Durum Wheat Seminal Root Traits within Modern and Landrace Germplasm in Algeria

Ridha Boudiar [1,2,*], Juan M. González [3] , Abdelhamid Mekhlouf [1], Ana M. Casas [4] and Ernesto Igartua [4,*]

[1] Laboratoire d'Amélioration et de Développement de la Production Végétale et Animale (LADPVA), University of Ferhat ABBAS (UFAS-Sétif1), Setif 19000, Algeria; mekhloufa@yahoo.fr
[2] Biotechnology Research Center (CRBt), UV 03 BP E73, Nouvelle Ville Ali Mendjli, Constantine 25016, Algeria
[3] Department of Biomedicine and Biotechnology, University of Alcalá, 28805 Alcalá de Henares, Spain; juanm.gonzalez@uah.es
[4] Estación Experimental de Aula Dei, EEAD-CSIC, Avenida Montañana 1005, 50059 Zaragoza, Spain; acasas@eead.csic.es
* Correspondence: boudiarreda@yahoo.fr (R.B.); igartua@eead.csic.es (E.I.); Tel.: +213-(31)-775037/39 (R.B.); +34-976716092 (E.I.)

Abstract: Seminal roots are known to play an important role in crop performance, particularly under drought conditions. A set of 37 durum wheat cultivars and local landraces was screened for variation in architecture and size of seminal roots using a laboratory setting, with a filter paper method combined with image processing by SmartRoot software. Significant genetic variability was detected for all root and shoot traits assessed. Four rooting patterns were identified, with landraces showing overall steeper angle and higher root length, in comparison with cultivars, which presented a wider root angle and shorter root length. Some traits revealed trends dependent on the genotypes' year of release, like increased seminal root angle and reduced root size (length, surface, and volume) over time. We confirm the presence of a remarkable diversity of root traits in durum wheat whose relationship with adult root features and agronomic performance should be explored.

Keywords: proxy traits; genetic resources; root screening; root architecture

1. Introduction

The root system of wheat includes two main types, seminal (embryonic) and nodal roots, also known as the crown or adventitious roots [1,2]. Both types of roots play a crucial role in plant growth and are active throughout the whole plant life. Seminal roots, however, could be more important under specific circumstances, like drought conditions, as they penetrate deeper into the soil layers than nodal roots, making water in deep layers accessible to the plant [3–5]. Seminal roots also play a capital role during crop establishment, as they are the only roots existing before the emergence of the fourth leaf. Seminal roots include one primary root, two pairs of symmetric roots at each side, and, at times, a sixth central root [6].

The main features of root systems are encompassed under two categories, root system architecture (RSA) and morphology. RSA is related to the whole, or a large subset, of the root system, and may be described as topological or geometric measures of the root shape. Root morphology, as defined by J. Lynch, refers to "the surface features of a single root axis as an organ, including characteristics of the epidermis such as root hairs, root diameter, the root cap, the pattern of appearance of daughter roots, undulations of the root axis, and cortical senescence" [7]. The traits often used to describe

wheat roots are total root length, root surface area, root volume, root angle, number of roots, and root diameter [8–10].

Roots are difficult to measure readily in natural conditions. Root trait determination has become accessible through the development of phenotyping methods in artificial systems, for instance, gel chambers [11], rolled germination paper [12], clear pots and growth pouches [13], "Termita" chamber and Whatman paper system [14], or growth pouches system [15]. Seminal roots can be phenotyped early and easily compared to the root system of mature plants [16,17], and for this reason, they have been proposed as good candidates to act as proxy traits in wheat [18] and maize [19,20]. Nevertheless, phenotyping these traits could be of interest only if they are useful to predict root growth and functioning in adult plants [21,22]. Indeed, several studies have found useful associations with traits in adult plants of wheat species [23–26]. For instance, the seminal root angle was correlated with nodal root angle [5,27], and with grain yield under drought conditions [28]. The seminal root number was correlated with thousand kernel weight (TKW) under stress, while the primary root length at the seedling stage was correlated with TKW under wetter conditions [25]. A steeper angle between the outermost roots and a higher root number in wheat seedlings have been linked to a more compact root system with more roots at depth in wheat [11,24,29].

Genotypic variation in root architecture has been reported within genotypes of different crop species [30–32], including wheat [13,21,25,33]. The presence of variation for the trait of interest is an essential requirement to improve the adaptability of crops under changing environmental conditions [34].

Local landraces are considered well adapted to the region where they were grown and contain large genetic diversity useful to improve crops like durum wheat [35]. These landraces were replaced by high yielding but more uniform semi-dwarf cultivars, better adapted to modern agriculture. However, scientists are convinced that local landraces still constitute a genetic resource useful to improve commercially valuable traits [36]. It is assumed that root traits enhance response to drought stress [37], but the realization of their contribution to superior grain yield depends on the type of drought and the agro-ecological conditions [38]. A deep rooting ideotype ("steep, cheap, and deep") was proposed by [39] to optimize water and N acquisition, building on the assumption that deeper rooting genotypes will use water that is beyond reach for shallower rooting genotypes. Modern breeding has caused some shifts in the root system architecture of durum wheat, from shallower and densely rooted systems in landraces of Mediterranean origin to deeper and more evenly distributed systems throughout the soil depth in cultivars worldwide [5].

