td>36</td>
<td>12</td>
<td>4</td>
<td>179</td>
<td>33</td>
<td>0.89</td>
<td>***</td>
<td>*</td>
<td>*</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>*</td>
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<tr>
<td>Grain weight (g)</td>
<td>GW</td>
<td>14</td>
<td>4</td>
<td>2</td>
<td>41</td>
<td>27</td>
<td>0.60</td>
<td>***</td>
<td>**</td>
<td>***</td>
<td>*</td>
<td>ns</td>
<td>**</td>
<td>**</td>
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<tr>
<td>Harvest index</td>
<td>HI</td>
<td>0.4</td>
<td>0.1</td>
<td>0.1</td>
<td>0.9</td>
<td>22</td>
<td>0.72</td>
<td>***</td>
<td>ns</td>
<td>*</td>
<td>ns</td>
<td>*</td>
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<tr>
<td>1000-kernel weight</td>
<td>TKW</td>
<td>41</td>
<td>7</td>
<td>19</td>
<td>67</td>
<td>17</td>
<td>0.46</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
<td>ns</td>
</tr>
<tr>
<td>Seminal root angle (°)</td>
<td>SRA</td>
<td>49</td>
<td>13</td>
<td>9</td>
<td>116</td>
<td>12</td>
<td>0.64</td>
<td>***</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<td>–</td>
</tr>
</tbody>
</table>

*,**,** Significant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† CV, coefficient of variation.
‡ H², broad-sense heritability.
§ ns, no significant effects.
effect in determining agronomic performances, five field trials were conducted in two drought-prone environments and under three types of irrigation (drip, flood, and supplemented via sprinklers). All below- and aboveground traits measured on single-plant studies were tested via correlation against the values measured in the field (Supplemental Table S1) and did not provide significant associations, with the following exceptions. Thousand-kernel weight in Kfardan was positively affected ($r = 0.55$) by deeper roots (RR 10–13 cm) and positively correlated to TKW measured in well-watered trial in the space-planted experiment. Grain yield in MKZ15 was negatively correlated to TRN and RB for belowground traits and negatively associated with TN, SN, SPN, and SB for aboveground traits. However, the correlation results were not consistent among dry or irrigated environments and thus were of limited use overall. Hence, these correlations and their meaning are not discussed further.

**Determination of Root Classes based on Multiple Traits and their Field Responses**

Single-trait characterization could not be used to identify meaningful classes of agronomically different genotypes when tested in the field. Therefore, other methods that combine multiple traits were tested. The first method assessed for multitrait prediction of classes used PCA among RR 2 to 8 cm, RR 8 to 10 cm, and RR 10 to 13 cm values (Fig. 3). The first two axes together explained 99% of the total variation. The angle of the trait vectors indicated that the two traits RR 2 to 8 cm and RR 10 to 13 cm were negatively correlated with one another and perpendicular to the vector of RR 8 to 10 cm. These associations among...
the three classifiers were consistent with the Pearson correlations (Fig. 2). The three root categories derived using this method indicated that the ICARDA Genebank (IG) landrace IG:79509, as well as the modern lines Bellaroi and Jabal2, were the closest to the RR 2 to 8 cm vector and hence constitute a group of genotypes that produce a larger portion of the roots in the upper soil layer. In contrast, genotypes including IG:85620, IG:85026, Amina, Bezaghras, Ouassara, Icadesful, and Icamoram7 formed another group that concentrate their roots in the medium layer of the soil. All the remaining genotypes were more associated with RR 10 to 13 cm developing a deep root system.

The second method of multitrait classification was done via LSD test (Fig. 4), and it revealed four major classes. The first class contained genotypes colonizing the superficial soils; the second class occupies the first and second layers; the third class is semideep, and its roots colonize the second and third layers, and the fourth class primarily develops roots in the third layer, exploring the deeper part of the soil.

