

# Identification of homeologous *TdDRF1* gene in wheat wild relatives *Triticum urartu* and *Aegilops speltoides*

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## INTRODUCTION

Drought is one of the major constrain that affects the yield of crop quantitatively and as well as qualitatively, leading to severe starvation of food and feed. Several genes have been discovered related to drought tolerance. Among them few are transcription factors that regulate the expression of many other downstream genes, which play vital role in drought tolerance. The *TdDRF1* gene identified in *Triticum durum* is a transcription factor belonging to DREB gene family (Latini et al., 2007). Homologous *TdDRF1* genes have also been identified in the progenitors of durum and bread wheat, *Triticum urartu* and *Aegilops speltoides*. *Triticum urartu* (2n=2x=14) belongs to the einkorn wheat group and comprises the genome A for all polyploid wheat. It was originated from South West Asia and presents similar morphology with *Triticum monococcum*. Although *Triticum urartu* possesses the genome A, its genetic potential has not been investigated as much as the donor species of genome D to the bread wheat (MacFadden and Sears, 1946; Dvorak, 1988). *Aegilops speltoides* (2n=2x=14) is a diploid progenitor of B genome for the modern wheat (Dvorak and Zhang, 1990), which belongs to sitopsis section. This species is naturally present in South Eastern Europe, Asia temperate, and Western Asia. Most of the evidences and recent research suggest that *Aegilops speltoides* genome (S genome) could be the B genome, progenitor of wheat (Friebe et al., 2000). However the origin of modern wheat B genome is still controversial. The exploitation of wild relatives for plant breeding could be helpful to enhance the resistance to abiotic stress like drought in modern wheat. The *TdDRF1* homeologous gene is being analyzed focusing principally on single nucleotide polymorphisms (SNPs) to decipher the genetic diversity among the natural populations of these two species and to detect possible associations between DNA sequence variations and heritable phenotype. In this work we report the preliminary analysis of inter and intra species genetic diversity based on SNPs molecular marker.



Fig1. *Aegilops speltoides* and *Triticum urartu* in green-house and geographical distribution of the species

DNA was extracted using CTAB. *TdDRF1* gene specific primers for the amplification of the exon 4, as E4for (5'-GTCCACCATTTGATCTTCATT-3') and E4rev (5'-TGATCCACAGGGTGCAA-3'). After the PCR, the DNA bands with the expected size were purified, cloned into TOPO vectors and subsequently sequenced. Sequence analyses were done by using Codon Code Aligner and other bioinformatic tools like Clustal W, Motif Scan and Scan prosite.

## RESULTS AND DISCUSSION

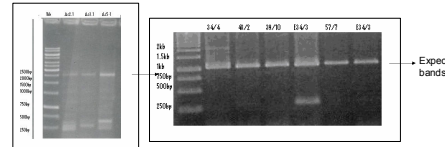


Fig2. Gel pictures showing expected bands: a) PCR amplification of 2.3kb fragment from exon1 to exon 4, 5' terminal region, b) 1Kb fragment of exon4 region

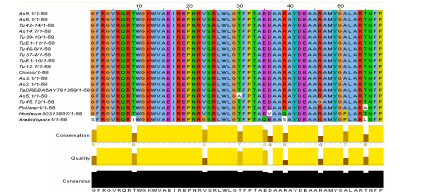
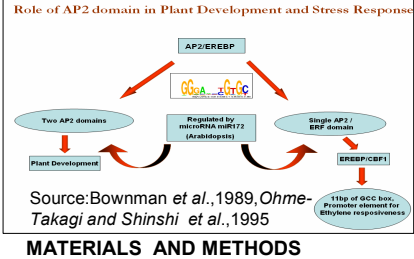


Fig3. The AP2 domain alignment of *Aegilops speltoides*, *Triticum urartu* with other species. *TdDRF1*, NCBI N: EU089820, *HvDRF1* NCBI N: AY223807, *ZmDREB2A* NCBI N: AB218832, *OsDREB* gene NCBI: AY196209, *AtDREB2A* NCBI N: AB016570, *TuDRF1* EU197053, *AsDRF1* NCBI N: EU197054



## MATERIALS AND METHODS

Plant genotypes were obtained from ICARDA, as follows:

Name of the species	Origin	ICAG Nr	IG	Accession
<i>A. speltoides</i>	Syria	403040	117905	21.8
<i>A. speltoides</i> var. <i>speltoides</i>	Turkey	403360	126225	9.1
<i>A. speltoides</i> var. <i>speltoides</i>	Syria	403006	116071	8.1
<i>Aegilops speltoides</i> var. <i>hispida</i>	Iraq	402444	48994	2.1
<i>Aegilops speltoides</i> var. <i>hispida</i>	Turkey	401390	47948	3.1
<i>A. speltoides</i> var. <i>speltoides</i>	Syria	402924	110757	5.1
<i>A. speltoides</i> var. <i>speltoides</i>	Syria	400730	47280	12.1
<i>T. urartu</i>	Turkey	600626	46115	45/7
<i>T. urartu</i>	Turkey	600627	46116	46/8
<i>T. urartu</i>	Armenia	500254	45213	E31/3, E31/2
<i>T. urartu</i>	Turkey	500148	45107	E1/1, E1/1
<i>T. urartu</i>	Lebanon	500530	45489	E33/6, E33/7, E33/13, E33/14
<i>T. urartu</i>	Syria	60009	45488	E35/1, E35/2
<i>T. urartu</i>	Syria	500333	45292	E36/5, E36/11, E36/12
<i>T. urartu</i>	Syria	50002	45205	E37/2, E37/10, E37/4
<i>T. urartu</i>	Iraq	500532	109084	B42/14
<i>T. urartu</i>	Syria	600392	45521	E11/2