The current study aims at evaluating the diversity of seminal root traits, including root angle and depth, during early growth of a set of durum wheat genotypes, consisting of modern cultivars and local landraces which are representative of the germplasm adapted to the mostly semi-arid conditions of Algerian cereal-growing regions before and after the advent of modern breeding. The study aims to reveal morphological diversity that could have agronomic relevance and, therefore, interest breeders.

2. Materials and Methods

2.1. Plant Material

We studied thirty-seven genotypes (landraces and modern cultivars), representative of durum wheat (*Triticum turgidum* ssp. *durum* Desf.) grown in Algeria. Geographical origins were varied (Algeria, France, Italy, Spain, Tunisia), and included genotypes produced at international breeding programs addressing semi-arid areas, namely the International Maize and Wheat Improvement Center (CYMMIT), the International Center for Agricultural Research in the Dry Area (ICARDA) and the Arab Center for the Studies of Arid zones and Drylands (ACSAD). These genotypes are representative of different periods of agriculture in Algeria, before and after the Green Revolution (Table 1).

Table 1. Name, type of cultivar, origin, and year of release of 37 genotypes of durum wheat used in the experiment.

N°	Genotype	Type/Pedigree	Origin	Year of Release
1	Beliouni	Landrace	Algeria	1958
2	Bidi 17	Landrace	Algeria	1930
3	Djenah Khotifa	Landrace	North Africa	1955
4	Gloire de Montgolfier	Landrace	Algeria	1960
5	Guemgoum R' khem	Landrace	Algeria	1960
6	Hedba 3	Landrace	Algeria	1921
7	Langlois	Landrace	Algeria	1930
8	Mohammed Ben Bachir (MBB)	Landrace	Algeria	1930
9	Montpellier	Landrace	Algeria	1965
10	Oued Zenati 368	Landrace	Algeria	1936
11	Acsad 65	Gerardo-vz-469/3/Jori-1// Nd-61-130/Leeds	ACSAD	1984
12	Altar 84	Ruff/Flamingo,mex// Mexicali-75/3/Shearwater	CYMMIT	1984
13	Ammar 6	Lgt3/4/Bicre/3/Ch1// Gaviota/Starke	ICARDA	2010
14	Bousselem	Heider//Martes/ Huevos de oro	ICARDA	2007
15	Boutaleb	Hedba 3/Ofanto	Algeria	2013
16	Capeiti	Eiti*6/Senatore-Cappelli	Italy	1940
17	Chen's	Shearwater(sib)/(sib)Yavaros-79	CYMMIT	1983
18	Ciccio	Appulo/Valnova(f6)// (f5)Valforte/Patrizio	Italy	1996
19	Cirta	Hedba-3/Gerardo-vz-619	Algeria	2000
20	Core	Platani/Gianni	Italy	2008
21	GTA Dur	Crane/4/Polonicum PI185309//T.glutin enano/2* Tc60/3/Gll	CIMMYT	1972
22	INRAT 69	Mahmoudi/(bd-2777)Kyperounda	Tunisia	1969
23	Korifla	Durum-dwarf-s-15/Crane//Geier	ICARDA	1987
24	Mansourah	Bread wheat/MBB	Algeria	2012
25	Massinissa	Ofanto/Bousselem	Algeria	2012
26	Megress	Ofanto/Waha//MBB	Algeria	2007
27	Mexicali 75	Gerardo-vz-469/3/ Jori(sib)//Nd-61-130/Leeds	CIMMYT	1975
28	Ofanto	Ademelio/Appulo	Italy	1990
29	Oued El Berd	Gta dur/Ofanto	Algeria	2013
30	Polonicum	Triticum polinicum/Zenati boulette 1953-58	France	1973
31	Sahell	Cit"s"/4/Tace/4*tc//2*zb/ wls/3/aa"s"/5/Ruff"s"/Albe"s"	CYMMIT	1977
32	Simeto	Capeiti-8/Valnova	Italy	1988
33	Sitifis	Bousselam/Ofanto	Algeria	2011
34	Vitron	Turkey77/3/Jori/Anhinga//Flamingo	Spain	1987
35	Waha	Plc/Ruff//Gta's/3/ Rolette	ICARDA	1986
36	Wahbi	Bidi 17/Waha//Bidi 17	Algeria	2002
37	ZB × Fg	Zb/fg"s" lk/3/ko 120/4/Ward cs 10604	Algeria	1983

*: Backcross.