A third multitrait methodology was based on the comparison of the SRA and adult RA. Since these two traits are not correlated, their combined study is of interest to determine root behavior (Fig. 5). Four classes could be determined, with the first and the third group showing wide and narrow angles in both seedling and adult plants, respectively. The second group comprised genotypes that start their growth with a narrow SRA but then expand the exploration of superficial soils as the plant ages, whereas the fourth group comprised genotypes with the RA changing from wide at the seedling stage to narrow at maturity. The two methods identified statistically significant differences (Table 4) for grain size among the dry environments, with PCA and LSD root classes explaining 34.3 and 25.9% of the TKW variation, respectively. In case of PCA, the medium and deep classes reached 36, 37, 29, and 30 g in Kfardan and Marchouch, respectively, which was 20, 23, 16, and 20% above the TKW recorded for the shallow class in the same environments (Supplemental Table S2). In case of LSD, the deep class reached 38 and 31 g in Kfardan and Marchouch, respectively, which was 35, and 29% above the TKW recorded for the shallow class in the same environment (Supplemental Table S2). Instead, the third classification method based on SRA vs. RA identified significant differences (Table 4) between classes of TKW combining the three irrigated environments and explained 16.4% of the variation. Considering individual environments, this difference was evident only in Melk Zehr under drip irrigation, where the shallow-rooted (shallow to shallow) plants reached 61 g of TKW, 24, 13, and 9% higher than the deep to shallow, deep to deep, and shallow to deep classes, respectively.
The three multitrait methodologies were combined to derive a single score of root behavior via hierarchical clustering (Fig. 6). Two and five classes were defined to explain 90 and 60% of the variation, respectively. The two main classes incorporated 11 genotypes with preference for exploring the superficial soil layers, and 13 genotypes with deeper rooting patterns. This classification method identified significant differences between classes ($p < 0.05$) for GY when the genotypes were field tested in environments exposed to terminal droughts, but not for TKW or irrigated environments. The highest variance of 90.9% was explained by the class effect, followed by 1.9% for environment and 7.1% for class $\times$ environment interaction effect (Table 4). Genotypes belonging to the deep-rooting class had a mean of 2883 and 2475 kg ha$^{-1}$ for KFD16 and MCH16, respectively, which was 42 and 28% higher than

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**Fig. 4.** Exploration of three different soil levels by the roots of the 25 genotypes tested, expressed as root ratio of the total. The values are best linear unbiased estimates (BLUEs) calculated over two replications, two treatments, and 2 yr. Gray bars indicate genotypes with values within two LSD levels from the maximum of that soil level. Vertical dashed red lines guide the visual distinction into four root categories.

**Fig. 5.** Variation in genetic control of root angle at seedling and mature stages. The dashed line denotes the average value of root angle on each axis.

**Determination of Root Classes by Hierarchical Combination of Multiple Traits**

The three multitrait methodologies were combined to derive a single score of root behavior via hierarchical clustering (Fig. 6). Two and five classes were defined to explain 90 and 60% of the variation, respectively. The two main classes incorporated 11 genotypes with preference for exploring the superficial soil layers, and 13 genotypes with deeper rooting patterns. This classification method identified significant differences between classes ($p < 0.05$) for GY when the genotypes were field tested in environments exposed to terminal droughts, but not for TKW or irrigated environments. The highest variance of 90.9% was explained by the class effect, followed by 1.9% for environment and 7.1% for class $\times$ environment interaction effect (Table 4). Genotypes belonging to the deep-rooting class had a mean of 2883 and 2475 kg ha$^{-1}$ for KFD16 and MCH16, respectively, which was 42 and 28% higher than
the mean of the shallow-rooted class in the same environments (Table 5). These two mega-classes were further divided into two subclasses each to determine differences between extreme types (Fig. 6). The shallowest group included Jabal2, 79509, and Bellaroi, whereas the deepest included Miki3, Icarasha2, Bezater, Icamator, 86075, Margherita2, Secondroue, Omrabi17, Kofa, and Creso. To reduce bias, three genotypes (Icamator, Margherita2, and Omrabi17) were selected as the most representative of the deepest root class and were compared with the same number of entries of the shallowest class. Figure 7 shows the GY performances of these two groups over different environments (rainfed and irrigated), with the deep-rooting types achieving a yield advantage of +1194 and +1225 kg ha$^{-1}$ in MCH16 and KFD16, respectively. However, the shallow-rooting types were superior (+2000 kg ha$^{-1}$) in

