

## ROOT SYSTEM ANALYSIS OF DURUM WHEAT UNDER DROUGHT STRESS USING HIGH-THROUGHPUT PHENOTYPING METHOD

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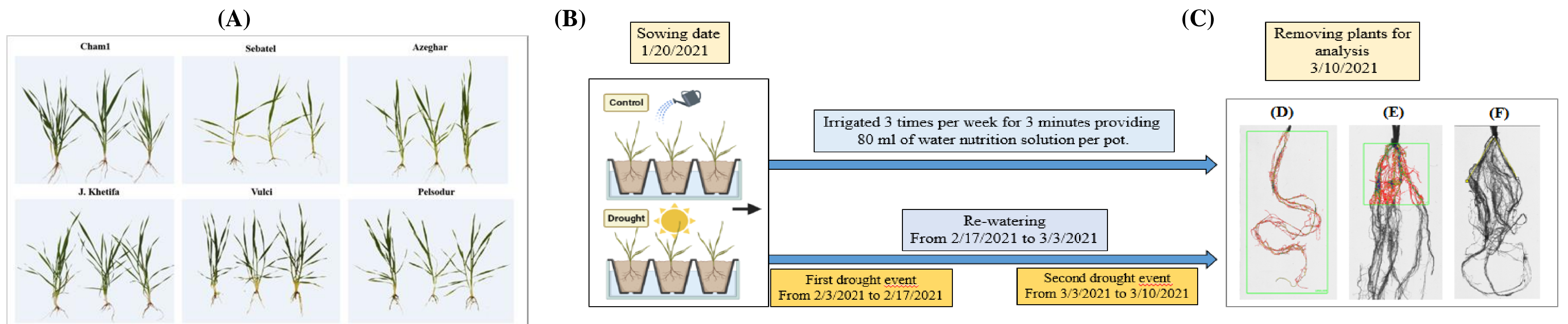
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### Aim of the study

The aim of the study was to analyze roots system diversity in six contrasting durum wheat genotypes using the high-throughput phenotyping software Win-RHIZO, to identify root traits associated with drought tolerance, and genetic diversity using SSR markers.

