

Study of the function of the TdDRF1 proteins

A. Latini¹, M. Sperandei¹, P. Galeffi¹
¹ENEA-Casaccia, BAS-BIOTEC GEN, Rome, Italy
 E-mail of presenter author: arianna.latini@casaccia.enea.it

ABSTRACT: The DREB proteins are important plant transcription factors that induce the expression of a set of abiotic stress-related genes. The durum wheat *TdDRF1* (Latini *et al.* 2007) is an *AtDREB2A*-homologous gene (Shinozaki & Yamaguchi-Shinozaki 1997), but it shows a different genomic structure producing three alternative splicing variants (1.1, 1.2 and 1.3). At the moment no direct information regarding the *TdDRF1* encoded proteins and their actual function is available, even though it was already established that TdDRF1.1 and TdDRF1.3 are putative transcriptional activators. Hence, we are attempting to elucidate the function of the three gene products. In plant functional genomics studies, the gene overexpression represents a very useful strategy to determine the function and molecular partners of a gene of interest, in particular for regulatory proteins that are low abundant into the cell. A heterologous transient expression system, by means of a Potato Virus X (PVX)-derived expression vector, was considered suitable for getting preliminary indications regarding the *TdDRF1* gene function. The three full-length cDNAs were isolated and three plasmid constructs for the transient overexpression in *Nicotiana benthamiana* and *Nicotiana tabacum* were engineered. The comparative analysis of both control and overexpressing plants, by microarray (transcriptome analysis) and/or by 2 dimensional gel electrophoresis of proteins (proteome analysis), will allow determining the changes that each transcript produces in the total plant expression profiles. Furthermore, in order to obtain specific antibodies and crystal structures, the three full-length cDNAs were expressed in bacteria for the production and purification of the TdDRF1 proteins.

5' AND 3' RACE, CHARACTERISTICS OF FULL-LENGTH cDNAs AND CODIFYING REGIONS (CDSs) OF TdDRF1 GENE

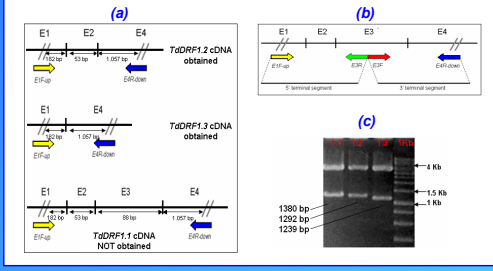
RNA extracted from **Ciccio** durum wheat variety was used to isolate the full-length cDNAs by 5' and 3' RACE (Rapid Amplification of cDNA Ends) and the CDSs of the three mRNAs. Their characteristics are reported in the table below:

Transcript	Full length cDNA length (bp)	Full length cDNA completion and UT (Exons-Introns) (bp)	CDS length (bp)	Predicted M.W. of the protein (kDa)
TdDRF1.1	1590 bp	5' UTR: 132 bp E1: 38 bp E2: 53 bp E3: 38 bp E4: 93 bp 3' UTR: 279 bp	393 aa	4231.9
TdDRF1.2	1502 bp	5' UTR: 132 bp E1: 38 bp E2: 53 bp E4: 93 bp 3' UTR: 279 bp	63 aa	7036.0
TdDRF1.3	1449 bp	5' UTR: 132 bp E1: 38 bp E2: 53 bp E3: 38 bp E4: 93 bp 3' UTR: 279 bp	369 aa	40185.9

Gene Bank Accession numbers:
Ciccio TdDRF1.1: DQ013204
Ciccio TdDRF1.2: DQ011890
Ciccio TdDRF1.3: DQ013205

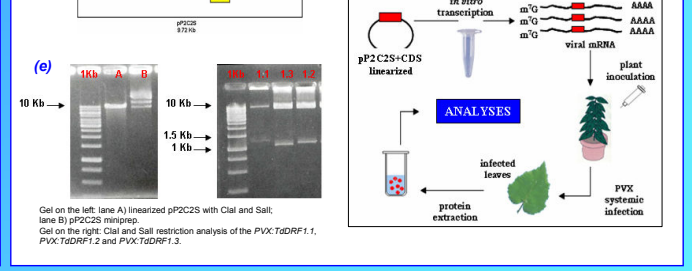
CLONING OF THE COMPLETE CDSs OF TdDRF1.1, TdDRF1.2 AND TdDRF1.3

The CDSs of TdDRF1.2 and TdDRF1.3 were amplified by using *E1F-up* (5'-CAGCACTCTCCCAACCTCTC-3') and *E4R-down* (5'-GGTCCACCATTTGATCTTCATT-3') primers (a). To selectively amplify the TdDRF1.1 CDS, a 5' terminal and a 3' terminal segments were amplified and joined together (b). The resulting PCR products were cloned into TOPO vectors (Invitrogen) (c).



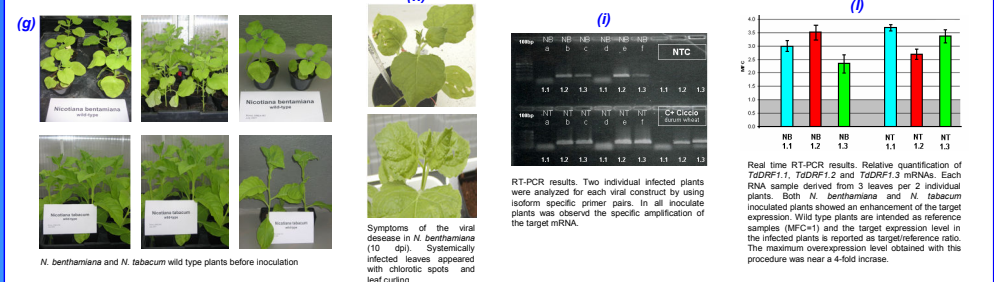
ENGINEERING OF THE CONSTRUCTS FOR TRANSIENT OVEREXPRESSION AND INFECTION IN PLANT

The heterologous overexpression of the three transcripts in *N. benthamiana* and *N. tabacum* plants was achieved by using the PVX-derived viral vector pP2C2S (Chapman *et al.* 1992) (d). The three CDSs were cloned between *Clal* and *Sall* restriction sites (e) and the final chimeric constructs were sequenced before their use in subsequent *in vitro* transcription and plant infection, as schematically shown (f).