2.2. Root Phenotyping

2.2.1. Preparation of Seeds

Twelve seeds of uniform size and healthy aspects were visually selected from each genotype and surface sterilized in a sodium hypochlorite solution (1.25% + one detergent drop, Mistol Henkel Iberica®). Seeds of each genotype were soaked and shaken in the solution for 15–20 min. Then,

they were rinsed four times with sterile deionized water, in sterile conditions. Twelve seeds of each genotype were placed in Petri dishes, each with two filter papers soaked with 4 mL of sterile water. Then the Petri dishes were placed in a dark room at 4 °C for four days, and then at 22 °C/18 °C in a growth chamber with a 12 h light/darkness photoperiod for about 16 h.

Finally, the pre-germinated grains were transferred to the rhizo-slide system, described in detail in the next section and Figure S1. The experiment was carried out at the Laboratory of Cellular Biology and Genetics, Department of Biomedicine and Biotechnology of the University of Alcalá, Spain.

2.2.2. The Rhizo-Slide System

The rhizo-slide system was constructed as a sandwich made with glass plate, black cardboard, filter paper, and a black plastic sheet. Sheets of A4-size black cardboard (180 g/m^2, www.liderpapel.com) and filter papers were previously sterilized in an autoclave and then soaked in the nutritive solution Aniol [40]. The nutritive solution was prepared by dissolving 0.5550 g of Ca Cl$_2$, 0.8215 g of KNO$_3$, 0.6352 g of MgCl$_2$·6H$_2$O, 0.0165 g of (NH$_4$)$_2$SO, 0.0400 g of NH$_4$NO$_3$ in 100 mL of distilled water, to which 500 µL/L of Plant Preservative Mixture (PPM^{TM}, Plant Cell Technology) at pH 5.8 was added. Each 8 mL was used to prepare 1 L of nutritive solution. Black cardboard with a nick made at the top center was placed on a glass plate with the same dimensions; then the pre-germinated grain (with embryonic part downward) was positioned just below the nick and covered by a filter paper. A black plastic sheet was used to cover the filter paper to ensure obscurity for roots, shifted ~2 cm upwards to allow better contact of the cardboard, and filter paper sheets with the nutritive solution. Two rhizo-slides were confronted to each other by the glass plate side, and the set was placed vertically in a glass box (internal dimensions of 32.2, 22, and 16 cm, length, width, and height) with two liters of the nutritive solution at the bottom, and then secured with two paper clips. Each glass box held 6 glass plates with two rhizoslides each, for a total of 12 seedlings, consisting of two genotypes, 6 seedlings for each (Figure S1). In total, each genotype was replicated 12 times. More details on the system are found in Ruiz et al. (2018) [25].

Once placed in the rhizoslides and the glass boxes, the seedlings were grown in a growth chamber for 7 days at 22/18 °C and 12/12 h photoperiod, day/night. The 37 genotypes were processed in batches of 6. Pre-germinated seeds of each 6 genotypes were placed into six glass boxes, each holding 6 seeds of two different genotypes. A complete batch comprised six boxes, three glass boxes prepared each Monday, and three each Thursday, every week. In total, 7 batches (14 runs) were performed until the experiment was completed (accounting for some seedlings that had to be replicated for various reasons). The set of genotypes for each run was selected randomly.

During the experiment, the boxes were replenished with distilled water every two days, to refill to the initial solution level. At the same time, to minimize seedling failure, each single seedling received 10 mL of the nutritive solution, applied with a pipette, near each seed. On the eighth day, the rhizo-slides were opened and shoots were immediately collected. The fresh roots were scanned using a Canon "LiDE210" scanner at 300 ppi to capture the first image then overlapped roots were manually separated and a second scan was done. The individual plant shoot dry weight (SDW) was obtained after oven-drying at 80 °C for six hours.

2.2.3. Image Analysis

The two images of a rhizo-slide were analyzed using SmartRoot software v.3.32 [41] plugin for ImageJ1.46R (http://imagej.nih.gov/ij/download.html). The first image was used to measure only root angles and the second one to assess the other root traits using manual and semi-automatic SmartRoot procedures. Each root of the seedling was traced, semi-automatically, and then SmartRoot automatically generated the corresponding traits. In total, ten variables from the Smartroot output were recorded for each seedling: total root length (TRL), primary root length (PRL), mean length of the other seminal roots (MRL), total root surface area (Surface), mean root diameter (Diameter), total root volume (Volume), root number (RN), and shoot dry weight (SDW). The root angle was determined

for each root with respect to the vertical (90°). From this determination, we extracted the maximum vertical angle (MVA) represented by the root growing with the steepest angle, the least vertical angle (LVA) represented by the root growing with the widest angle, and mean vertical angle (MRA) of all the roots, for each seedling.

