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Study of Variability in Root System Architecture of Spanish *Triticum turgidum* L. Subspecies and Analysis of the Presence of a MITE Element Inserted in the *TtDro1B* Gene: Evolutionary Implications

Juan M. González [1,*], Rodrigo Cañas [1], Alejandra Cabeza [1], Magdalena Ruiz [2], Patricia Giraldo [3] and Yolanda Loarce [1]

[1] Department of Biomedicine and Biotechnology, University of Alcalá, 28805 Alcalá de Henares, Spain; rodricober@gmail.com (R.C.); acabeza@eead.csic.es (A.C.); yolanda.loarce@uah.es (Y.L.)
[2] Plant Genetic Resources Centre, National Institute for Agricultural and Food Research and Technology, CSIC, 28805 Alcalá de Henares, Spain; mruiz@inia.es
[3] Department of Biotechnology and Plant Biology, School of Agricultural Engineering, Polytechnic University, 28040 Madrid, Spain; patricia.giraldo@upm.es
* Correspondence: juanm.gonzalez@uah.es

Abstract: We analysed nine traits of the root system of 223 genotypes of *Triticum turgidum* (2n = 4x = AABB) subspecies *dicoccoides*, *dicoccum*, *turgidum*, *durum* and *polonicum*, finding a large intra and interspecific variability in both the number and size of roots, as well as in their spatial distribution. We studied the presence of an incomplete MITE (Miniature Inverted-repeat Transposable Element) inserted in the *TtDro1B* gene, which is present in some genotypes of *dicoccoides*, *dicoccum*, and *turgidum*, but not in *polonicum* and the 97.9% of the *durum* accessions. Comparison between genotypes shows that genotypes with the MITE element have smaller and shallower roots. Since *Aegilops* is considered to be the donor of the wheat B genome, the presence of the same MITE element was analysed in 55 accessions of the species *Aegilops speltoides*, *searsii*, *bicornis* and *longissima,* and in no case was it detected. We propose that after the emergence of *T. turgidum* subsp. *dicoccoides*, the insertion of the MITE element probably occurred in a single plant. Subsequent domestication resulted in genotypes of *dicoccum* with and without the MITE element, which after selection gave rise to the subspecies *turgidum*, and *durum* and *polonicum,* respectively. The MITE element can be used to differentiate *turgidum* from the *durum* and *polonicum* with high reliability.

Keywords: *durum* wheat; germplasm; plant genetic resources; RSA; transposon

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1. Introduction

The genus *Triticum* includes both diploid (2n = 14), tetraploid (2n = 4x = 28) and hexaploid (2n = 6x = 42) species [1,2], some of which are of great economic importance [3]. Thus, durum wheat (*T. turgidum*, 2n = 4x = 28; genomic constitution AABB) and common wheat (*T. aestivum*, 2n = 6x = 42; genomic constitution AABBDD) are two of the species that occupy the largest global cultivated area and provide a high percentage of animal feed and human food [4,5]. Common wheat is used to produce bread, noodles and biscuits, and durum wheat for pasta and couscous and other semolina-based staples [6].

T. turgidum is an allotetraploid species that originated from hybridisation between the diploid species *T. urartu* donor of genome A, and *Ae. speltoides* or a closely related species, which would have contributed genome B [2,7–9], resulting in the emergence of wild emmer wheat (*T. turgidum* L. subsp. *dicoccoides* (Korn. ex Asch. and Graebn.) Thell.) that around 12,000–10,000 years ago was domesticated by ancient farmers to give rise to emmer wheat (*Triticum turgidum* L. subsp. *dicoccum* (Schrank ex Schübl.) Thell.) [10,11],

with hulled grains and brittle ears [12]. For a historical review of the origin of durum wheat, see Martínez-Moreno et al. [13].

The selection from *dicoccum* evolved the naked type subspecies tetraploid wheats such as durum (*T. turgidum* L. subsp. *durum* (Desf.) Husn.), rivet wheat (*T. turgidum* L. subsp. *turgidum* (Desf.) Husn.) and the polish wheat (*Triticum turgidum* subsp. *polonicum* (L.) Thell.), resulting in different landraces, many of which have been lost because of modern breeding programmes with the consequent reduction in genetic variability [14,15]. Fortunately, the gene banks conserve many accessions, including a high proportion of local varieties that show a great diversity of high value for genetic improvement, which could be used to increase adaptability to low-input systems yield, resistance to biological agents, and tolerance to environmental stresses in the current scenario of climate change [6]. In order to facilitate the management of these large collections, a core collection (CC) is obtained. The CC contains a much smaller number of entries but maintains the variability of the whole collection. This is the case of the Spanish core collections of barley [16], durum wheat [17] and common wheat [18].

The roots allow plants to anchor themselves to the soil and capture mineral nutrients and water, and the set of all roots and their spatial configuration is called the Root System Architecture (RSA) [19], being variable between different species and genotypes. For instance, shallow roots allow the plant to capture nutrients such as P, while deep roots allow it to capture N and deep soil water and, therefore, the root is one of the plant organs with the biggest potential to influence crop productivity [20–23]. However, due to the difficulty of studying the roots, they have hardly ever been considered in breeding programmes [24].

Ruiz et al. [25] analysed the root system of seedlings of Spanish durum wheat CC [17] and found that the three subspecies included showed a high variability. Thus, the subspecies *dicoccum* has small and shallow roots while *durum* has the longest and deepest roots, and *turgidum* has an intermediate phenotype between the other two subspecies, reflecting an adaptation to different growing areas. Other authors have studied the variability in the RSA of different wheat varieties and landraces and have also observed a large variability and potential for modifying root structure to increase yield or drought tolerance [26–29].

