



FROM GENE TO GENOMICS: THE *DRF1* GENE

Karthikeyan Thiyagarajan¹, Arianna Latini¹, Domenico Di Bianco^{1,2},
Cristina Cantale¹, Karim Ammar² and Patrizia Galeffi¹

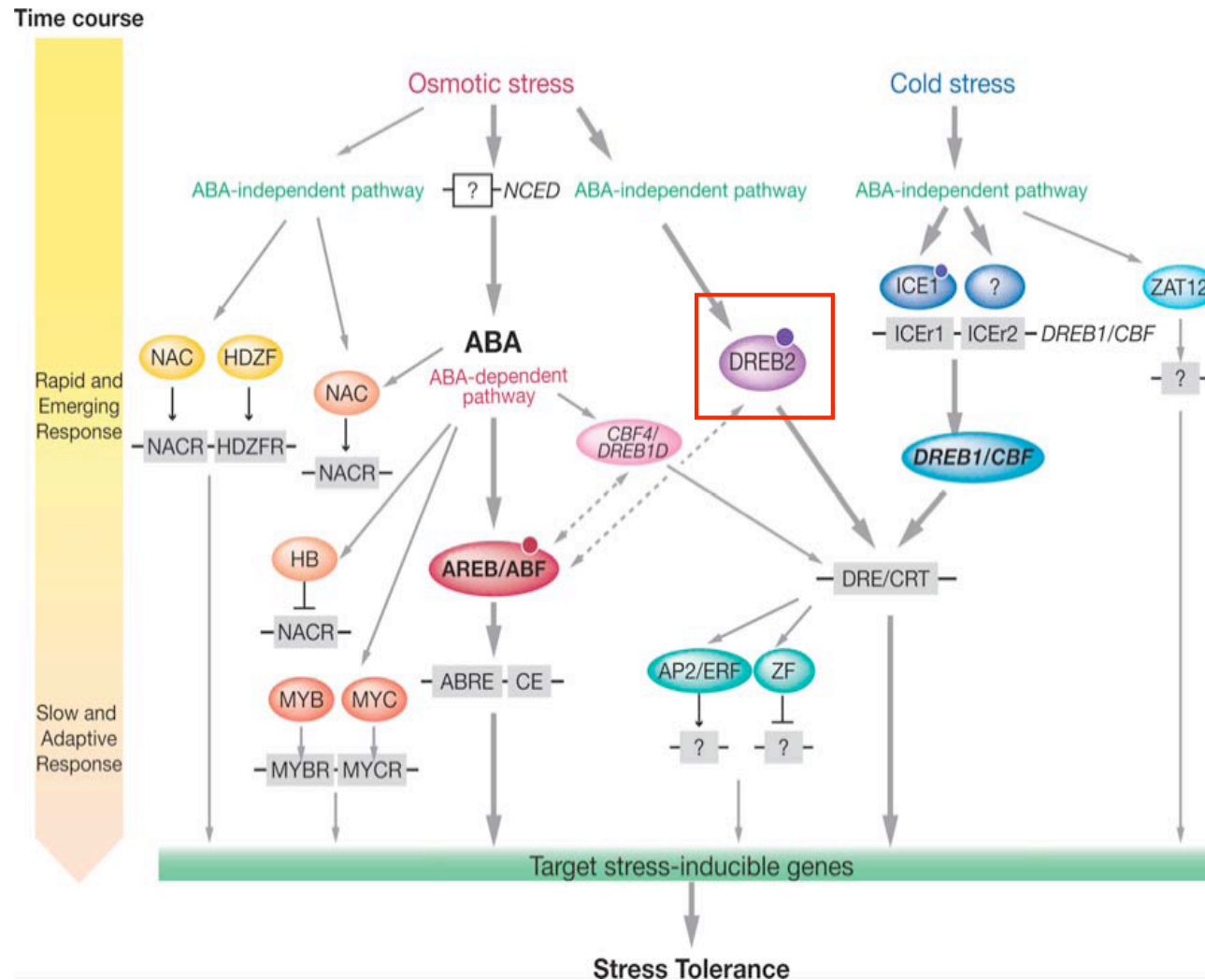
¹ENEA CR Casaccia, Rome, Italy

²CIMMYT (Centro Internacional de Mejoramiento de Maíz y Trigo), México



THE CONTEXT

CLIMATE CHANGES AND DESERTIFICATION



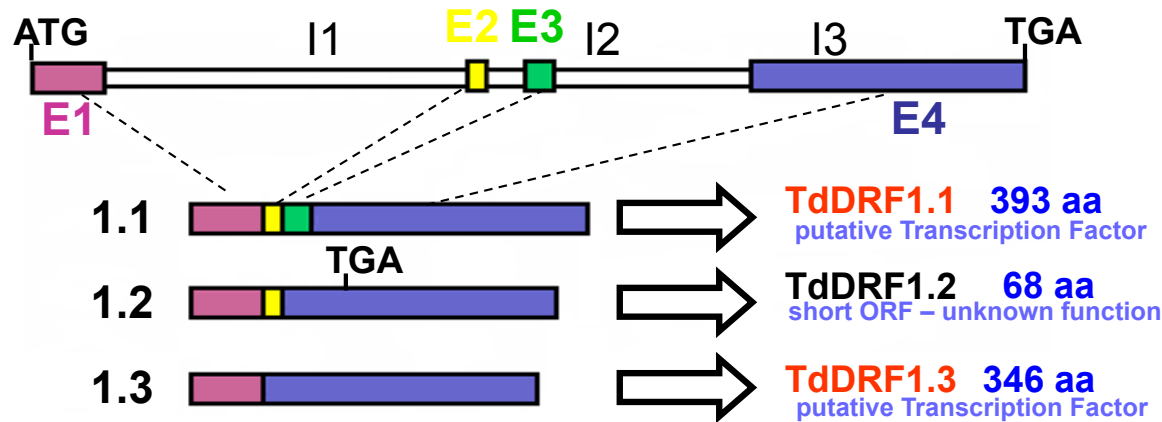
➔ **CIS-AGENT ELEMENT:**
DRE sequence
(Dehydration Responsive Element: TACCGACAT), in the promoters of several stress-inducible genes

➔ **TRANS-AGENT ELEMENT:** DREB proteins bind the DRE sequence in the promoters of the target genes

DRF1:

A DREB2-FAMILY GENE IN DURUM WHEAT

TdDRF1 GENE STRUCTURE



Facts:

~ 3.4 kb
promoter ~1200bp

The gene is located on Chr 1 in the two genomes, A and B, and is present also in Chr 1 of genome D, as revealed searching in IWGSC Sequence Repository at URGI: (<http://urgi.versailles.inra.fr/Platform>).