Table 4. Statistics of different root types (classes) for grain yield and thousand-kernel weight (TKW) in dry and favorable environments using different classification methods.

| Classification method† | Source of variation | Rainfed environments | | Irrigated environments | | |
|------------------------|----------------------|----------------------|----------------------|----------------------|----------------------|
|                        |                      | Grain yield          | TKW                  | Grain yield          | TKW                  |
|                        |                      | Variation p value    | Variation p value    | Variation p value    | Variation p value    |
| Multitrait PCA classification | Class               | %                    | %                    | %                    | %                    |
|                        | Env†                 | 62.3 0.58            | 25.9 0.01*           | 7.3 0.62             | 3.2 0.38             |
|                        | Class × Env         | 28.0 0.78            | 1.3 0.78             | 7.9 0.90             | 3.5 0.70             |
| LSD classification     | Class                | 4.9 0.99             | 34.3 0.00**          | 7.9 0.73             | 5.9 0.30             |
|                        | Env                  | 9.5 0.69             | 64.3 0.00***         | 67.0 0.00**          | 90.2 0.00***         |
| Biplot of SRA vs. RA   | Class                | 20.3 0.96            | 6.8 0.66             | 18.1 0.41            | 16.7 0.00**          |
|                        | Env                  | 10.7 0.69            | 92.8 0.00***         | 68.8 0.00**          | 73.9 0.00***         |
| Combined multitrait classification | Class              | 69.1 0.79            | 0.4 0.99             | 13.2 0.91            | 4.0 0.76             |
|                        | Env                  | 7.0 0.59             | 81.6 0.00**          | 51.7 0.04*           | 94.3 0.00***         |
|                        | Class × Env         | 7.1 0.32             | 3.2 0.45             | 46.6 0.05            | 5.2 0.45             |

*,**,** Signiﬁcant at the 0.05, 0.01, and 0.001 probability levels, respectively.
† PCA, principal component analysis; SRA, seminal root angle; RA, root angle.
‡ Env, environment.
MKZ16 under drip irrigation in sandy soils and in Tessaout (+1095 kg ha$^{-1}$) under gravity irrigation.

**DISCUSSION**

Plant behavior belowground is deemed of fundamental importance for water uptake and nutrient acquisition, particularly in water- and resource-limited environments (de Dorlodot et al., 2007; Lynch and Wojciechowski, 2015). Here, an attempt was made to adapt low-cost and scalable methodologies to reliably characterize root behavior in durum wheat. The clear pot method has already been used to study other crops and was most recently adapted to durum wheat (Alahmad et al., 2018). Its scalability and reliability have been discussed in depth elsewhere (Richard et al., 2015). The pasta strainer method under upland-field conditions was first presented in rice (Uga et al., 2009; Uga, 2012), demonstrating a variation in root morphology among different rice genotypes. Some minor modifications were necessary to adapt it to the characterization of mature roots of durum wheat and assess its reliability.

**Interaction between Root Traits and Water Regimes**

Significant genotypic variation was observed for all assessed traits, which demonstrated the usefulness of this panel for root genetic research. The heritability for root fractions measured at different soil depths—RR 2 to 8 cm (0.82), RR 8 to 10 cm (0.52), and RR 10 to 13 cm (0.87)—was extremely high, and the water treatment imposed after flowering had no significant effect. These results support the hypothesis that root preference for shallow or deep soil layers is under strong genetic control, and hence that traits measured under space-planted controlled conditions could well represent the root behavior under true field conditions in different soil and moisture types. This hypothesis is further tested here.

Instead, in the case of RB, which was also revealed to be a highly heritable trait (broad-sense heritability = 0.9), the amount of moisture did have a significant effect on the root behavior. Thus, this trait is more affected by the environment and hence it would be harder to predict its behavior under true field conditions using data from space-planted experiments. However, strong correlation was found between RB and SB, suggesting that above- and belowground behavior have shared genetic controls (López-Castaneda and Richards, 1994). Further, this above- and belowground connection provides a simple proxy for breeders to select for high RB, without physically measuring the belowground component of the plant.