2.3. Statistical Analysis

The experiment was considered a completely randomized design, with 12 replicates per genotype. Statistical analyses were performed using the REML (Restricted maximum likelihood) procedure with Genstat 18 [42]. Genotypes were considered as fixed factors and replications were considered as a random factor. The "Genotype" factor (n-1 degrees of freedom) was broken down into a single degree of freedom comparison of landraces vs. cultivars (named "Type" effect), and a "within type" factor (n-2) which corresponds to the variation of genotypes within each type. Multiple means separation was carried out using LSD at 0.05 level, for variables in which the F-value for "Genotypes" was significant. A principal component analysis (PCA) and a hierarchical cluster analysis (HC) were performed using the R package FactoMineR [43]. The hcut function was used for tree cutting levels truncation. The R package Factoextra [44] was employed for extracting and visualizing the results. Broad-sense heritability (h^2) was calculated on an entry mean basis using the REML procedure, as follows:

$$h^2 = \sigma^2_g/(\sigma^2_g + (\sigma^2_e/r)) \tag{1}$$

where σ^2_g is the genotypic variance, σ^2_e is the error variance and r is the number of replications.

3. Results

3.1. Genotypic Variability

We found remarkable genetic variability for all measured traits, as revealed by the highly significant differences among genotypes in the analyses of variance (Table 2). Significant differences were also found in the "type" comparison for most traits, except for Diameter and RN (Table 2). For the other traits, the mean squares for type were 4 to 12 times larger than those for genotypes.

The means of landraces showed higher or equal mean values compared to cultivars for all traits, except root angle (MRA, LVA, and MVA), which was higher in cultivars (Figure 1). It is worth mentioning that the landrace group presented higher root depth (PRL) than the cultivars.

All traits but SDW were root-related traits so, henceforward all the traits will be referred to generally as root traits unless stated otherwise. All traits (except RN) showed a near-normal distribution (Figure 1) which denotes their polygenic control. A wide range of phenotypic values was observed for most traits (Table 2). The landrace group showed a larger range of variation for TRL, Surface, Volume, and SDW than the cultivars. For the other traits, the cultivars had higher ranges of variation (Tables S1 and S2).

The coefficients of variation (CV) ranged from small values like 5.19 (Diameter) to 24.60 (MRA, Table 2). The exception was the large CV found for MVA, 59.82. When calculated separately for landraces and cultivars, slightly higher CV for most traits were found in landraces compared to cultivars (Table S2). All the traits exhibited high broad sense heritability (h^2), ranging from 0.80 for MVA to 0.98 for MRA (Table 2).

Table 2. Descriptive statistics, broad sense heritability (h^2), ANOVA summary, and correlation coefficients for the root traits assessed in 37 durum wheat cultivars.

Traits	Descriptive Statistics					ANOVA		Correlation									
	Min	Mean	Max	CV	h^2	Genotype	Type	TRL	Surface	Volume	Diameter	PRL	SDW	MRA	LVA	MVA	RN
TRL (cm)	54.28	98.49	137.22	13.51	0.90	***	***	1	***	***	ns	***	***	***	**	***	***
Surface (cm^2)	8.25	16.77	24.82	14.74	0.90	***	***	0.95	1	***	***	***	***	***	ns	***	***
Volume (cm^3)	0.0962	0.2326	0.3721	17.03	0.90	***	***	0.83	0.97	1	***	***	***	***	ns	**	***
Diameter (cm)	0.0447	0.0538	0.0620	5.19	0.87	***	ns	0.08	0.38	0.59	1	***	***	***	*	**	***
PRL (cm)	14.32	26.51	32.06	10.76	0.90	***	***	0.55	0.55	0.52	0.17	1	***	ns	**	***	ns
SDW (g)	5.70	14.72	23.50	18.66	0.94	***	***	0.55	0.63	0.64	0.40	0.58	1	ns	**	*	ns
MRA (°)	1.20	30.36	45.69	24.60	0.98	***	***	−0.39	−0.32	−0.23	0.20	−0.01	0.00	1	***	***	ns
LVA (°)	20.40	42.71	61.47	17.81	0.92	***	***	−0.28	−0.23	−0.18	0.12	0.08	0.01	0.76	1	***	ns
MVA (°)	0.00	14.10	43.49	59.82	0.80	***	***	−0.25	−0.20	−0.15	0.12	−0.27	−0.13	0.62	0.25	1	**
RN (no.)	4.00	5.32	6.00	9.13	0.87	***	ns	0.37	0.32	0.26	−0.22	−0.01	0.06	−0.09	0.02	−0.14	1