The depth that the roots can reach depends on their length and on the inclination. Thus, the smaller the angle with respect to the vertical of the soil, the greater is the depth they can reach. Some genes that influence the angle of root development have been identified, such as the *Dro1* gene that was studied in rice by Uga et al. [30] and subsequently identified in other species [31–34]. Loarce et al. [35] analysed the *TtDro1A* and *TtDro1B* genes in durum wheat and found some differences in their sequence and expression. The most striking difference was the identification of an incomplete MITE (Miniature Inverted-repeat Transposable Element) of the *Tc1-Mariner* transposon class, in the *TtDro1B* gene in accessions belonging to subspecies *turgidum* but not in those belonging to subspecies *durum*, and they associated these differences with the inclination of the roots of the seedlings of both subspecies.

MITEs are non-autonomous DNA transposons (TEs), which are abundant in many plant genomes, and they are mainly characterised by their short sequence (up to a few hundred bases), their structural similarity, and their conserved terminal inverted repeats [36,37]. It has been observed that their distribution in plant genomes is not homogeneous, highlighting their presence in intragenic regions [38]. Despite being non-autonomous elements, the activity of MITEs has been demonstrated in rice [36,37]. TEs have been widely studied as agents for the creation of new genetic variability, since in addition to the disruption of pre-existing sequences TEs have been widely studied as a source of genetic variability. These elements can vary gene expression by integrating into new regions of the genome, generating new alternative mRNA splicing, new promoters, premature termination of transcription or even modifying the state of the surrounding chromatin. New epigenetic marks can extend outside TEs and affect the expression of nearby gene regions. [39]. It has been shown that stress events such as cell culture, protoplast transformation, temperature changes, radiation or polyploidisation events can trigger the mobilization of TEs that were

previously inactivated by plant genomic defense processes because the methylation marks would disappear, allowing their reactivation [36,37,40,41].

The aims of the present work are: (i) to study the RSA of a collection of 223 genotypes belonging to 5 tetraploid durum wheat subspecies. (ii) to identify the presence of a truncated MITE element insertion in the *TtDro1B* gene in the above collection, to know if there are differences between the subspecies and its possible relationship with the structure of the root system, (iii) to study the presence of the MITE element in a collection of 55 genotypes belonging to 4 species of the genus *Aegilops*, to provide information on the evolutionary origin of tetraploid wheat subspecies, (iv) to assess whether the MITE element can serve as a subspecies marker.

2. Materials and Methods

2.1. Plant Materials

The materials used in this study included wild species, landraces and commercial varieties of a set of 278 accessions from 5 subspecies of *Triticum turgidum* and 4 species of *Aegilops*, provided by the National Plant Genetic Resource Center of Spain (CRF, INIA, CSIC) and the Leibniz Institute of Plant Genetics and Crop Plants Research (IPK) of Germany (Table S1). The total number of accessions of each species and subspecies are: *Triticum turgidum* subsp. *durum* (143), subsp. *turgidum* (37), subsp. *polonicum* (10), subsp. *dicoccum* (14) and subsp. *dicocoides* (19); *Aegilops speltoides* (41), *Ae. bicornis* (4), *Ae. longissima* (5) and *Ae. searsii* (5).

2.2. RSA Analysis

The RSA study was carried out using the rhizoslide technique according with Ruiz et al. [25] and Boudiar et al. [29]. Briefly, 12 seeds from each of the genotypes analysed were disinfected with sodium hypochlorite solution (1.25%) during 15 min and rinsed 4 times with sterile distilled water. Seeds were placed in Petri dishes with two sheets of filter paper moistened with 4 mL of distilled water were kept at 4 °C for 2 days, and then put in the rhizoslide system and grown in a chamber at 22 °C–18 °C with a photoperiod of 12 h of light for 1 week. The seedlings were then removed from the rhizoslide and the roots were scanned with a Canon "LiDE210" scanner at 300 ppi. Next, the roots of each seedling were manually separated, and a second scan was performed. The first image was used to measure the angles of each root with respect to the vertical, and the second image was used to measure the length, diameter, surface area and volume of each root. All measurements were carried out with the SmartRoot software v.3.32 that is a plugin for ImageJ1.46R (http://imagej.nih.gov/ij/download.html) (Accessed on 1 July 2020). For each seedling, the following variables were obtained or calculated from the different measurements: total root length in cm (TRL), total root surface area in cm^2 (S), total root volume in cm^3 (V), mean root diameter in cm (D), primary root length in cm (PRL), number of roots (NR), mean vertical angle of all the roots in sexagesimal degrees (MRA), the maximum vertical angle in sexagesimal degrees (MxAV) and the most least vertical angle in sexagesimal degrees (MAV).

2.3. DNA Extraction

The DNA was extracted from young leaves with the "NZY Plant/Fungi gDNA Isolation kit" (NZYTech, Lisbon, Portugal) following the instructions specified by the manufacturer.

2.4. MITE Detection in Triticum turgidum Subspecies

We used 25 ng of DNA for PCR amplification of the specific region of the *TtDro1B* gene containing the MITE sequence with these primers: TtB1F (5'TGCTCCTCCGAAAAGGGAAT3'), and TtB1R (5'GCTTAGTTGTTGACAGCCTGACTTAT3') designed from *T. turgidum TtDro1B* sequences (Genbank accession: MZ151532 and MZ151533). Reactions were carried out in a final volume of 25 µL with NZYTaq II 2x Green Master Mix (Nzytech^{TM}) according to the manufacturer's specifications. The PCR reaction consisted of 1 cycle of 5 min at 94 °C

followed by 35 cycles at 94 °C 30 s; 55 °C 30 s; 72 °C 1 min 30 s followed by 1 cycle at 72 °C 7 min. We used 15 µL of the PCR reaction in a restriction reaction with *Hae*III restriction enzyme, and the digestion products were separated on a 1.5% SB-agarose gel.

