

Candidate Gene Trees

Ortholog or paralog?

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Ortholog or Paralog?

- Ortholog
 - identical by descent
 - function conserved across species
 - same member of gene family in species X as Y
- Paralog
 - other member of a gene family arising from duplication
 - function may or may not be conserved



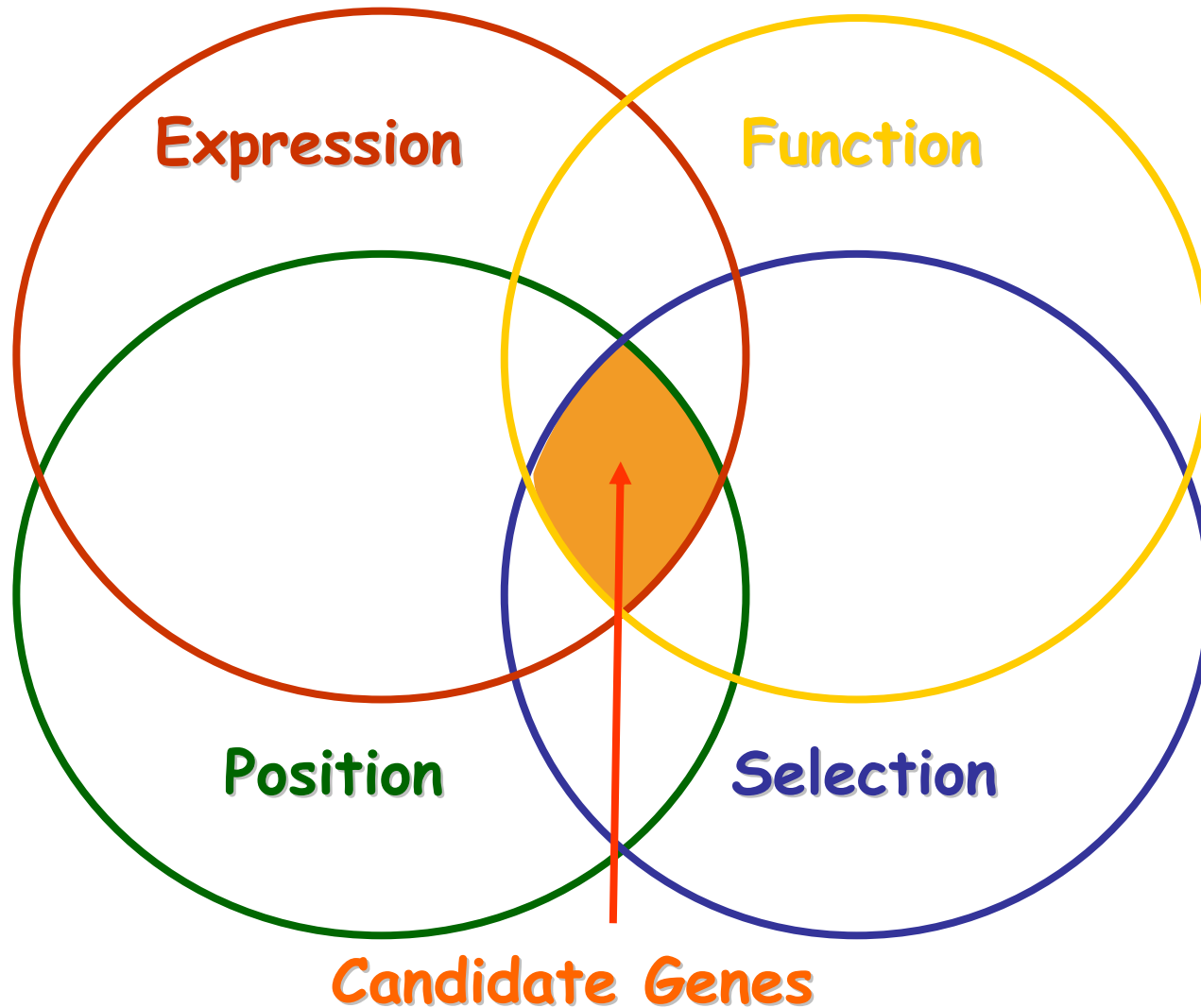
Multi-gene families

- determining orthologous loci is confounded by complexity of family
- current annotation of sequences may not reflect status of orthology/paralogy
- annotation is frequently based on sequence similarity without considering structure of family