<http://urgi.versailles.inra.fr/Platform>

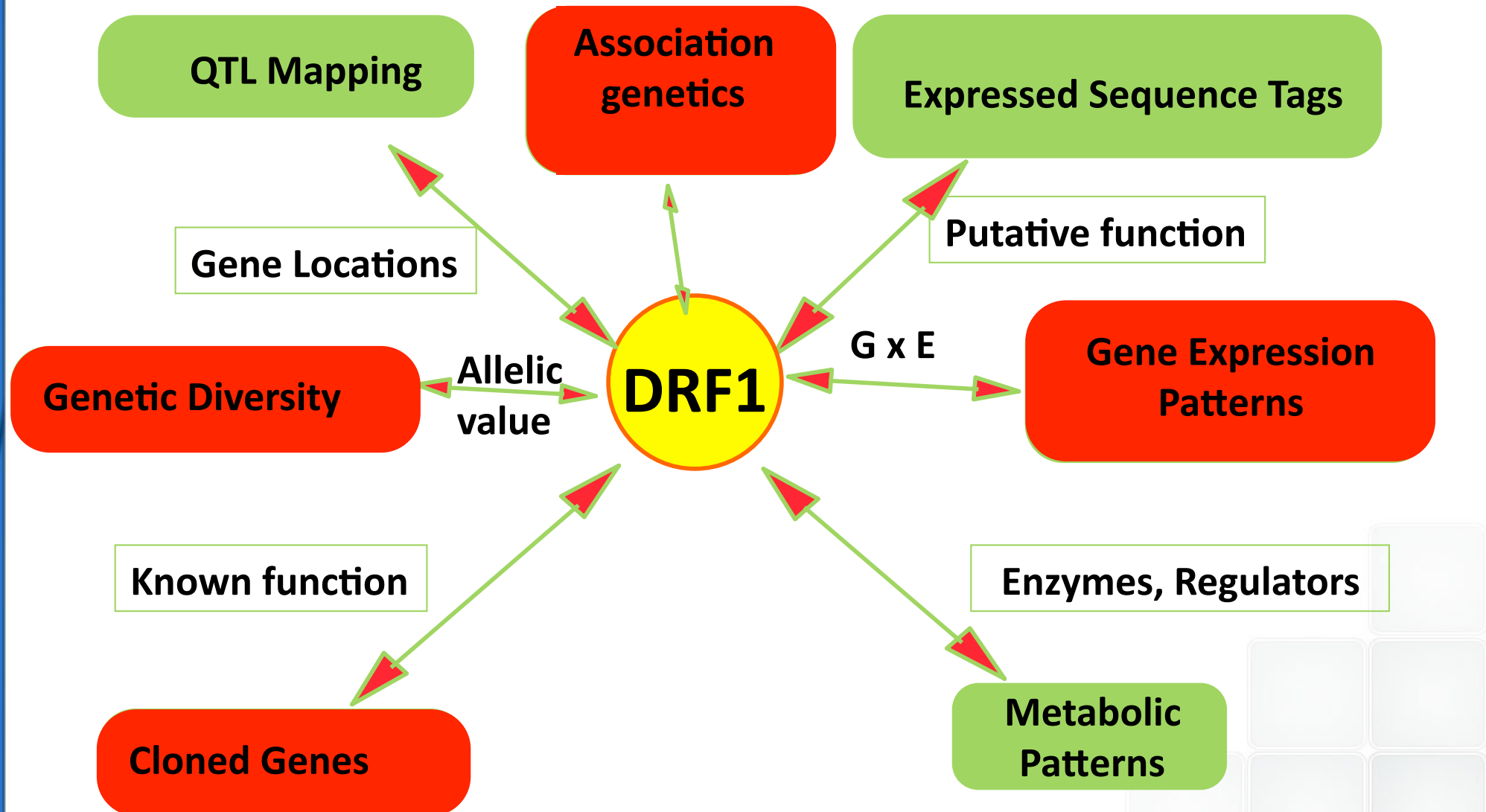
The gene expresses three transcripts through alternative splicing and this gene structure is shared in Liliopsida (monocotyledons) class

DREB2 gene structure in Arabidopsis (dicotyledons) consists of one single CDS

Latini A, Rasi C, Sperandei M, Cantale C, Iannetta M, Dettori M, Ammar K, Galeffi P 2007 Identification of a DREB-related gene in Triticum durum and its expression under water stress conditions. *Ann. Appl. Biol.