2.5. MITE Detection in Aegilops Species

We used 25 ng of DNA in two independent PCR reactions with TtB1R primer and two different forward primers B1MITEinF (5′CATGTATAAGCTACTCCCTC3′) with the 3′ region inside the MITE sequence and B1MITEoutR (5′ATGCCAGATGAAGCATGT3′) with the whole sequence outside the MITE element. The PCR reaction consisted of 1 cycle of 5 min at 94 °C followed by 35 cycles at 94 °C 30 s; 55 °C 30 s; 72 °C 1 min 30 s followed by 1 cycle at 72 °C 7 min. The PCR products were separated by electrophoresis on 0.8% TAE-agarose gels.

2.6. Statistical Analysis

The means of the RSA variables were compared between subspecies, and between the set of genotypes presenting or not the MITE element. Variables showing equality of variances were compared by ANOVA, and the least significant difference (LSD) test was used to detect differences between pairs of means. The Kruskal–Wallis non-parametric test was used for variables that did not show equality of variances, and the Tukey test was used for comparison between pairs of means. Statistical calculations were performed with StatGraphics plus v.5.1 software.

3. Results and Discussion

The evolutionary origin of durum wheat is complex, involving one or more hybridisation and polyploidisation events, which have resulted in different lines of the oldest wild relative of durum wheat, *T. turgidum* subsp. *dicoccoides* [1,12,42,43]. Domestication of *T. turgidum* subsp. *dicoccoides* took place between 10 and 12,000 years ago, giving rise to cultivated emmer wheat *T. turgidum* subsp. *dicoccum* [10–12,44], that is considered a valuable gene source to improve the elite durum wheat cultivars [45–47]. Artificial selection of *dicoccum* gave rise to other subspecies and a large number of landraces, many of which are maintained in plant germplasm banks. The core collection (CC) obtained from these large collections allows genetic studies and breeding programmes to be carried out [48].

3.1. Study of the RSA in T. turgidum Subspecies

The CC of Spanish durum wheat includes 94 accessions of the subspecies *durum*, *turgidum* and *dicoccum* [17]. The analysis of the root system architecture (RSA) of this collection showed great variability in the length, number and diameter of the roots, as well as in the angle of inclination in relation to the vertical of the soil [25]. In the present work, we have extended the RSA study to a total of 223 genotypes belonging to 5 of the 8 subspecies of *T. turgidum* [49]. Table 1 shows a statistics summary of the nine variables related to RSA in the five subspecies of *T. turgidum*. Figure 1 shows the means and the confidence intervals for each variable in every subspecies. Variables related to root size characteristics (TRL, S, V, D, PRL and NR) show similar coefficients of variation in the different subspecies, ranging from 4.77 for D in subsp. *polonicum* to 25.09 for V in subsp. *dicoccoides*. Nevertheless, the three variables related to root inclination angles (MRA, MAV and MxAV) have larger and more diverse Coefficients of Variation (CVs), with a maximum value of 65.53 for MAV in subsp. *polonicum*. The nine RSA variables were compared taking subspecies as an independent factor. The variables V, D, PRL, NR, MRA and MAV showed equal variances, and TRL, S and MxAV did not. ANOVA was used for the first group of variables and the Kruskal–Wallis test for the second, and all results showed significant differences ($p < 0.05$). Comparison between pairs of means was done using the LSD or Tukey test, depending on whether the variables had equal variances or not. Figure 1 shows the means of each genotype for each of the variables and 95% confidence intervals of the LSD or Tukey tests.

Table 1. Summary statistics of the RSA variables analysed in the five subspecies of *T. turgidum*: Total root length in mm (TRL), total root surface area in mm^2 (S) total root volume (V) in mm^3, mean root diameter in mm (D), primary root length in mm (PRL), total number of roots (NR), mean vertical angle of all the roots in (MRA), the maximum vertical angle in (MxAV) and the most least vertical angle in (MAV). SD (Standard deviation) CV (Coefficient of variation). Min (Minimum value). Max (Maximum value). n = number of accessions analysed.

Subspecies	Variable	Mean	SD	CV	Min	Max
dicoccoides (n = 19)	TRL	58.8	10.41	17.68	42.53	75.34
	S	9.70	2.06	21.22	5.96	13.19
	V	0.13	0.03	25.09	0.07	0.19
	D	0.05	0.00	6.89	0.04	0.06
	PRL	20.6	4.03	19.51	12.64	27.84
	NR	3.91	0.67	17.18	3.00	5.33
	MRA	20.8	5.59	26.83	12.35	29.57
	MAV	9.07	4.00	44.10	2.30	17.53
	MxAV	32.7	8.72	26.60	20.75	51.50
dicoccum (n = 14)	TRL	72.3	12.08	16.71	51.53	91.31
	S	12.3	2.43	19.61	9.35	16.57
	V	0.18	0.04	23.50	0.13	0.25
	D	0.05	0.00	6.32	0.05	0.06
	PRL	20.0	2.17	10.80	16.99	23.22
	NR	4.83	0.31	6.37	4.08	5.42
	MRA	33.1	7.50	22.60	20.21	43.23
	MAV	18.2	6.12	33.49	11.22	32.83
	MxAV	47.8	8.93	18.68	31.83	58.75
turgidum (n = 37)	TRL	83.8	10.46	12.47	53.55	101.63
	S	14.5	1.87	12.88	10.08	18.20
	V	0.21	0.03	14.50	0.14	0.27
	D	0.06	0.00	7.55	0.05	0.07
	PRL	21.5	2.17	10.08	15.70	25.29
	NR	5.02	0.25	5.05	4.08	5.50
	MRA	27.1	6.44	23.76	13.78	42.76
	MAV	13.1	4.23	32.22	3.83	21.17
	MxAV	43.1	7.68	17.83	30.14	64.33
polonicum (n = 10)	TRL	86.3	10.98	12.72	75.24	108.15
	S	14.4	1.59	11.01	12.97	18.29
	V	0.20	0.02	11.55	0.17	0.26
	D	0.05	0.00	4.77	0.05	0.06
	PRL	21.7	3.10	14.29	16.15	26.36
	NR	5.23	0.36	6.95	4.75	6.00
	MRA	19.0	5.53	28.99	11.78	30.57
	MAV	9.48	6.21	65.53	1.75	20.40
	MxAV	31.2	7.46	23.85	20.00	46.63