Candidate Gene Examples

- Oxalate oxidase / germin-like proteins
(response to oxidative stress, required for lignification during HR)
- 14-3-3 family (signal transduction, protein-protein interactions, and protein phosphorylation)
- Trehalose-6-phosphate phosphatase / synthase
- Drought responsive element binding factors
(transcription factors)
- Aquaporin / membrane channel proteins
(water uptake and transport)

Convergent evidence identify candidate genes



Evidence for choice

- OxOx and 14-3-3 gene family member(s) cosegregate with QTL for quantitative blast tolerance
- TPP, DREB1, and aquaporin cosegregate with drought-related QTL
- TPP, DREB1 known to increase tolerance to drought stress in transgenic rice

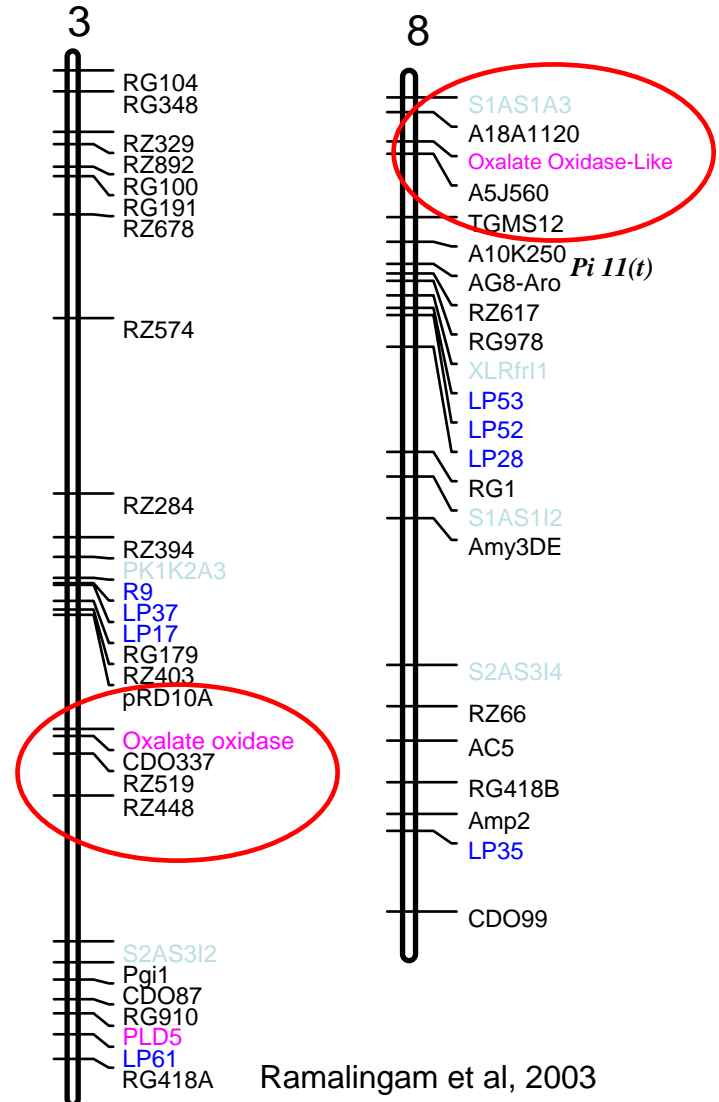
- TPP, aquaporin undergo changes in gene expression during water stress
 - TPP up regulated in Azucena
 - Aqp Nod26-like up regulated in IR64 panicles
 - Aqp Pip2-like up regulated in IR64 roots but not Moroberekan (RT-PCR)

Strategy Employed (classical)