*, 150:187-195

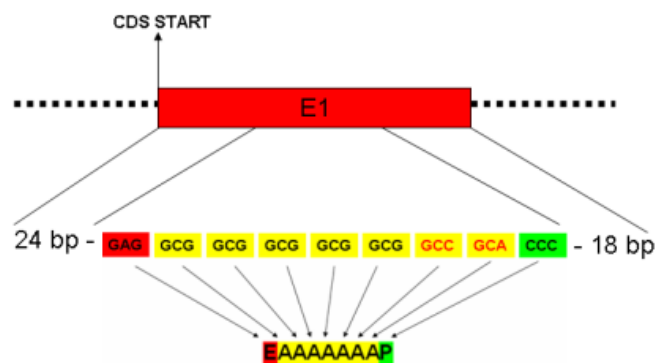
Gene sequence NCBI Accession EU089819

VARIOUS APPROACHES FROM MOLECULAR GENETICS



Analysis of the Genetic Variability of *DRF1* gene in durum wheat varieties, including wild ancestors species, a RIL population and triticale lines

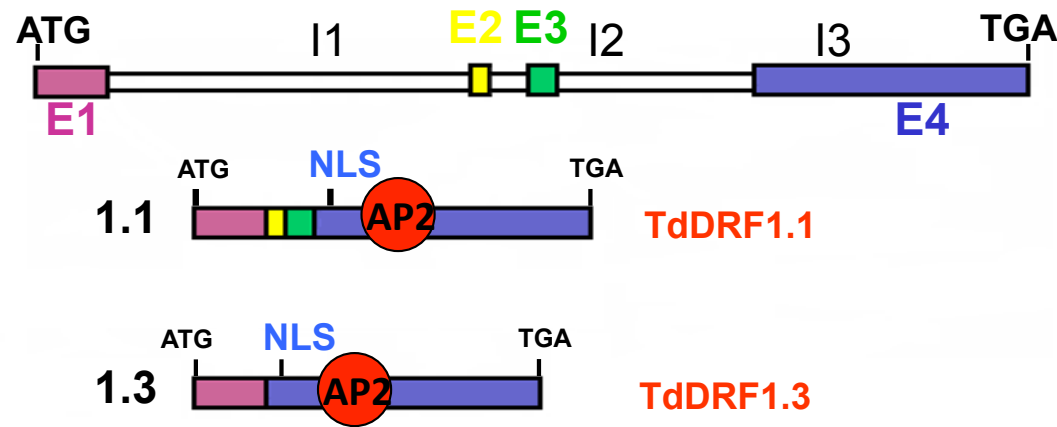
SNPs in the whole gene including UTRs a translated SSR in Exon 1