PLANT INOCULATION AND mRNA EXPRESSION ANALYSES OF THE INFECTED PLANTS

Both *Nicotiana* species, inoculated with PVX:*TdDRF1.1-1.2-1.3* viral constructs, developed typical viral symptoms on leaves after 7-10 days post inoculation (dpi) in contrast to the mock inoculated plants (g), indicating a systemic infection of the plants (h). To prove for virus replication and *TdDRF1.1-1.2-1.3* transcripts accumulation, samples were collected at 7 dpi and total RNA was extracted for RT-PCR (i) and real-time RT-PCR (j) analyses. To discriminate the exogenous from the endogenous expression, the relative abundances of the transcripts in wild type and in infected plants were compared by means of real-time RT-PCR.



CONCLUSIONS

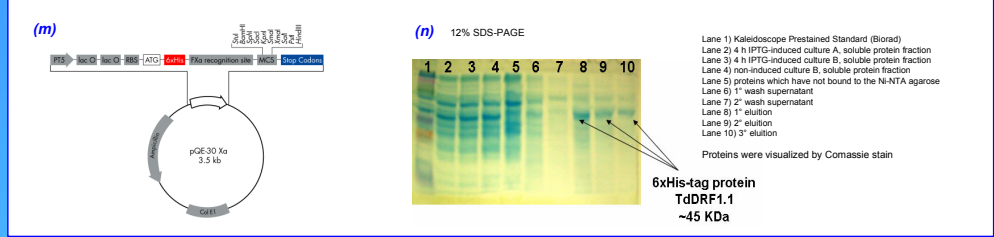
- 1) The three full-length *TdDRF1* transcripts were isolated and cloned. It is worth to note that the cloning of the 1.1 variant has not been reported and the studies on barley and bread wheat are based on the 1.3 variant.
- 2) The viral constructs were obtained for the three isoforms and the successful infection of *N. benthamiana* and *tabacum* plants was demonstrated by detection/quantification of the target mRNAs.
- 3) The three CDSs for the putative TdDRF1 proteins were cloned in a bacterial expression/purification vector. At the moment the TdDRF1.1 protein was observed in the soluble native fraction. Generally, the recombinant protein solubility is indicative of a good conformational quality and biological activity.

FUTURE WORK

- 1) Comparative proteomic and transcriptomic analyses between overexpressing and wild type plants to investigate the molecular pathway.
- 2) TdDRF1 proteins isolation from plants for further functional genomics studies (DNA-binding, partners of interaction, post-translational modification, etc.).
- 3) Investigation focused on the TdDRF1.2 isoform, whose function is completely unknown.
- 4) Set-up of a large-scale bacterial protein extraction and purification from the soluble protein fraction under native conditions.
- 5) Antibody anti-TdDRF1 proteins production.
- 6) Set-up of the heterologous protein crystallization, in view of a X-ray structural analysis. This would be highly desirable in the near future, given the scarcity of deposited 3D plant protein structures.

EXPRESSION AND PURIFICATION IN E. COLI OF TdDRF1 PROTEINS

The three TdDRF1 CDSs were also cloned into a vector for the heterologous expression in bacteria. For this purpose the pQE-30 Xa vector (Qiagen) was chosen, because it is provided of a tag (6xHis) for the purification and, in addition, it presents a recognition site for the Xa protease, which allows the elimination of the His-tag (m). Vector and complete CDS were joined together by *Stul* and *HindIII* ends. After cloning and sequencing, the expression was induced in M15 *E. coli* strain by adding 1mM IPTG in the liquid culture. Non induced cultures (-) and induced ones (4 hours after induction) were used for protein extraction. Preliminary results concerning TdDRF1.1 protein are showed below (n).



SPEIAL THANK'S

C. Cantale and E. Palmieri (ENEA-Casaccia, BAS-BIOTE GEN, Rome, Italy)
 Dr. M. Iannetta (ENEA-Casaccia, BAS-BIOEC GEN, Rome, Italy)
 Dr. K. Ammar (CIMMYT, Mexico)

FUNDINGS

FRIDE project (ENEA DES) 2003-2006
 FRUMISIS (2004-2006)
 MAE Relevant Projects Italy-Mexico (2007-2009)

REFERENCES

Chapman *et al.* Potato Virus X as a vector for gene expression in plants. *Plant Journal*, 1992.
 Latini *et al.* Identification of a DREB-like gene in Triticum durum and its expression under water stress conditions. *Annals of Applied Biology*, 2007.
 Latini *et al.* TdDRF1: expression profile analyses under greenhouse conditions and genotype characterization in some drought tolerant cultivars (*Triticum turgidum* L. and *Triticale*). Submitted to *Theoretical and Applied Genetics* (April 10, 2008).
 Sakuma *et al.* Functional analysis of an *Arabidopsis* transcription factor, DREB2A, involved in drought-responsive gene expression. *Plant Cell*, 2006.
 Shinozaki & Yamaguchi-Shinozaki. Gene xpression and signal transduction in water-stress response. *Plant Physiology*, 1997.