A major achievement of the Green Revolution was to maximize harvest index by converting biomass into grains (Manschadi et al., 2006, 2008; Hammer, 2006; Kirkegaard et al., 2007), so it does create a challenge for breeders, as a targeted increase in RB might ultimately result in a reduced harvest index and a detrimental loss in yield (Richards et al., 2007; Rebetzke et al., 2012). Perhaps selection for higher root number may be better suited for breeding. This trait is strongly correlated to the TN and also to GW per plant. Both these characteristics are sought after by breeders, hence targeting an increase in root number does not appear to be linked to any negative effect in terms of productivity. Therefore, aboveground selection for TN could represent an ideal proxy to also achieve rapid genetic gain for root number.
Field Variation for Grain Size based on Differences in Root Behavior

It has been observed in several crop species that large diversity exists for root characteristics through the use of different root phenotyping methods (Nakamoto et al., 1991; Manschadi et al., 2006, 2008; Chen et al., 2016). These authors came to the conclusions that the plasticity of root architecture increases adaptability, which in turn should improve productivity when the best fit is deployed for specific soils and moisture conditions. In cereals, the root growth angle is useful for predicting root distribution in the soil layers (Nakamoto et al., 1991; Oyanagi, 1994); therefore, this a convenient proxy to predict the mature root system architecture without digging to the lower soil layers.

The ability of roots to penetrate deep into the soil or to fully occupy the superficial layer is an adaptive mechanism to maximize the amount of moisture absorbed by the plant to be then converted into biomass and grains. As mentioned, extending water availability after anthesis is normally linked to a prolonged grain-filling period before drying out. In turn, this extra time and moisture should normally be used by the plant to better fill its grains (Kirkegaard et al., 2007; Vadez et al., 2013; Vadez, 2014). All traits connected to RA show very high heritability and were not affected by the simulated water scarcity after anthesis, which as indicated above, supports the case for strong genetic control. However, it cannot be concluded that other water treatments not tested here could have a more significant effect on rooting behavior, especially if water scarcity was to occur before flowering. Still, it was already demonstrated that the presence of the gene DEEPER ROOTING 1 (DRO1) in rice cultivars affords the crop yield stability under drought, and it does not cause any penalty under irrigated conditions (Uga et al., 2011, 2013). This finding is also in agreement with Voss-Fels et al. (2017), who found that VERNALIZATION 1, a key gene influencing flowering time and aboveground development in wheat, also has an important pleiotropic role on root system architecture and alone controls 8% of RA total variation.

In the experiment described here, RA traits were correlated with each other but did not show any strong and direct dependence on any aboveground trait. Hence, no simple proxy could be identified to replace the hard job of physically measuring the belowground feature of the plant. However, none of the single-trait classifications measured in space-planted trials could be correlated to the actual field performances. Therefore, to determine the field-level response of root behavior in terms of preferred depth of colonization, multitrait and combined multitrait methods were assessed and used to identify difference in agronomic response in the field. Two of the multitrait methods (LSD and PCA classifications) were capable of capturing part of the variation for grain size (TKW) in field yield trials with an increase of 16 to 35% for the deep-rooting classes under rainfed conditions, but they could not predict any change in GY (Supplemental Table S2). Among the multitrait analyses, the PCA method relies on the simplest trait to measure, as it does not need imaging software or even removing the roots from inside the basket. The second method used an LSD determination of differences using the same trait as Method 1. Method 3 was the most time consuming, as it required conducting a separate seminal root experiment by clear pots, as well as imaging each root crown to determine the angle at maturity. Interestingly, the clear pot and pasta strainer methods reached good agreement of RA for 11 of the tested genotypes, whereas the results were different for the remaining entries. The observed differences between the two stages could be explained by trait adjustment during the life cycle of the plant. A previous study in rice showed that RA changed from early to mature stages to follow moisture in water-scarce conditions (Uga et al., 2013). Hence, it is of interest to conduct analysis using both methods. In fact, there could be a specific interest in these more plastic genotypes that change root behavior over their lifespan, but more detailed studies are required to better understand these types. Still, the SRA vs. mature RA method could only determine significant differences for TKW under drip-irrigated trials, with the shallowest types (shallow to shallow) outperforming the other classes by 9 to 24%.