Table 1. *Cont.*

Subspecies	Variable	Mean	SD	CV	Min	Max
durum (n = 143)	TRL	91.9	10.98	11.94	47.35	115.89
	S	15.5	1.99	12.85	8.69	20.43
	V	0.21	0.03	15.22	0.11	0.30
	D	0.05	0.00	5.32	0.04	0.06
	PRL	22.3	2.33	10.40	12.77	28.05
	NR	5.34	0.40	7.41	4.50	6.58
	MRA	23.1	5.25	22.69	11.78	37.27
	MAV	10.8	3.87	35.86	4.25	24.92
	MxAV	37.0	7.21	19.47	18.83	58.12

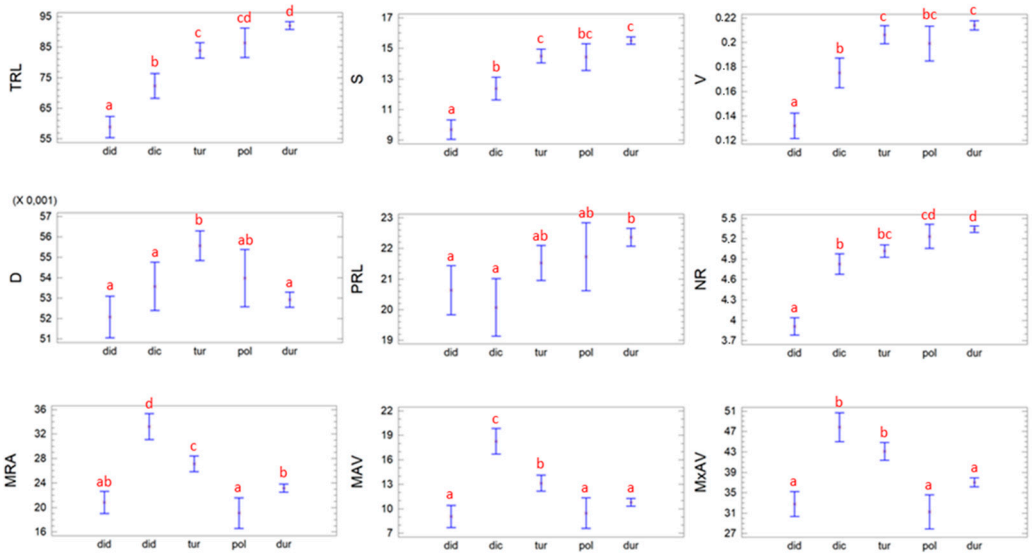

Figure 1. Mean and 95% confidence intervals of the LSD or Tukey tests of the nine RSA variables analysed in the five subspecies of *Triticum turgidum* analysed. Total root length (TRL), total root surface area (S) total root volume (V), mean root diameter (D), primary root length (PRL), total number of roots (NR), mean vertical angle of all the roots (MRA), the maximum vertical angle (MxAV) and the most vertical angle (MAV). In X axis subspecies abbreviations: did, *dicoccoides*; dic, *dicoccum*; tur, *turgidum*; pol, *polonicum* and dur, *durum*. Subspecies with the same letter have no statistically significant differences ($p > 0.05$).

The subspecies *dicoccoides* has the lowest values for roots' total length, surface area and root volume, which can be explained by the lower number of roots (3.91) compared to the other four subspecies (4.83–5.84). In contrast, the subspecies *durum* and *polonicum* have a more developed root system. However, the primary root length is similar in the 5 subspecies analysed, ranging from 20.07 to 22.26 cm. Regarding to the angles formed by the roots, the subspecies *polonicum*, *dicoccoides* and *durum* have the most vertical roots, while *dicoccum* and *turgidum* have the shallowest roots. (Table 1 and Figure 1). These results could indicate a greater similarity between the latter two subspecies, as reported by Pascual et al. [50]. According to our data, the domestication of *dicoccoides* to give rise to *dicoccum* involved an increase in the number of seminal roots, total length, surface and volume of the root system, and in angles of the roots making them more horizontal. This agrees with Gioia et al. [11], who found an increase in stem and root development when moving from wild to domesticated emmer wheat and then to *durum* wheat.

The selection by early farmers of *dicoccum* is likely to have resulted in the subspecies *turgidum*, *polonicum* and *durum*, whose root systems would have been selected according to the different cultivation areas. Thus, the subspecies *turgidum* has long and shallow roots,

and is mainly grown in more temperate and humid areas [25], while the subspecies *durum* and *polonicum* have long and deep roots. The latter phenotype allows the subspecies *durum* to be cultivated in extensive hot and dry regions, and the subspecies *polonicum*, although it has interesting nutritional characteristics, is currently cultivated only in marginal areas of southern Spain and Italy, Algeria and Ethiopia [51,52].