| | | |
|------------------|-----|--|
| EAAAAAAP | 34% | cDNA from many durum cultivars, as Ciccio, and some pseudogene forms from <i>Aegilops speltoides</i> |
| ESAAAAALP | 21% | cDNA from pseudogene form from Ciccio durum cultivar |
| EAAAAAAP | | cDNA from <i>Leymus chinensis</i> |
| EAAAAAATP | 16% | cDNA from many durum cultivars, as Cannizzo, and some gDNA from <i>Triticum urartu</i> accessions |
| EAAAAAAP | 26% | cDNA from many durum cultivars as Karalis, Duilio, Creso and Colosseo |
| ETAAAAAP | 4% | cDNA from Triticale Pollmer |
| EAAAAAP | 4% | gDNA from <i>Aegilops speltoides</i> and <i>Aegilops tauschii</i> accessions |
| EAAAAAP | 2% | gDNA from <i>Aegilops speltoides</i> |
| EAAATP | 12% | gDNA from many <i>Triticum urartu</i> accessions |
| VAAAP | | gDNA from <i>Brachypodium distachyon</i> |

| AMINOACIDIC FORM | NUCLEOTIDIC SEQUENCE |
|------------------|-------------------------------------|
| 7 A | GAG GCG GCG GCG GCG GCG GCC GCA CCC |
| 6 A + 1 T | GAG GCG GCG GCG GCG GCG GCG ACG CCC |
| 1 S + 6 A | GAG TCG GCG GCG GCG GCG GCC GCA CCC |
| 6 A | GAG GCG GCC GCG GCG GCG GCA CCC |
| 1 T + 5 A | GAG ACG GCG GCG GCG GCG GCA CCC |
| 5 A | GAG GCG GCC GCG GCG GCG CCC |
| 5 A | GAG GCG GCC GCG GCG GCG CCC |
| 5 A | GAG GCG GCG GCG GCG GCG CCC |
| 4 A | GAG GCG GCG GCG GCG CCC |
| 3 A + 1 T | GAG GCG GCG GCG ACG CCC |