**Theoretical analysis of amino acids:** *Triticum durum* (AABB), *Triticum urartu* (AA) and *Aegilops speltoides* (BB) sequences were compared with other crops and Arabidopsis. Clustal W alignment results reveal that there is no much variation in the AP2 domain. Even though some amino acid changes occur across the species, secondary structure prediction using HNN software reveals that there is no much structural change in AP2 domain. Some of the amino acids such as Alanine has been replaced by Threonine in *A. speltoides* accession 5.1 whereas in other *A. speltoides*, threonine remain the same. Likewise in *Triticum urartu* 45/7, Arginine was replaced by Glycine. Valine and Glutamic acid are conserved in ERF/AP2 domain. Valine plays an important role in DNA binding (Sakuma Y. et al 2002). In our analysis we did not find any replacement for these two amino acids. So the change in other amino acids, hypothetically, does not affect the DNA binding. A single amino acid change in the AP2 domain like valine (important for DNA binding) will cause enormous variation, if the mutation is non synonymous. We could observe less amino acid variation in Arabidopsis than in other Poaceae family plants concerning the AP2 domain.

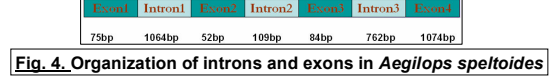


Fig. 4. Organization of introns and exons in *Aegilops speltoides*

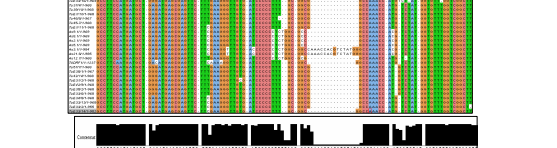


Fig. 5. Nucleotide alignment of Exon 4 region, where we found more SNPs and duplications

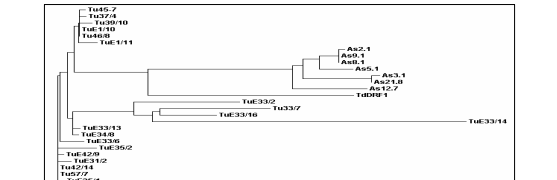


Fig. 6. Phylogenetic analysis of nucleotide sequences -exon 4 part

**Nucleotide analysis and SNPs variations:** *Aegilops speltoides* DRF1 and *Triticum urartu* DRF1 gene is about 3.2 kb. It consists of four exons and 3 introns (See Fig. 4 for *As* gene organization). A part of exon 4 region code for AP2 domain and it is highly conserved across all the species. Fig. 5 show the results of nucleotide alignment from 950 to 1060 bp near by 3'UTR region, which is the most variable region of the whole exon. Several SNPs were observed. At the position of 963, in just one genotype of *As*, Alanine is replaced by Glycine, while at the position 981, cysteine and threonine are present with the same probability in both *Aegilops speltoides* and *Triticum urartu* species. Some accessions of *Aegilops speltoides* (2.1 & 3.1) show the duplication of some nucleotides near by 3'UTR, at the positions from 1010 to 1027. Phylogenetic analysis (Fig. 6) show that the species *Triticum urartu* DRF1 TuE33/4 is the most diverging accession, *Aegilops speltoides* DRF1 accessions make a cluster with *Triticum durum* DRF1 and all the *Triticum urartu* accessions make further clusters.

Concerning *Aegilops speltoides*, some bands of 500 bp, corresponding to isoforms of *TdDRF1* gene devoided of introns, were collected, showing that further mechanism can be occurred during the evolution of this species.

At nucleotide level, several SNPs were observed throughout the gene, while in well conserved region, like AP2 domain, just a few SNPs were identified

**Gene sequence analysis in Plant Genome Database:** Sequence of *AsDRF1*, *TuDRF1* and *TdDRF1* were compared with other species in the Plant Genome Database. We found *AsDRF1* is more similar to *Triticum aestivum*, concerning the size of both the introns and the exons.

## CONCLUSION AND WORK IN PROGRESS:

We analyzed the whole gene in different accessions of *Triticum urartu* and *Aegilops speltoides*. Our attention was particularly focused on the exon 4, since it is coding for AP2 domain, involved in DNA recognition. We found significant SNP variations and duplication of some regions in exon 4 and variations in other part of the gene, as well. More genotypes will be analyzed shortly to show variations among further accessions and other species, from diverse geographical regions.

We think that the analysis of the SNP variations in these species can be fruitful, for gaining information about the evolution of modern wheat and possibly addressing the controversy concerning the B genome donor and finally for the identification of useful ancestor genes related to tolerance.

We want to acknowledge ICARDA and WGGRC gene banks for providing the genotypes used in this study.

## REFERENCES

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