3.2. Analysis of a MITE Element

One of the characteristics of the cereal genome, and in particular of durum and common wheat, is the presence of many transposable elements, accounting for 85% of the total nuclear genome [43]. Among the identified transposons are the MITE elements, which are typically located less than 2kb upstream and downstream of the genes [53]. Loarce et al. [35] isolated and determined the sequence of the *TtDro1A* and *TtDro1B* genes from accessions BGE045630 and BGE048497 conserved in the CRF, belonging to the *durum* and *turgidum* subspecies of *T. turgidum*, respectively, which showed very different RSAs. Comparison of the sequences of these two genes between the two subspecies showed some differences, the most obvious being the insertion of a fragment of a MITE element in the 5'UTR region of the *TtDro1B* gene in *turgidum* subspecies.

In the present work, we have analysed the presence of the MITE element in the collection of 223 durum wheats of the subspecies *dicoccoides*, *dicoccum*, *turgidum*, *polonicum* and *durum*. To detect the presence of the MITE fragment, the region containing the MITE element was amplified by PCR. The sizes of the amplified fragments are 1435 bp with the MITE element and 1397 bp without the MITE element, respectively. These fragments showed 2 RFLPs in *Triticum turgidum* when digested with the *Hae*III restriction enzyme, 1 of 180 bp in the accessions without the MITE element and another of 226 bp in the accessions with the MITE element (Figure 2).

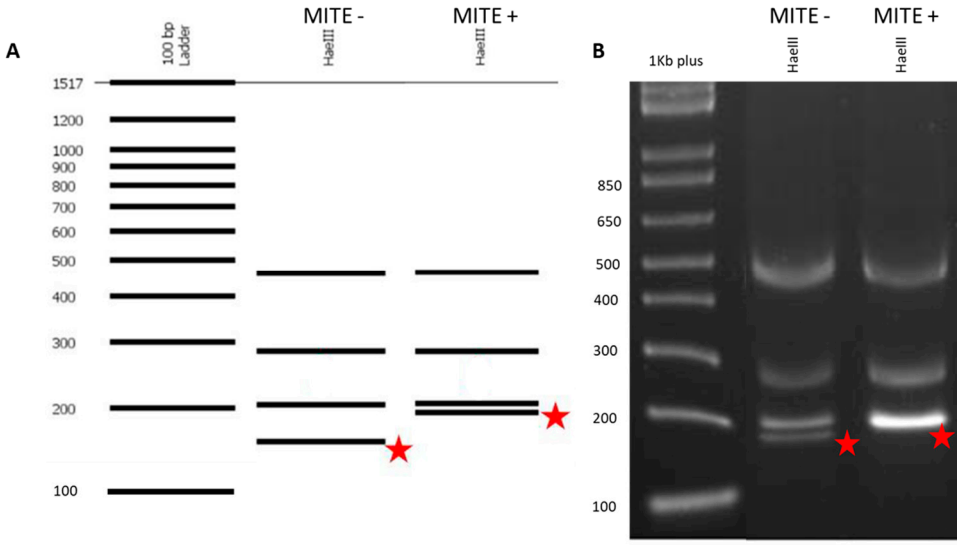

Figure 2. (**A**) *Hae*III digestion patterns of the *TtDro1B* fragment containing the MITE element. (**B**) MITE-RFLPs in SB-Agarose gel. Stars indicate polymorphic bands. The polymorphic *Hae*III bands generated in samples with MITE migrate together on the agarose gel like a single and higher intensity band.

The results obtained show than the insertion of the MITE element is present in 36.84% of the accessions of the subsp. *dicoccoides*, in 64.29% of subsp. *dicoccum*, in 91.89% of the accessions of subsp. *turgidum*, in 2.09% of subsp. *durum*, and in none of the accessions of subsp. *polonicum* (Supplementary Table S1).

Genomic modifications following polyploidy processes have been studied by several authors [41,43,54–56]. For instance, Hao et al. [57] identified the 4AL-5AL-7BS translocation in eight subspecies of *T. turgidum*. The 4AL-5AL translocation is present in the diploid species *T. urartu* and *T. monococcum* (2n = 14, AA) [58], indicating that the translocation

with chromosome 7BS must have arisen when *T. turgidum* subsp. *dicoccoides* originated. In this scenario, several hybridisation events could have occurred, giving rise to different lines of *T. turgidum* subsp. *dicoccoides.* Some might have the translocation and some might not, but as there are currently no *T. turgidum* without the translocation, this would imply that the translocation conferred a major evolutionary advantage that would have resulted in the disappearance of the non-translocated cytotypes [57].

Transposons have also been shown to be activated by stresses, including the emergence of new species through hybridisation and chromosome duplication [36,37,41,59]. The presence of the fragment MITE insertion in the *TtDro1B* gene in some genotypes of the *dicoccoides* and *dicoccum* subspecies could be due to hybridisations with different genotypes of *Aegilops speltoides* (or a related species of the section Sitopsis (S-genome species) that are considered to be the donor of the wheat B genome, which would have the MITE insertion or be absent of it. In an attempt to explain this hypothesis, we analysed the presence of the MITE element in the *Dro1B* gene in 55 genotypes belonging to the species *Ae. speltoides* (41), *Ae. searsii* (5), *Ae. bicornis* (4) and *Ae. longissima* (5), respectively.

The detection of the MITE element in the *Aegilops* species required a new strategy because the *Hae*III pattern could not distinguish between the presence and absence of the element in these genotypes. The primers B1MITEinF, with part of its sequence outside the MITE element and the 3′ region inside, and the primer B1MITEoutF, with the whole sequence outside the MITE element, were designed. The absence of the MITE element resulted in no amplification in reactions with the B1MITEin primer and a smaller band size than the control MITE-DNA in reactions with B1MITEout (Figure 3). In none of the accessions of the *Aegilops* species studied was the presence of the MITE element detected.