Considering that two of the three multitrait methods could distinguish differences in TKW under water-scarce field trials, it could be advisable to use Methods 1 or 2, as these are less time consuming and do not demand imaging software for processing. Instead, Method 3 becomes advantageous only when aiming for adaptation to drip irrigation.

Deeper Rooting Types Have Higher Yields under Terminal Droughts

The three multitrait methodologies were combined via hierarchical clustering to identify two mega-classes, one of shallower rooting genotypes, and one of deeper rooting types. This type of grouping reduces the amount of detailed classification of the single methodologies but allows combining of all measured traits. The two root mega-classes showed significant increases for GY under drought conditions, where water access is critical for wheat production. The genotypes allocating more roots at depth had on average 28 to 42% higher yield, probably due to a better capacity to capture deep soil moisture during grain filling (Lynch and Wojciechowski, 2015; Yu et al., 2015).

Since breeders are normally more interested by extreme types, a second distinction was made to identify five subclasses, two of which represent the shallowest and deepest rooting genotypes, and the remaining three the intermediate ones. Within the deepest rooting group are...
included the genotypes Margherita 2 and Omrabi 17, two elites that have been identified by several countries as most suited for environments prone to terminal drought, a type of condition most suitable for deep-rooting genotypes. Instead, the shallowest group includes Bellaroi and Jabal 2. The first is an Australian cultivar that performs particularly well in the southern region, where topsoil rarely exceed 60 cm in depth and the amount of rainfall received in short periodic intervals during crop growth period is higher than in northern and western regions. Also, Jabal2 finds its most appreciation in the Atlas Mountains of Morocco, where the impenetrable rock layers are located just 50 cm below the surface. In both cases, these appear as ideal conditions for shallow-rooted genotypes. Together, these considerations provide good support for the correct distinction into classes. Considering only the lines displaying extremely narrow and wide root phenotypes, the deepest types had a 37 to 38% yield increase under low moisture. However, under drip irrigation in sandy soils (MKZ16) and gravity irrigation in heavier calcic xerosols (TES16), the deepest rooted types had a significant yield disadvantage of 20 to 40%. Hence, there might be a cost associated with seeking deeper soil layers when moisture is abundant. Therefore, breeders could make important yield gains by selecting for the root architecture that better fits the specific environment. For instance, Uga et al. (2013) showed yield gains in rice of 44 and 63% when genotypes carrying the narrow angle allele at the DRO1 quantitative trait locus (QTL) were field tested under moderate and severe drought, respectively. Also, in wheat, many QTLs have been detected for RA that collocated with QTLs for GY and TKW in multienvironment studies (Canè et al., 2014; Maccaferri et al., 2016). Similarly, Richard et al. (2018) showed that it was possible to shift the allele frequency for RA in wheat, with good benefit for the lines combining favorable alleles for each root ideotype.

CONCLUSION
In this study, GY advantages of up to 40% could be obtained in both moisture-rich and -poor environment by selecting for the ideal root pattern. Further, grain size could be increased by up to 34% under moisture stress by selecting for deep-rooting types. Hence, it can be said that each root architecture is suitable to particular environmental scenarios and selecting and breeding for root system architecture is a cost-effective strategy to increase crop productivity and adaptation (Siddique et al., 2001).

The combination of methods presented here was confirmed as a suitable practice to identify these differences in durum wheat genotypes and can be used for breeding selection. The pasta strainer method is low cost, as it requires only the purchase of extremely affordable punctured plastic containers. In addition, the maintenance of the experiment occurs directly in the field, which reduces the investment in greenhouse maintenance. The most time-consuming aspect is certainly the removal of the plastic containers from the ground to study the fully developed mature root system of durum wheat. However, even this step is relatively simple when only the extruding roots are measured (PCA and LSD method), without the need for angle imaging. In addition, the RA showed strong heritability and therefore appeared as a trait of choice for rapid genetic gain through breeding. Together, the affordability, scalability, strong genetic control, and high effect on GY make the suggested methodology a protocol of choice for further use in breeding new cultivars that are well adapted to different moisture conditions.

Conflict of Interest
The authors declare that there is no conflict of interest.

Supplemental Material Available
Supplemental material for this article is available online.

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