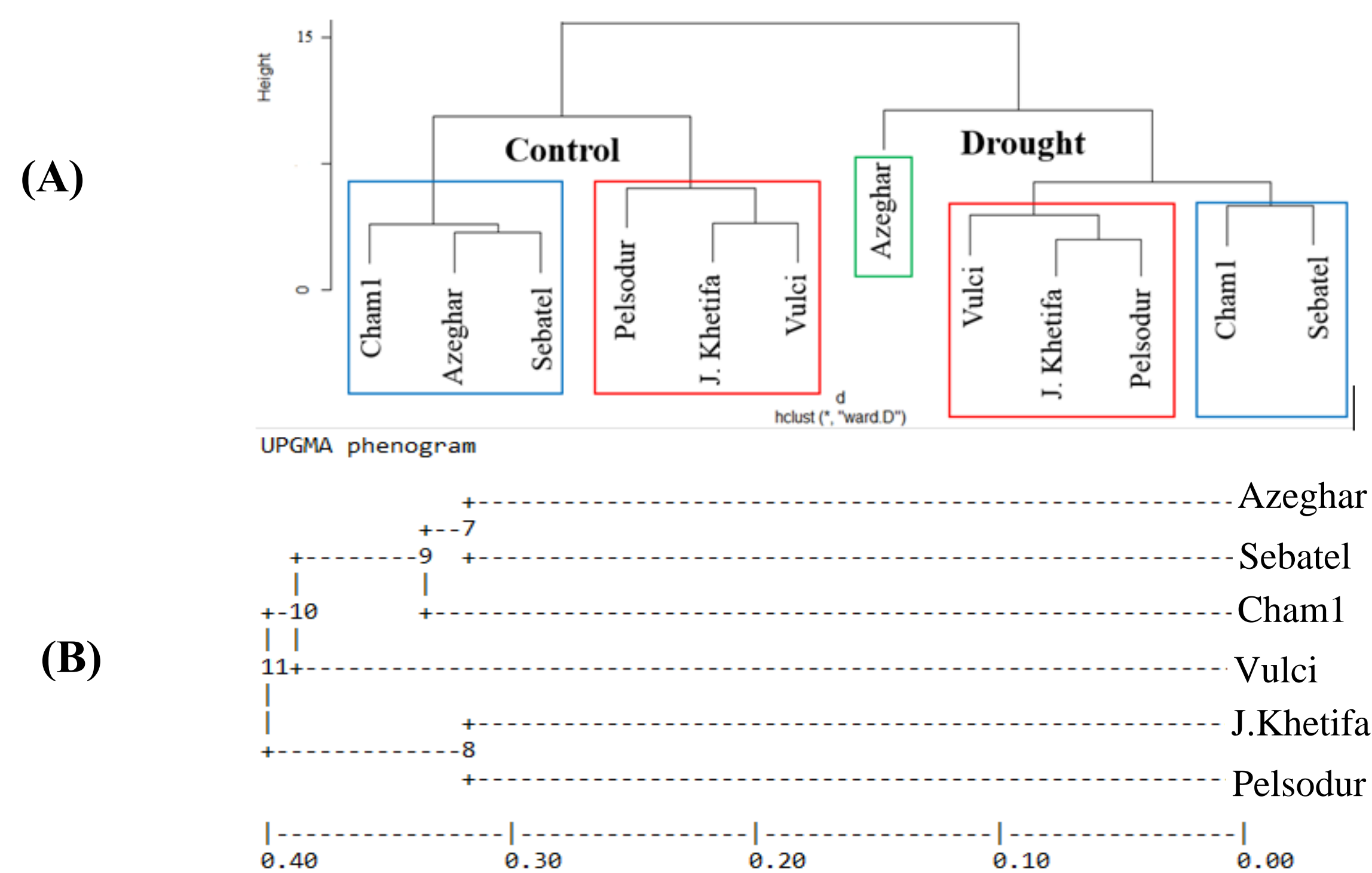
### Material and methods

Two drought events were applied on six durum wheat genotypes in 49 days from seedlings stage (Figure 1A,B). The morphological traits of the roots were recorded both for the whole root system and separately in the first 5 cm below ground (Figure 1C). Root phenotyping data were validated with results from plants grown under rainfed field conditions (Figure 1A). The PCR amplifications were carried out, on genomic DNA extracted from fresh leaves of each genotype, with SSR markers associated with root traits (Tables 1 and 2).



**Figure 1.** A) differences among genotypes used, B) experimental design, C) morphological traits for whole roots (D), topsoil (E) using Win-RHIZO system such as root length, surface area, root volume, number of tips, forks, and crossings, and root angle (F) using ImageJ (°).

**Results:** This study highlighted the presence of a great variability among the analyzed genotypes, the two hydric stress-tolerant genotypes, Cham1 and J.Khetifa, showed different root system ideotypes and rooting patterns under drought conditions. J. Khetifa, Pelsodur and Vulci showed a steep and long root system ideotype that led to maintain the highest root biomass, length, and volume under drought conditions, while Cham1 and Sebatel were distinguished by a wider root system ideotype, increasing the root volume in topsoil under drought conditions (Figure 2A). The genetic distance clustering using the UPGMA phenogram model (Nei, 1972) by selected SSR markers, and the observed phenotypic data under drought conditions, discriminate the tested genotypes almost into the same three clusters (Figure 2B). Moreover, a significant positive correlation was obtained between the root angle of plants grown under greenhouse conditions and natural field conditions.



**Figure 2.** The hierarchical analysis (A) of phenotypic data under drought conditions, and dendrogram (B) of the genetic relationships among six durum wheat genotypes based on SSR markers.

The co-dominant SSR markers associated with specific morphological traits were used to determine the genetic characteristics such as Na-No. of Alleles, I-Shannon's Information Index =  $-1 * \sum(\pi * \ln(\pi))$ , Ho-Observed Heterozygosity, He-Expected Heterozygosity, uHe-Unbiased Expected Heterozygosity, PIC-polymorphism information content (Table 1). Six SSRs markers from our study, wms5, gwm636, wmc727, gwm427, and gwm499 were found positioned onto the durum wheat reference genome (Svevo physical map). These marker locations are overlapped with previously reported root-related QTLs (Table 2).

Table 1. Genetic diversity in durum wheat based SSR markers.

Locus	Na	I	Ho	He	uHe	PIC	Size, bp
wms5	3	1.011	0.000	0.611	0.667	0.536	179-190
gwm427	5	1.561	0.000	0.778	0.848	0.744	201-255
gwm499	4	1.330	0.000	0.722	0.788	0.671	147-197
gwm636	3	1.078	0.500	0.653	0.712	0.579	110-132
wmc727	2	0.679	0.833	0.486	0.530	0.368	88-96
gwm573.2	5	1.314	0.500	0.667	0.727	0.622	194-240

Table 2. Markers associated with QTL from GrainGenes database.

Marker	Chr.	QTL	Traits
wms5	3A	QTL1778,	Root tips
		QTL1530	primary root diameter
		mtaq-3A.5	primary root length, and mean of lateral root diameter
		mtaq-3A.6	
		mtaq-3A.7	total lateral root surface, and volume
		QTRN1-3A	total root number
gwm427	6A	QTL1567	total root length
		QTL1568	root growth angle
		QTL1569	total and average root length
		QTL1570	primary root length
		QTL1571	primary root surface
		QTL1463	root tips
gwm499	5B	QTL1464	root length
		QTL1465	root surface area
		QTL0509	total root length
		QTL1514	lateral root number per primary root
gwm636	2A	QTL1515	total root number
		QTL0440	root growth angle
		QTL0504	
wmc727	5A	QTL1952,	average root length and primary root length
		QTL0653,	
		QTL0653,	

### Conclusion

This study highlighted that the analysis of phenotypic responses to drought stress using Win-RHIZO scanner together with molecular analysis can provide useful information to increase the efficiency of abiotic stress-tolerance breeding programs.

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