*, **, ***: sources of variation in the analyses of variance or correlation coefficients significant at $p < 0.05$, 0.01, and 0.001, respectively. TRL: Total root length, Surface: total root surface area, Volume: Total root volume, Diameter: mean root diameter, PRL: Primary root length, SDW: Shoot dry weight, MRA: Mean root angle, LVA: Least vertical angle, MVA: Maximum vertical angle, RN: Root number.
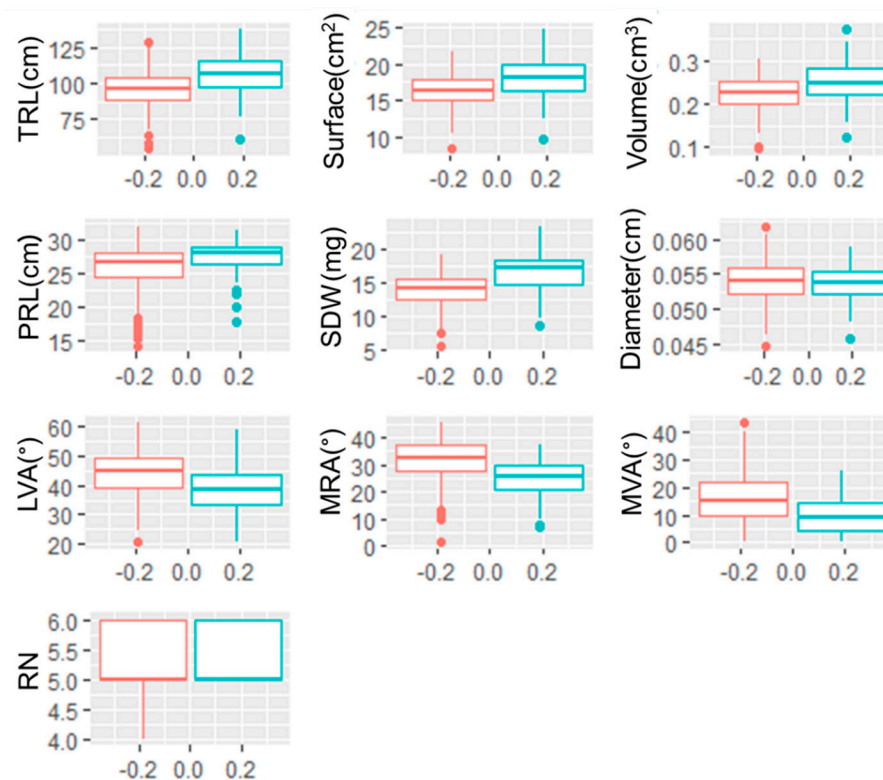
Figure 1. Boxplots for root traits for the cultivar (red) and landrace (blue) groups. Horizontal lines splitting the boxes indicate the median values; box limits indicate the 25th and 75th percentiles; whiskers extend 1.5 times the interquartile range from the 25th and 75th percentiles; outliers are represented by dots. Variable names coded as in Table 2. Genotype mean values are in Table S1.

3.2. Relationships between Traits

Highly significant correlations were found between most traits (Table 2). TRL, Surface and Volume were highly and positively correlated among them. There were moderate positive correlations between TRL, surface, and Volume, with PRL, SDW, RN, and negative ones with root angle variables (seedlings with higher TRL, Surface, and Volume tended to have steeper root angles). Seedlings with higher RN tended to have roots with thinner root diameter, indicating that there could be some kind of compensation between these traits (more roots with a finer diameter and vice versa). Interestingly, seedlings with higher primary root length produced more shoot biomass. Performing correlations between traits within each group (cultivars and landraces) showed, in general, similar patterns to the correlations performed for the entire dataset (Table S2). The moderate relationship of PRL with MVA and RN disappeared in the landrace group, compared to the cultivars and the whole dataset (Table S2).

3.3. Time Trends of Root Traits

When the genotypic means were plotted against year of release of the genotypes, different trends were observed (Figure 2 and Figure S2), in which, all the traits presented significant regression coefficients except Diameter, RN and PRL (Table S3). This trend was largely influenced by the comparison of landraces vs. cultivars because landraces are older. The trend was positive or negative depending on the trait. Overall, cultivars reduced their seminal root length and developed a shallower root angle compared to landraces (Figure 2). The root surface and volume of root presented the same trend as root length, as they were highly correlated, as mentioned above. MVA and LVA showed the same trend as MRA. No substantial variation was observed for RN, Diameter, and PRL. Regarding the shoot, a remarkable and steady reduction in SDW over the years was detected (Figure 1 and Figure S2).
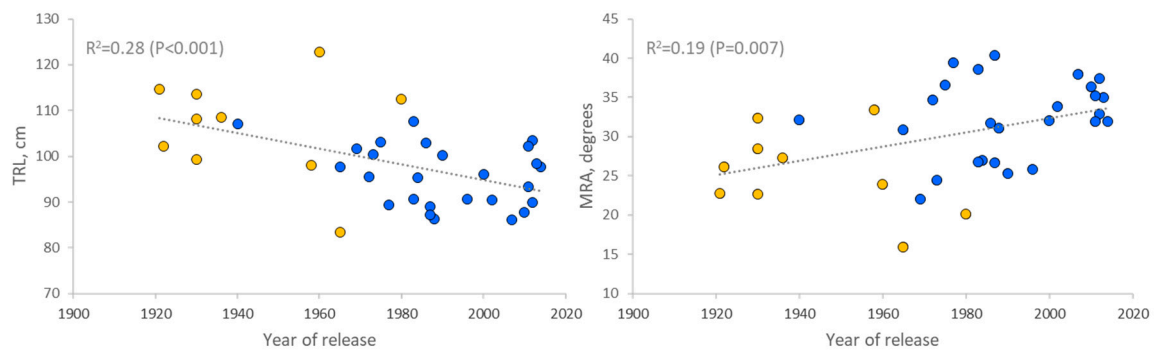
Figure 2. Time trends for total root length (TRL) and mean root angle (MRA) in seedlings of 37 durum wheat varieties. Yellow symbols correspond to landraces; blue symbols correspond to cultivars. The coefficients of determination (R^2) of the regression lines are indicated in each graph.