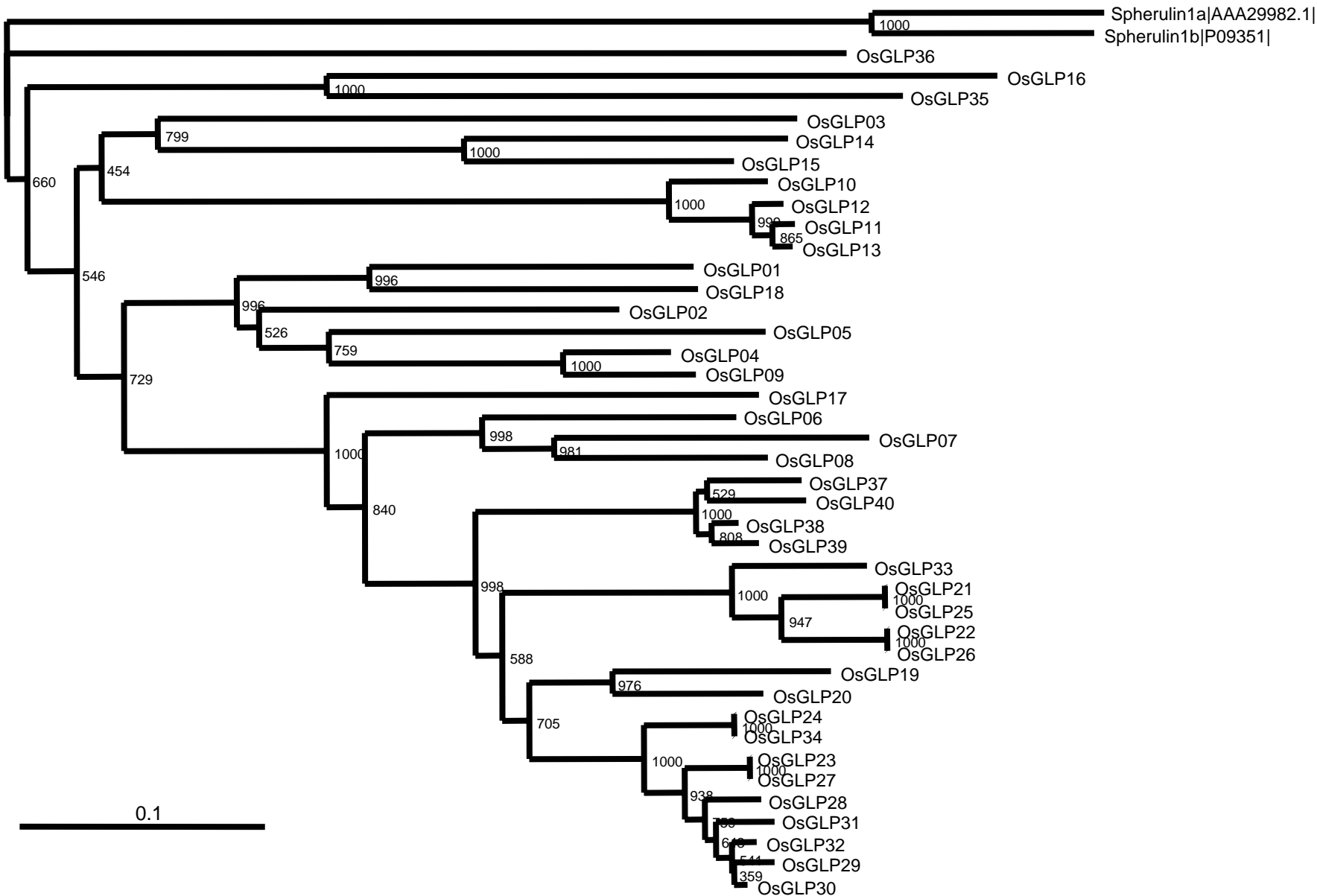
- Begin with target locus
- Identify "best" hits to protein alignment (Blast NCBI nr and TIGR pseudomolecules v2) factoring in depth of previous annotation
- Perform multiple sequence alignment using ClustalW
- Manually refine alignment (visual inspection, BioEdit)
- Use refined alignment for bootstrapped phylogenetic tree (UPGMA on protein distance)

Evidence for the role of oxalate oxidase in resistance to plant pathogens

- Increased oxalate oxidase activity in barley infected with powdery mildew (Zhou et al., 1995)
- Induction expression of germin gene in wheat infected with powdery mildew (Hurkman and Tanaka, 1996)
- Association of oxalate oxidase to partial blast resistance in Vandana X Moroberekan population (Wu et al., 2004)



Ramalingam et al, 2003



Phylogenetic relationships of rice germin-like proteins (GLP). The tree was rooted using outgroup of spherulin1A (AAA29982) and spherulin1b (P09351).