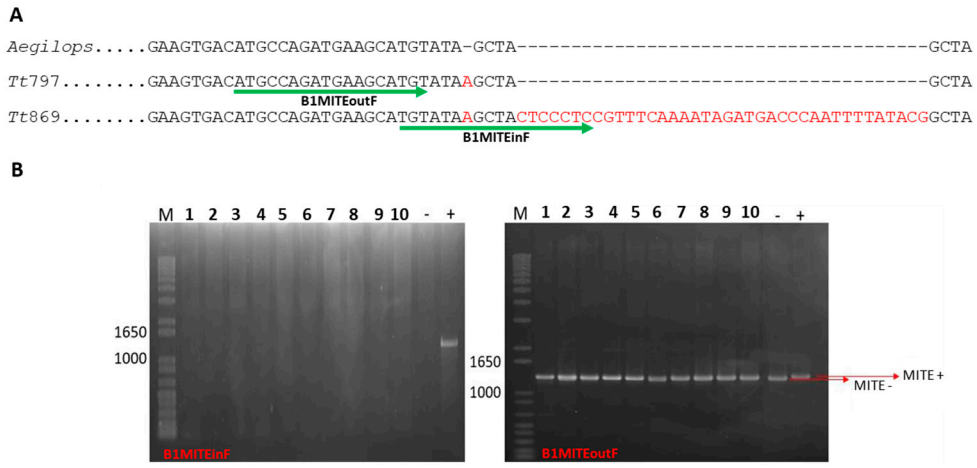

Figure 3. (**A**) Sequence alignment from *Aegilops* and *Triticum turgidum* 797 (BGE045630) and 869 (BGE048497) regions containing the MITE element (sequences in red) on which the B1MITEinF and B1MITEoutF primers were designed. (**B**) On the left, PCR amplifications from 10 *Aegilops* accessions (1–10) with the B1MITEin primer forward. On the right, PCR amplifications from 10 *Aegilops* accessions (1–10) with the B1MITEout primer forward. M (Molecular marker). (+) Positive control with MITE (*Tt*869). (−) Negative control without MITE (*Tt*797).

Previous studies have tried to find out whether *Triticum turgidum* originated after one or several hybridisation and chromosomal duplication events between *T. urartu* and *Ae. speltoides* or a related species, which would result in the synthesis of *T. turgidum* subsp. *dicoccoides* once or several times [1,2,6,7,13,43]. Our study does not allow us to differentiate between the two alternatives; however, the results we obtained with the Spanish landraces that were analysed indicate that a single plant carrying the insertion of the MITE element in the *TtDro1B* gene probably appeared because of transposition phenomena (Figure 4). This hypothesis is based on the observation that in all the accessions analysed, we detected the same insertion of the truncated MITE element. Subsequent evolution allowed the propagation and expansion of the plants, giving rise to *dicoccoides* genotypes with or

without the MITE insertion. The domestication process between 10,000 and 12,000 years ago [13,44,45], resulted in the emergence of different lines of *T. turgidum* subsp. *dicoccum*, some with and some without the MITE element, depending on the type of *dicoccoides* plant from which they originated. However, in order to have more evidence for this hypothesis, it would be interesting to extend the study to materials from other countries, especially from the Middle East.