3.4. Grouping of Genotypes According to Root Traits

The first two principal components explained 69.63% of the total variation (Figure 3A). The first component (46%) was most related to Surface, TRL, Volume, and SDW, with the respective contributions of 20.19, 19.04, 18.16, and 11.51 (Table S4). MRA, LVA, and Diameter had the highest loadings for the second component (PC2). Correlations between these traits are discussed above (Table 2). Thus, the first axis (PC1) was related to root size traits and the second one to root architecture traits.
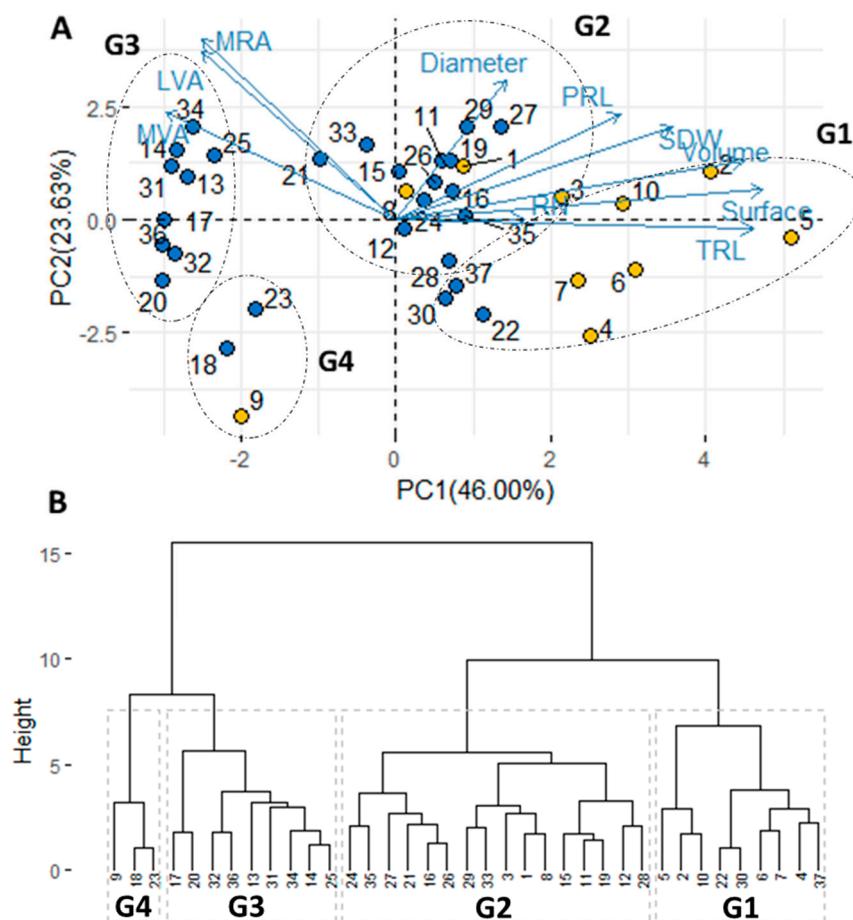
Figure 3. Biplot of the first two principal components (**A**) and dendrogram resulting from hierarchical clustering (**B**) based on seedling traits for 37 durum wheat genotypes. Ellipses in (**A**) encompass the individuals according to the clustering presented in (**B**). Yellow symbols correspond to landraces; blue symbols correspond to cultivars. Genotypes coded with numbers as in Table 1.

Genotypes were better distributed along with the first component, as a result of the contrasting position between landraces, many with large positive scores on PC1 (due to their higher root size and shoot weight) and the cultivars, with lower positive or negative scores in PC1, so the discrimination between these two groups was clear (Figure 3B). From the hierarchical classification, which was carried out based on the original data, four groups were created (G1 to G4) (Figure 3B). G1 was mostly formed by landraces. G2 was the largest one and was constituted by cultivars, and two landraces. This group was at a central position in the biplot graph (Figure 3B), presenting close to average values for most traits. G3 was located on the negative side of PC1, contrasting with G1 by having a relative smaller root size. Finally, the last group (G4) was formed by only three genotypes depicted on the negative quadrant, for both PC1 and PC2, having smaller values for both classes of root traits; fine, steeper root angle and reduced root traits related to biomass. This group included landrace Montpellier (genotype 9), which showed a special root system architecture compared to other landraces, with steeper root angle, and lower SDW, closer to two cultivars from Italy and ICARDA.