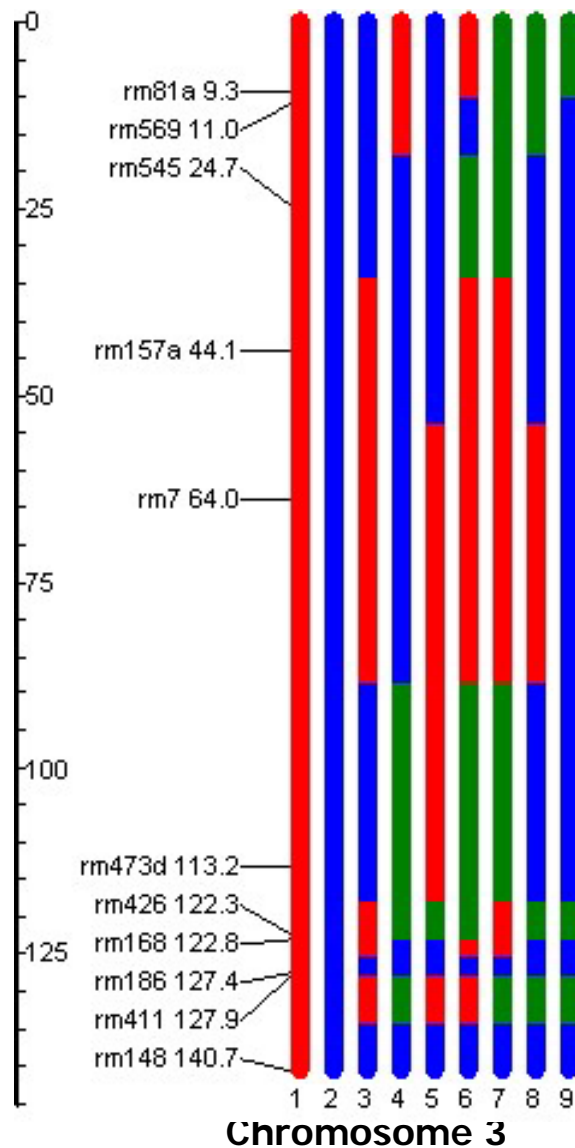
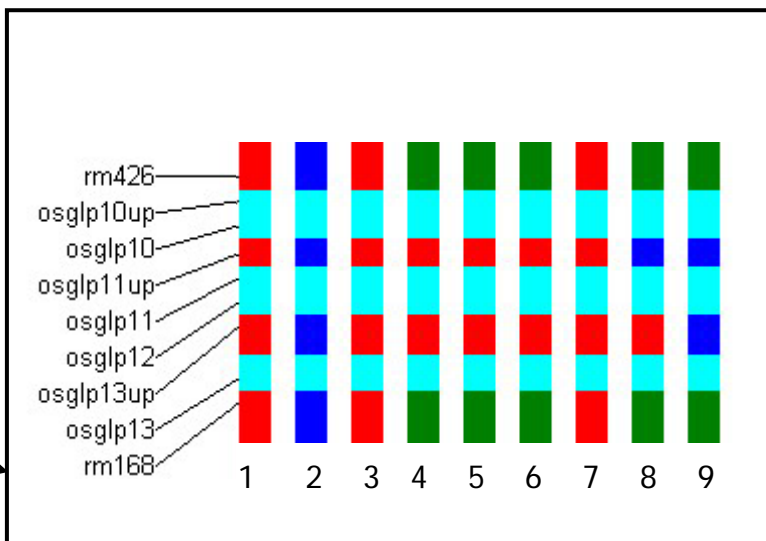


Figure 3. Detailed marker analysis of Chromosome 3 loci identified with 4 putative oxalate oxidases. PCR primers used were designed from the coding region of each gene. Primers were also designed in the 1000bp upstream region of each gene (OsGLP10UP, OsGLP11UP and OsGLP13UP). (1: Moroberekkan; 2: Vandana; 3: IR78221-19-6-56; 4: IR78222-20-7-148; 5: IR78222-20-1A-7; 6: IR78224-22-2-59; 7: IR78224-22-2-114; 8: IR78222-20-1A-18NB; 9: IR78222-20-2-7NB. Heterozygous loci are colored green. Monomorphic markers are colored light blue.



Synteny between rice CH3 and maize CH1

- A putative oxalate oxidase in maize CH1 AY108860 identified
- AY108860 has 80-82% identity with CH3 oxalate oxidases in rice