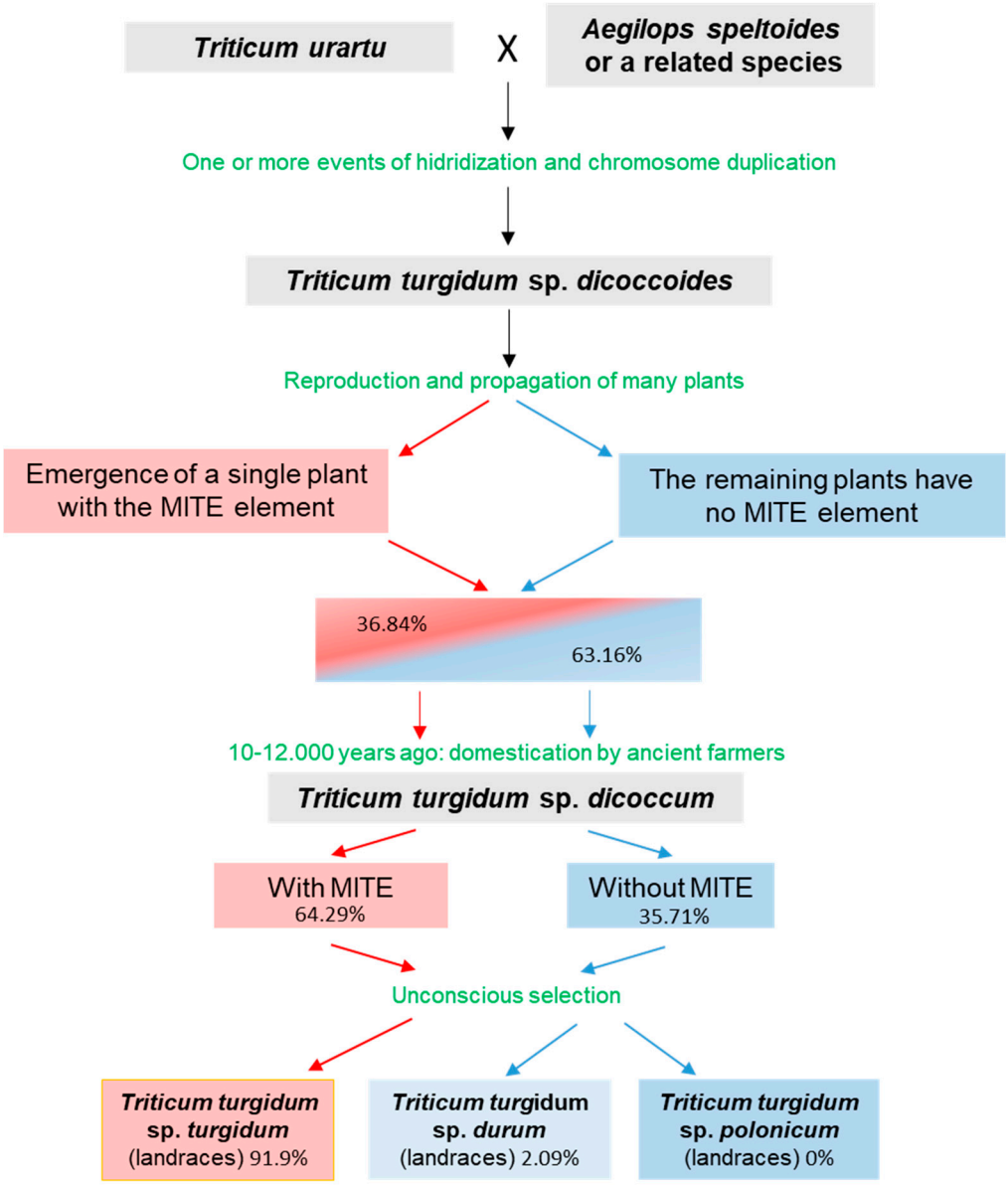

Figure 4. Proposal to explain the origin of some subspecies of *Triticum turgidum*. Percentages indicate the presence of the MITE element in each of the subspecies.

The selection undertaken by ancient farmers gave rise to the subspecies *turgidum*, *durum* and *polonicum*. Therefore, selection of *dicoccum* plants with the MITE element resulted in the different landraces of *T. turgidum* subsp. *turgidum*, suggesting that there is probably a link between the insertion of the MITE element and the adaptation of this subspecies to a warmer and more humid environment. Similar results were obtained by Muterko and Salina [41] when they analysed, in a collection of hexaploid and tetraploid wheat, the insertion of a transposon of a new family called M882 in the promoter region of the VRN-B3 gene. The selection from *dicoccum* plants without the MITE element resulted in different landraces of *T. turgidum* subsp. *durum* and *T. turgidum* subsp. *polonicum*. In

this case, the absence of the MITE element would be related to growth in warmer and drier environments. However, we have detected some accessions of subsp. *turgidum* and subsp. *durum* that do or do not have the MITE element, respectively. These discrepancies could be explained because of spontaneous crosses between genotypes of both subspecies and subsequent selection by farmers over many generations, resulting in the different landraces preserved in the Genebanks [14,15].

3.3. Relationship between the Presence of the MITE Element Insertion and the RSA

In rice, Uga et al. [13] showed that the *Dro1* gene is involved in the angle at which roots develop, and Loarce et al. [35] studied the expression of the *TtDro1A* and *TtDro1B* genes in eight genotypes of the subsp. *turgidum* and *durum* and found differences in expression of the two genes and in both subspecies. Thus, the *TtDro1A* gene is more highly expressed than the *TtDro1B* gene. Moreover, the *TtDro1A/TtDro1B* ratio is higher in the subspecies *turgidum* than in *durum*, so that the higher the ratio, the shallower the roots are. The latter authors proposed that the insertion of the MITE element in the *TtDro1B* gene of the subspecies *turgidum* leads to a decrease in the expression of this gene and to shallower roots. According to our results, surface roots could have been selected in cultivation areas with higher water availability, facilitating at the same time the acquisition of nutrients such as phosphorus, which accumulate in the surface layers of the soil [60].

We pooled the genotypes of the five subspecies of *T. turgidum* according to whether or not they had the MITE element inserted in the *TtDro1B* gene. A statistical summary is show in Table 2.

Table 2. Summary statistics of the root system architecture variables in *T. turgidum* genotypes without and with the MITE element: total root length in mm (TRL), total root surface area in mm^2 (S) total root volume (V) in mm^3, mean root diameter in mm (D), primary root length in mm (PRL), total number of roots (NR), mean vertical angle of all the roots in (MRA), the maximum vertical angle in (MxAV) and the most vertical angle in (MAV). SD (Standard deviation) CV (Coefficient of variation). Min (Minimum value). Max (Maximum value). n = number of genotypes.

MITE	Variable	Mean	SD	CV	Min	Max
Without (n = 170)	TRL	88.95	13.66	15.35	42.53	115.8
	S	14.98	2.47	16.51	5.96	20.43
	V	20.69	3.87	18.71	6.87	30.15
	D	52.97	2.97	5.60	42.14	60.49
	PRL	22.17	2.63	11.87	12.64	28.05
	NR	5.22	5.40	10.34	30.00	65.83
	MRA	23.14	5.63	24.33	11.78	39.99
	MAV	10.91	4.13	37.84	1.75	24.92
	MxAV	36.88	7.82	21.21	18.83	58.12
With (n = 53)	TRL	77.89	14.78	18.97	43.03	112.3
	S	13.35	2.65	19.84	6.99	18.20
	V	18.86	4.08	21.64	8.62	26.92
	D	54.70	4.01	7.33	48.23	72.34
	PRL	21.05	2.28	10.82	15.70	25.71
	NR	4.83	5.58	11.54	30.00	56.67
	MRA	27.01	7.72	28.58	12.35	43.23
	MAV	13.16	5.84	44.35	2.30	32.83
	MxAV	42.01	9.23	21.97	20.75	64.33

The RSA variable means differed significantly between the sets of genotypes without and with the MITE element ($p < 0.05$). Figure 5 shows the mean values and 95% confidence intervals of the LSD or Tukey tests.