Exon 4: the core of the functional putative transcription factors



DRF1 IS A DREB2-LIKE GENE, THUS IT BELONGS TO THE AP2/ERF FAMILY

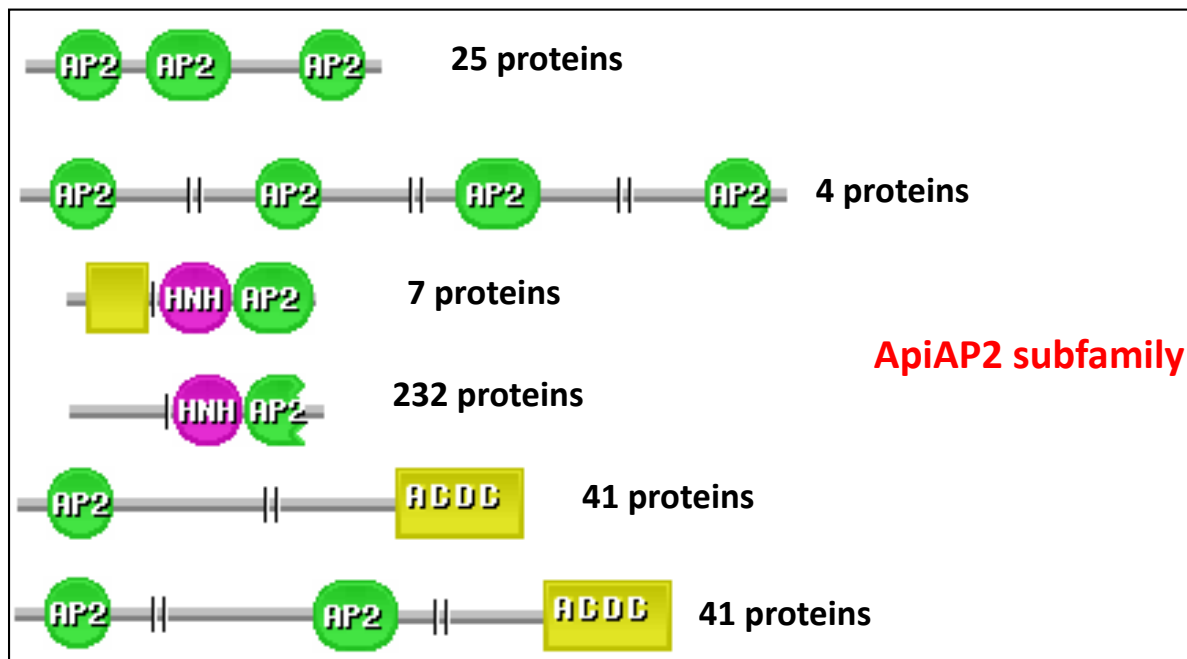
- AP2/ERF proteins contain short stretches of basic aa residues as nuclear localization signal (NLS) usually localized at N-terminus of AP2 domain
- AP2 domain recognizes *cis*-regulatory sequences having a 'CCG' core box on DNA



The AP2/ERF Family

Transcription factors involved in the regulation of developmental processes (flower development, spikelet meristem determinacy, leaf epidermal cell identity, embryo development) and responding to environmental stresses

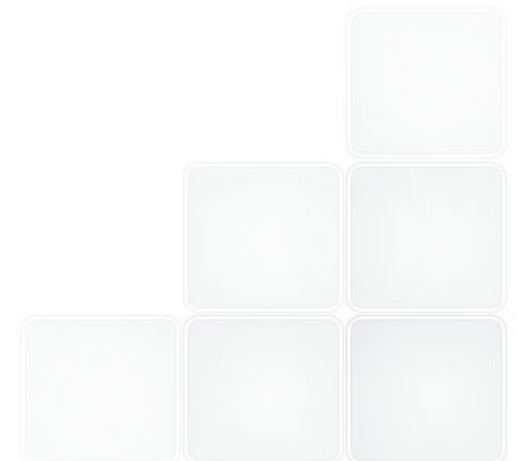
Previously considered exclusive of plants, recently found in ApiAP2 protein family from Apicomplexa, a large group of parasitic protists and in pathogen bacteria

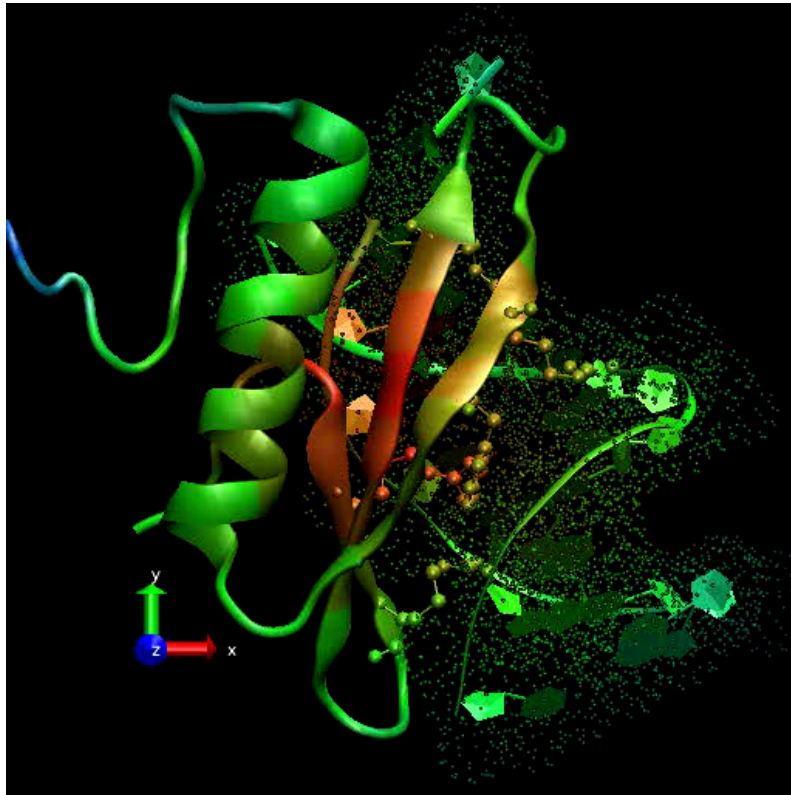


AP2/ERF family: 5868 proteins

Average length of the domain:
52.10 aa

Average identity of full alignment: 39%





Only a 3D structure from plant is available
for the AP2 domain:

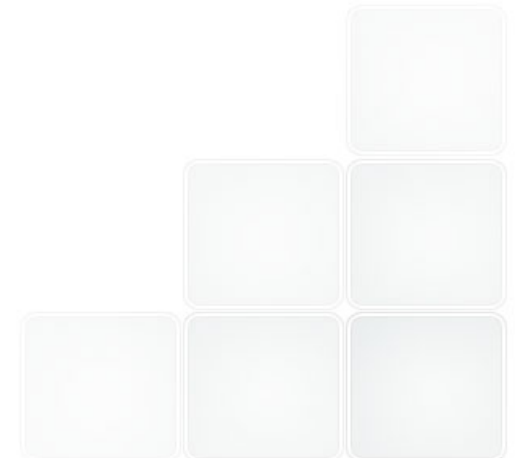
AP2 DOMAIN and its gcc-box DNA of AtERF1 from
Arabidopsis solved by NMR

Allen et al. 1998, Embo J. 17:5484–5496

Ap2 domain → anti-parallel three-stranded β -sheet packed against an α -helix
approximately parallel to the sheet.

Stabilized by extensive hydrophobic contacts of the side-chains

DNA contacts → R and W residues located in the β -sheet





A greenhouse time-course stress experiment

DRF1 expression profiles: RT-PCR of three transcripts in full and reduced irrigation:

- controlled greenhouse

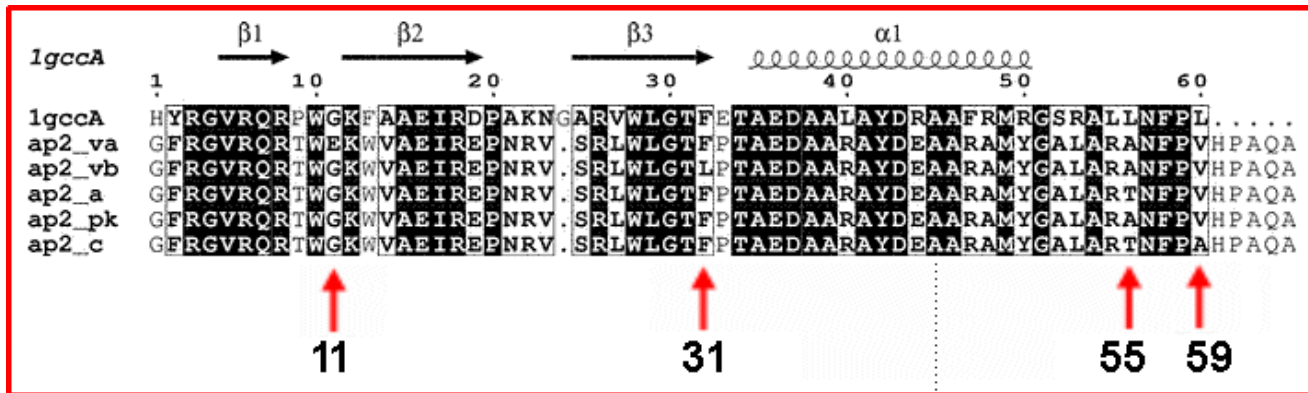
| Transcript | Time | Ciccio | Atil C2000 | Karalis | Vitromax | Pollmer TCL |
|-----------------|------|---------------|---------------|---------------|-------------------|-------------------|
| <i>TdDRF1.1</i> | T0 | 1 | 1 | 1 | n.d. ^a | n.d. ^a |
| | T4 | 6.6 (4.3-8.9) | 1.1 (0.9-1.4) | 1.4 (1.2-1.6) | n.d. ^a | n.d. ^a |
| | T7 | 1.4 (1-1.9) | 2.6 (2.2-3) | 1.1 (0.9-1.3) | n.d. ^a | n.d. ^a |
| <i>TdDRF1.2</i> | T0 | 1 | 1 | 1 | 1 | 1 |
| | T4 | 3.6 (3-4.2) | 1.7 (1.4-2.1) | 2.5 (2.0-3.0) | 0.8 (0.6-1.0) | 2.9 (2.4-3.4) |
| | T7 | 1.7 (1.4-2) | 1.4 (1.2-1.6) | 7.1 (5.5-8.8) | 0.02 (0.01-0.03) | 3.7 (2.9-4.6) |
| <i>TdDRF1.3</i> | T0 | 1 | 1 | 1 | 1 | 1 |
| | T4 | 4.3 (3.3-5.2) | 1.0 (0.7-1.3) | 1.8 (1.4-2.3) | 0.6 (0.48-0.76) | 2.7 (2.2-3.2) |
| | T7 | 1.7 (1.4-2.0) | 0.6 (0.4-0.8) | 9.7 (7.4-12) | 0.1 (0.08-0.12) | 2.3 (1.4-3.3) |