4. Discussion

The durum wheat collection used in this study was assembled to explore the seminal root variability present in a set of genotypes cultivated in Algeria, with a historical perspective on the possible changes caused by modern breeding. The method chosen enabled data acquisition and processing of 444 single plants, by one person, in two months. Its performance could be easily expanded by increasing the number of boxes and operators. Therefore, it is amenable to the scale needed for the type of studies carried out in plant genetics and breeding. Root number together with root length, the main results of this type of experiment, describe how extensively the seminal axes can potentially explore the rooting volume. These easily measurable traits at an early stage can have agronomic implications. For example, root spread angle is an additional feature whose variation can influence how crops cope with water-limited conditions and/or other environmental constraints, such as high pH, toxic ions, or low nutrient availability [45,46]. The root angular spread at an early growth stage can be used to predict the partitioning of root biomass in the soil profile at the adult plant stage [5,27,28], a feature relevant for water use efficiency in wheat [21,47]. Therefore, artificial systems are efficient at revealing phenotypic (and presumably genetic) variability, but its implications on agronomic performance must be validated later under field conditions.

4.1. Large Genotypic Variation for Seminal Root Traits

An overview of the results found in different studies sheds more light on the actual genetic variation available for seminal root traits, better than any single study. Differences among studies may be partly due to slight differences in the experimental methods, but also to the size and scope of the genetic material used. Nevertheless, some meaningful conclusions can be derived.

We found significant genetic variation for all traits. We found a range of values for the least vertical root angle (LVA) from 20.40° to 61.47°. Multiplying these values by two (range from 40.80° to 122.94°) allows the comparison of our study with others, in which the values of the total opening of the angle of the root system was reported. Our range was superior to those found by others in durum [47] and bread wheat [24,29]. Our wheat genotypes displayed similar low ranges of variation in mean root number as in similar studies in durum [47,48], with a slightly higher mean. In our genotypes, the sixth root was present in about a third of all genotypes, with no significant differences between landraces and cultivars. This is a similar proportion than found in a study of Mediterranean and North-American elite material [47], with the striking difference that in the former study they reported almost absence of the sixth seminal root in native Mediterranean materials [47]. Neither sample of landrace materials was large enough to derive definitive conclusions from these studies, but at least we can say that Algerian landraces are not more likely to lack the sixth seminal root than modern cultivars.

Based on the coefficients of variation, overall, landraces showed higher slightly variability for most traits, especially for root angle, even though the sample size was lower than for cultivars. Previous

reports indicate that native Mediterranean landraces are likely to provide additional genetic variability for root architecture [46], particularly in wheat accessions that experienced long-term natural selection in drought-prone environments [49], and in barley [50]. Overall, the Algerian landraces showed sizeable genetic variation for most traits, indicating that they harbor relevant root morphology variation that should be further investigated by geneticists and breeders.

4.2. Classification of Durum Wheat Genotypes According to Root Morphology

Overall, genotypes with higher root length tended to have larger root number, as found in a previous study [47], and a narrower root angle. Other authors [24,51] found no correlation between root angle and root number. Sanguinetti et al. [47] also found no correlation of root angle with other traits and suggested that the root angle was controlled by an independent set of genes. In our study, however, given the negative correlation between MRA and root size traits, we cannot rule out that these two traits are controlled by the same set of genes.

We found that higher root length and Diameter were associated with higher SDW (r of 0.55 and 0.40, respectively, Table 2), suggesting a size effect that affected the whole plant. Rather similar observations were done in the Spanish core collection of tetraploid wheat, but the plant size effect was visible for subsp. *dicoccon* and *turgidum*, but not for *durum* [25]. Correlation between root length and volume and SDW was also found in hexaploid wheat [52]. We found no correlation between RN and MRA, in agreement with previous studies [24,51]. It seems that an overall plant size effect that affects harmonically roots and shoots is common in wheat species.

Our genotypes displayed different seminal root system patterns, from vigorous and steep to a small and shallow root system. These root patterns may be related to phylogenetic relationships, regional origin, and functional plant adaptation to different environments, as indicated in previous studies [53]. There were differences in the length of the seminal roots of single plants. This was made evident by calculating the difference between the length of the primary root (PRL), and the average of the rest (MRL). G2 and G3 had a higher difference between PRL and the mean length of other roots (MRL), compared to groups G1 and G4, which had roots with more similar lengths (Table S5). G2 genotypes combined a significantly longer primary root (Table S5) with the largest difference between it and the other seminal roots (together with G3). This rooting pattern, based on dissimilar growth of the roots, could have an impact on overall soil exploring capacity that should be explored further, particularly its usefulness in semi-arid environments, to access to stored water at deep layers at critical periods (flowering and grain filling), while keeping enough shallow roots to take advantage of in-season precipitations.