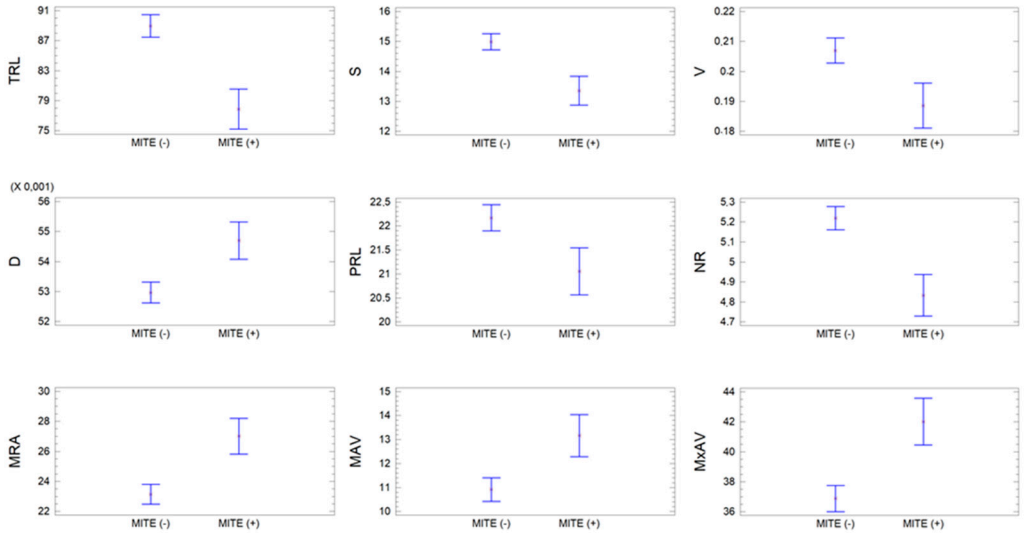

Figure 5. Mean value and 95% confidence intervals of the LSD or Tukey tests of the nine RSA variables analysed in the genotypes of the five subspecies of *Triticum turgidum*, depending on whether or not they have the MITE element inserted. Total root length (TRL), total root surface area (S) total root volume (V), mean root diameter (D), primary root length (PRL), total number of roots (NR), mean vertical angle of all the roots (MRA), the maximum vertical angle (MxAV) and the most vertical angle (MAV). All variables show statistically significant differences ($p < 0.05$) between the two groups of genotypes.

Genotypes with the MITE element inserted have less root system development, and the root angles are larger and therefore the roots grow more horizontal. This phenotype is mainly observed in the subspecies *dicoccum* and *turgidum* and confirms the similarity between them found by Pascual et al. [50] and suggests a closer resemblance of the subspecies *turgidum* to the more ancestral domesticated forms of durum wheat represented by *dicoccum*, while *polonicum* and *durum* would have appeared more recently. Our results confirm those obtained by Tang et al. [7], who studied the DMC1 gene and found that subsp. *turgidum* clusters with subsp. *dicoccum* are part of the same clade, while subsp. *durum* and subsp. *polonicum* are part of a different clade.

3.4. MITE as a Subspecies Marker

Molecular markers have been used in the genus *Triticum* to differentiate species with high phenotypic similarity. Thus, Czajkowska et al. [61] designed a test based on Ppd-1 gene variation that allows discriminating between the tetraploid species *T. turgidum* from *T. timophevii*, which are morphologically very similar and lead to misclassification errors. In our work, the subspecies *turgidum* and *durum* are morphologically very similar with naked and nonbrittle spikes. From a practical point of view, the identification of the MITE element insert has a useful application as most subsp. *turgidum* landraces have the insert (94.6%) while subsp. *durum* landraces lack it (97.9%), allowing the differentiation of the two subspecies with a high degree of accuracy.

4. Conclusions

There is great variability in the RSA of the Spanish subspecies *dicoccoides*, *dicoccum*, *turgidum*, *durum* and *polonicum*, the last two having the longest and deepest roots. The insertion of an incomplete MITE element in the 5′ UTR region of the *TtDro1B* gene has been identified in genotypes of *dicoccoides*, *dicoccum* and *turgidum* subspecies, but not in *polonicum* and only in the 2.09% of the *durum* accessions, and in none of the 55 genotypes of *Ae. speltoides*, *searsii*, *bicornis* and *longissima* studied. The results of this study seem to suggest that it is likely that the insertion of the MITE element occurred in a single plant from which all genotypes with the MITE element are derived. However, in order to have

more evidence for this hypothesis, it would be interesting to extend the study to materials from other countries, especially from the Middle East. Genotypes with the MITE element have shallower and less developed roots. The MITE element inserted in the *TtDro1B* gene serves to differentiate, in most genotypes, the subspecies *turgidum* and *durum*.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/agronomy11112294/s1, Table S1: List of accessions analysed including the species and subspecies, the GenBank accession number provided by The Spanish National Plant Genetic Resources Centre (CRF-INIA-CSIC Alcalá de Henares, Spain) and the Leibniz Institute of Plant Genetics and Crop Plant Research (IPK Gatersleben, Germany), the geographic origin, type of material, and if the fragment of the MITE element is not present (0), or present (1).

Author Contributions: Conceptualization, J.M.G. and Y.L.; methodology, R.C., A.C., Y.L. and J.M.G.; formal analysis, J.M.G. and Y.L.; resources, M.R.; writing—original draft preparation, J.M.G. and Y.L.; writing—review and editing, J.M.G., Y.L., M.R. and P.G.; project administration, P.G. and Y.L.; funding acquisition, Y.L., M.R. and P.G. All authors have read and agreed to the published version of the manuscript.

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