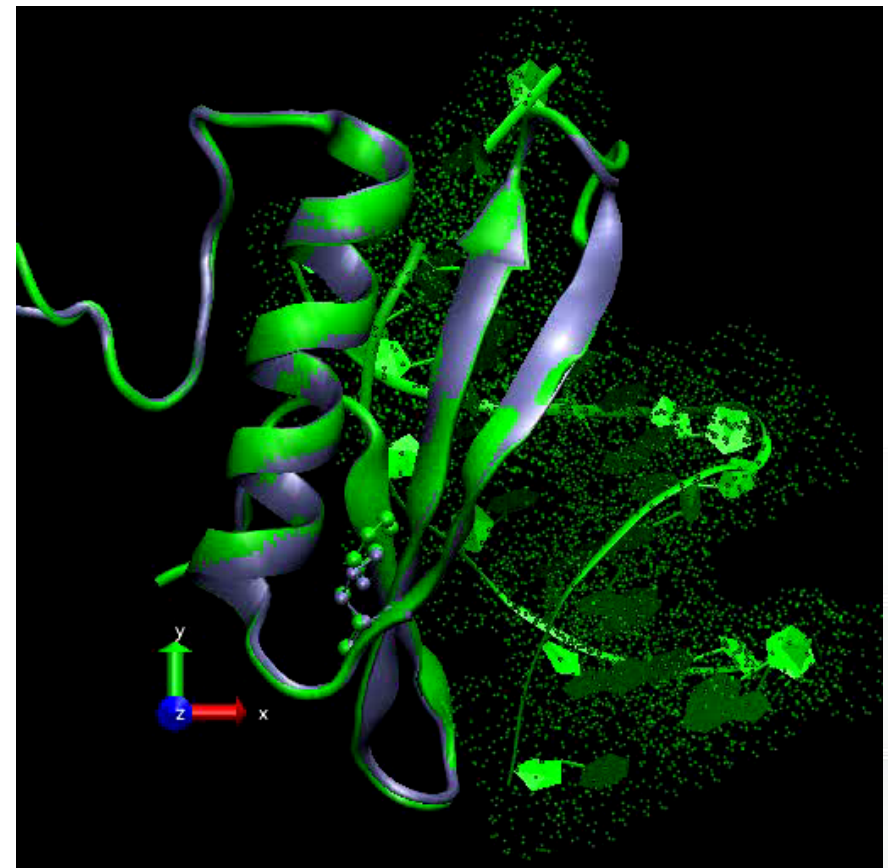
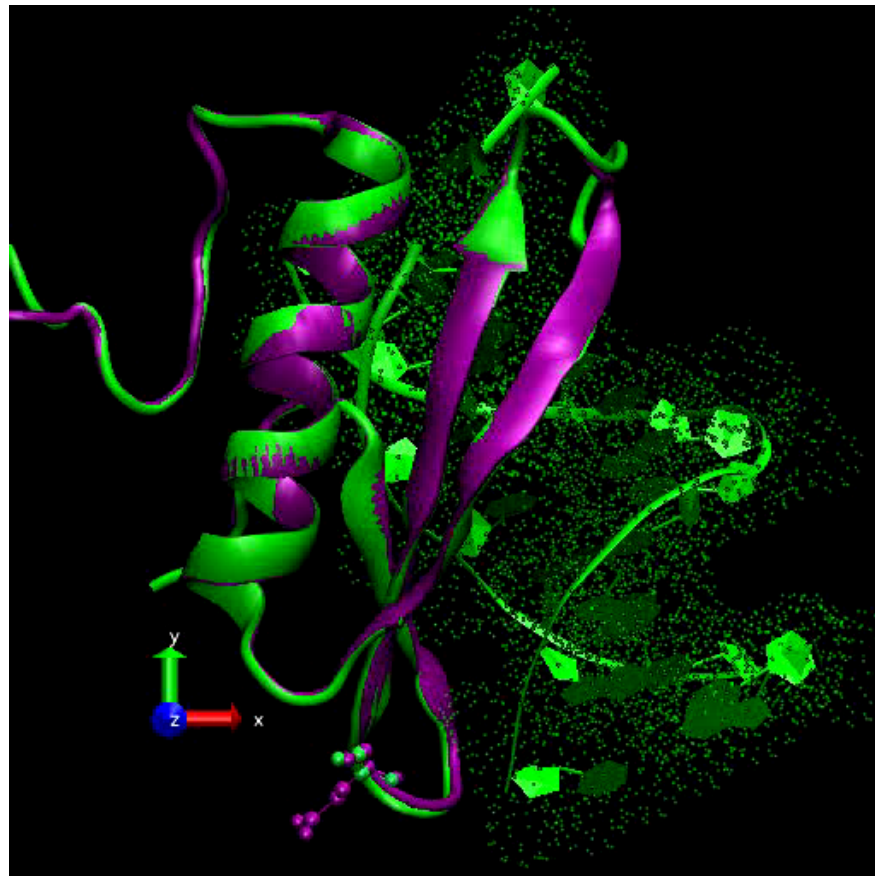
| VARIETY | ENEA GREENHOUSE | ENEA/CIMMYT FIELDS |
|--------------|-----------------|--------------------|
| ALTAR 84 | | + |
| ARCOBALENO | | + |
| ATIL C2000 | + | + |
| BOB WHITE | | + |
| BRADANO | + | + |
| CANNIZZO | + | + |
| CAPEITI 8 | + | + |
| CICCIO | + | + |
| CLAUDIO | + | + |
| COLOSSEO | + | + |
| CRESO | + | + |
| DULLIO | + | + |
| GIALLO-VERDE | + | + |
| GIANNI | + | + |
| IRIDE | + | + |
| JUPARE C2001 | + | + |
| KARALIS | + | + |
| LESINA | + | + |
| LIRON | + | + |
| MERIDIANO | | + |
| MEXICALI 75 | | + |
| PIETRAFITTA | | + |
| POLLMER | + | + |
| PORTORICO | | + |
| QUADRATO | + | + |
| RUSTICANO | | + |
| SIMETO | + | + |
| SOOTY | | + |
| STOT | | + |
| SVEVO | + | + |
| VERDI | + | + |
| YAVAROS 79 | + | + |
| TRITICALE H | | + |