Two groups (G1 and G3) showed the highest contrast in the multivariate analysis (Figure 3). G1, with a majority of landraces, displayed a vigorous seminal root system, in contrast with G3, formed entirely by cultivars with small root systems. Our finding was in agreement with the study of a collection of 160-durum wheat landraces [33] in terms of larger seminal root size. This study found that landraces coming from the eastern Mediterranean region (Turkey), the driest and warmest areas considered in the study, showed the largest seminal root size and widest root angle compared to landraces from eastern Balkan countries. The authors claimed that these differences were due to the adaptations of landraces to the contrasting environmental conditions of these two regions. The larger root size and wider root angle from Turkish landraces would allow better exploration of the full soil profile and better water capture. Among the four groups found in this study, no one combined the highest MRA and TRL, comparable to Turkish landraces. Therefore, there could be room for improvement for the root systems of durum wheat for Algeria. Crosses to combine these traits in a single genotype should be devised, and Turkish landraces could be tested in Algerian conditions, to assess their potential.

In our germplasm, the landraces showed on an average narrower angle and higher root size. Previous studies on Mediterranean durum wheat [33] found that the genotypes with the narrowest angle came from the western Mediterranean region and that they also had heavier grains [54,55].

Additionally, it was reported that *Triticum turgidum* subsp. *dicoccon* landraces coming from cooler and wetter zones had shallower seminal root systems than those from warmer and drier areas [25]. The subsp. *durum* landraces, developed in warmer and drier areas, tended to have larger and steeper root patterns than landraces coming from cooler and wetter zones. Accordingly, the root system architecture of the Algerian landraces would indicate adaptation to a warm and dry environment. Other studies have found different root morphologies in apparent adaptation to stressful conditions. For instance, the drought-tolerant bread wheat cv. SeriM82 has a compact root system [21], associated with a limited water use early in the season, facilitating access to stored water later in the reproductive phase. Contrary to our landraces, SeriM82 exhibited less vigorous shoot growth. In contrast with our findings, a study of bread wheat germplasm grown historically in the semi-arid northwestern of China [56] found that breeding caused a narrowing of the seminal root angle, reduced root number, and increase of primary seminal root length. In that study "newer cultivars produced higher yields than older ones only at the higher sowing density, showing that increased yield results from changes in competitive behavior." This view was confirmed and expanded later [57], confirming that the advantage of new Chinese wheat cultivars came from the attenuation of inter-plant competition and increased plasticity in root morphology. A seminal root architecture with fewer, longer seminal roots with narrower root angle, would overlap less with neighbors, leading to less competition between individuals [58], and these trends agree with the hypothesis of weakening of "selfish" traits [59].

The shift in root morphology observed in Algeria in the step from landraces to modern cultivars does not conform to the scenario described in those works. There was a reduction of overall root length and volume after the advent of modern breeding, which could be consistent with the reduction of inter-plant competition but combined with the widening of the root angle, which does not bode well with that hypothesis. It seems that wheat breeding may have resulted in different trends for root morphology in different parts of the world. This could be the result of the adaptation of Algerian landraces to agronomic conditions different from current agriculture. The difference in rooting patterns between landraces from different geographical areas and cultivars may lie in the agronomic environments in which they were developed. In general, modern durum wheat cultivars were bred under high plant densities [57], whereas landraces were grown in stands with density adapted to the environment. The morphology of Algerian landraces (long seminal roots growing in steep angles) conforms to the "steep, cheap, and deep root ideotype" [60], and could be the result of adaptation to accessing water in deep soil layers. Further studies with adult plants are needed to evaluate if root features of seedlings are maintained when the competition between individuals for root growth is increased (as the seminal and nodal roots require more space and resources than just the seminal roots of the seedlings). A shovelomics experiment is being carried out with the same genotypes, which could elucidate this issue at least for some measurable traits like root angle.

The high SDW of our durum landraces compared to cultivars could be related to the lack of dwarfing genes in the landraces. This hypothesis was already confirmed previously for bread and durum wheat for some height reducing genes [61], which reduced the first seedling leaf growth in *Rht* genotypes compared with the corresponding tall wheat lines.

4.3. Conclusions and Perspectives

We have found wide genetic variability in a collection of durum wheat genotypes cultivated in Algeria and unraveled a possible historic trend that sheds light on the outcomes of modern breeding. An important issue is to what extent this variability found at the seedling stage can reflect the variability in the field with the same genetic material, more precisely, which traits can be consistent across plant phases (seedling and adult plant), enabling the selection at the early seedling stage. If this relationship is not found, then the room for the testing of seminal root traits is very limited. Experiments to evaluate this relationship are ongoing.

Overall, landraces showed a larger root size and steeper root angle. These two traits could be involved in the adaptation of landraces to water-stressed environments. The dwarfing genes seem to

influence biomass partitioning; screening the current germplasm for these genes would elucidate this issue. The root size and shape in our data indicated some independence that would open opportunities to design cultivars with the desired combinations of traits.

Overall, the current genotypes present a diverse root system architecture, from compact deep-rooting to wide shallow one. This opens the opportunity to test the four different root ideotypes found (G1–G4) for functional implications under water and nutrient-limited environments. Based on the above results, we hypothesize that root architecture difference between cultivars, landraces (or steep deep vs. shallow root systems) may result in different strategies of adaptation to the availability of water and nutrients over the soil profile.

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