?

Isolation, sequencing, translation and alignment of DRF1 transcripts: variability inside AP2 domain



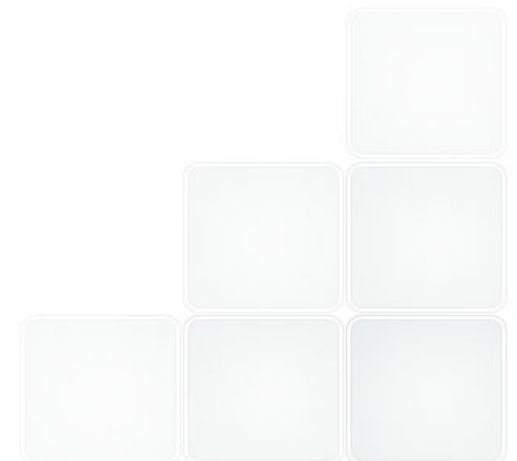
Latini *et al.*, 2013 *Planta* 237:967-978



CONCLUSION

The polymorphisms do not seem heavily affect the structural arrangement of the DNA-binding domains in the 5 cultivars.

However, a different binding specificity and affinity to DNA, due to the polymorphisms of putative AAs close to the DNA contacting β -sheet (position 11 and 31 of the AP2 domain), cannot be completely excluded.





FUNDING

**Italian National Project RIADE
IT-MX bilateral MAE project
COST TRITIGEN FA 0604
2012-2014 ENEA International Fellowship**

THANKS FOR YOUR ATTENTION