Eukaryotic Gene Orthologs

<http://www.tigr.org/tdb/tgi/ego/ego.shtml>

10 Orthologous groups of germin-like proteins in plants

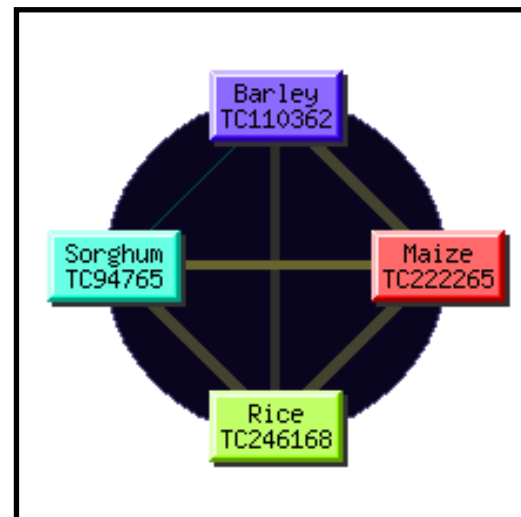
Tentative Ortholog 697734

Barley Y14203

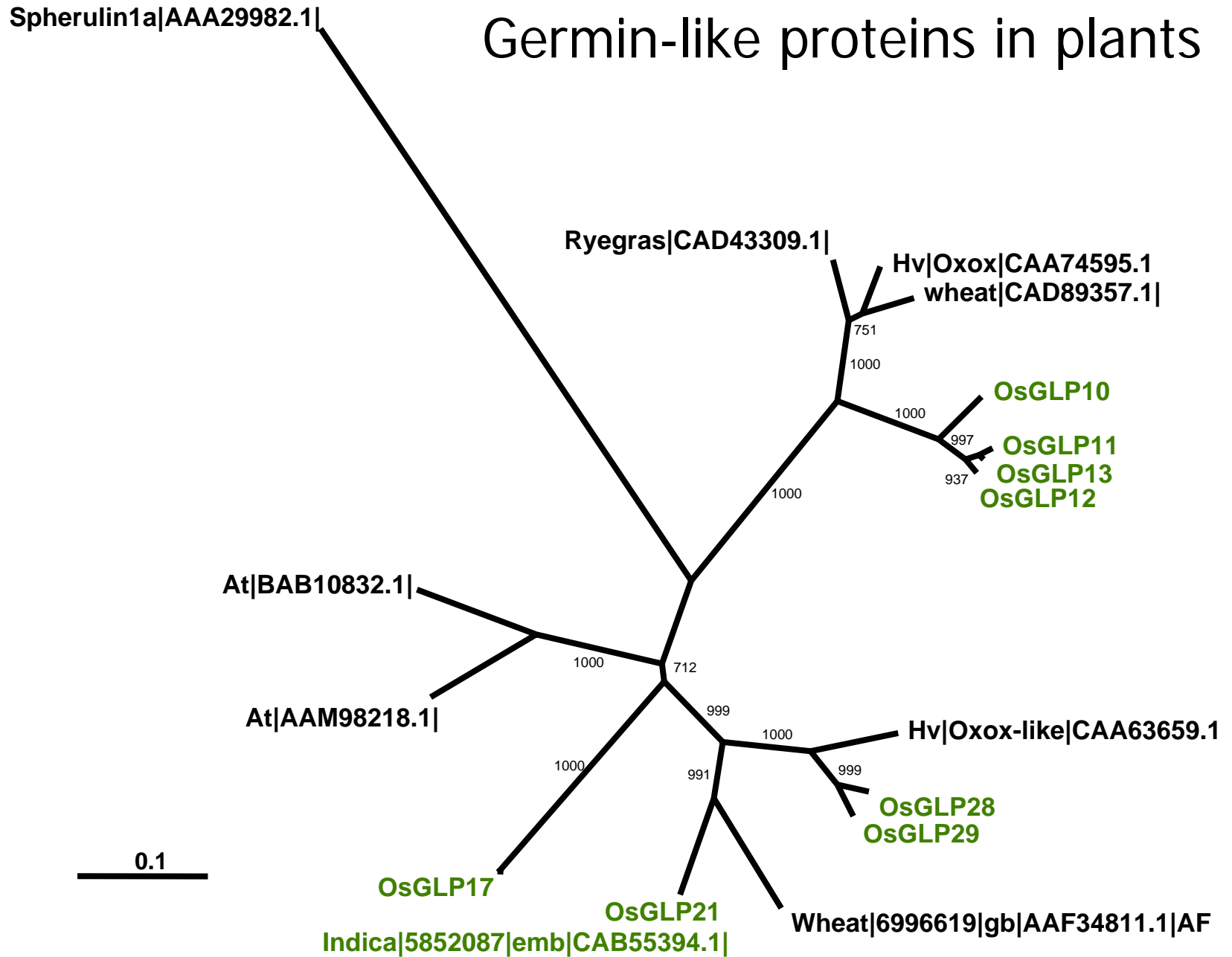
Maize AY108860

Rice OsGLP13/OsGLP11

Sorghum AW677167

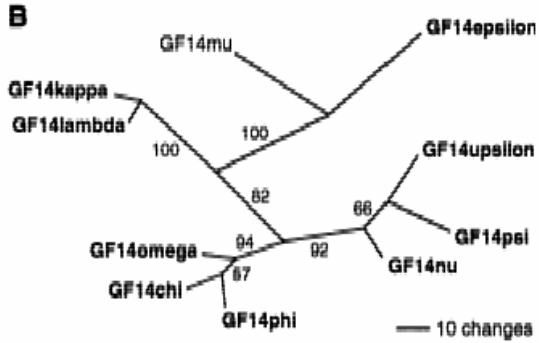


Germin-like proteins in plants

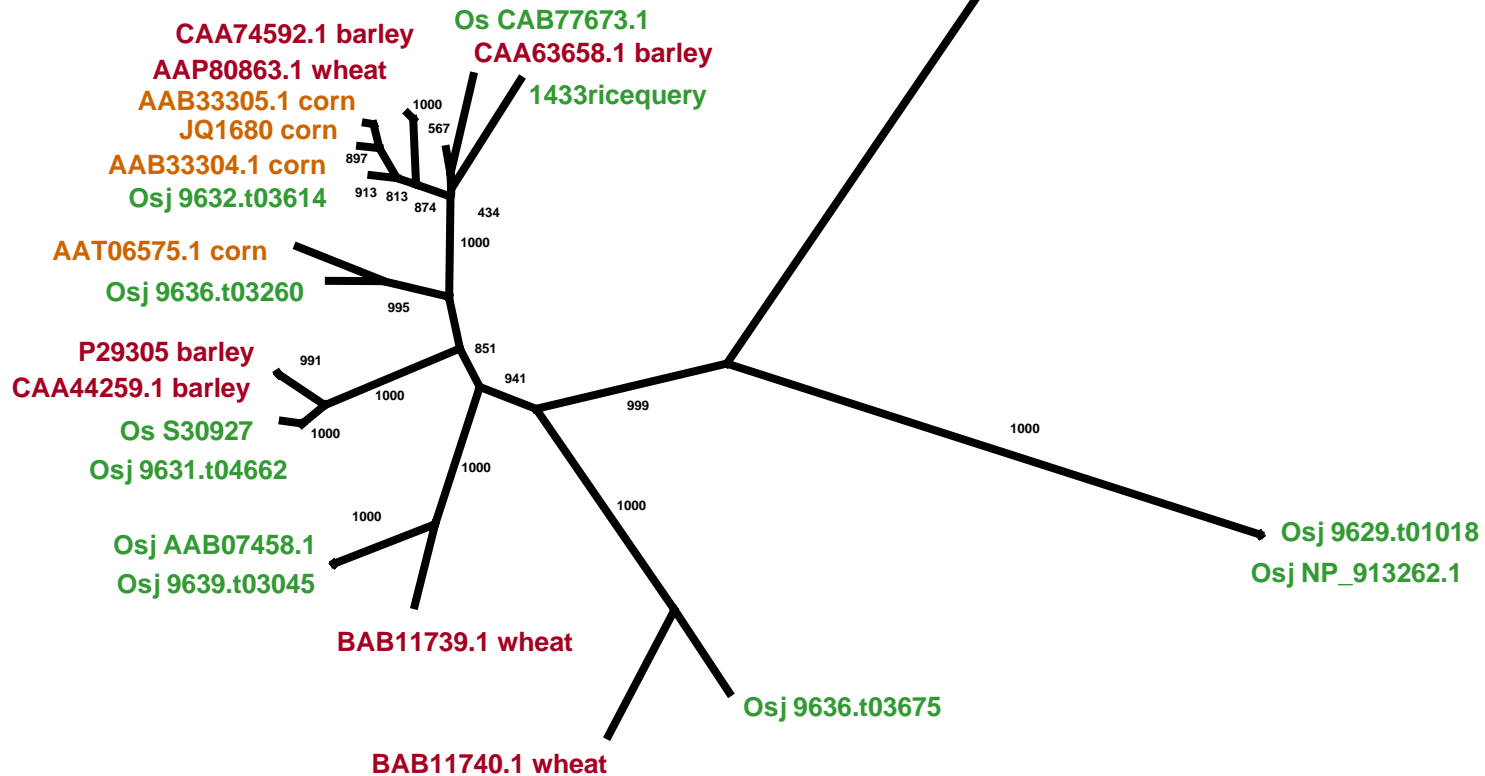


Arabidopsis thaliana

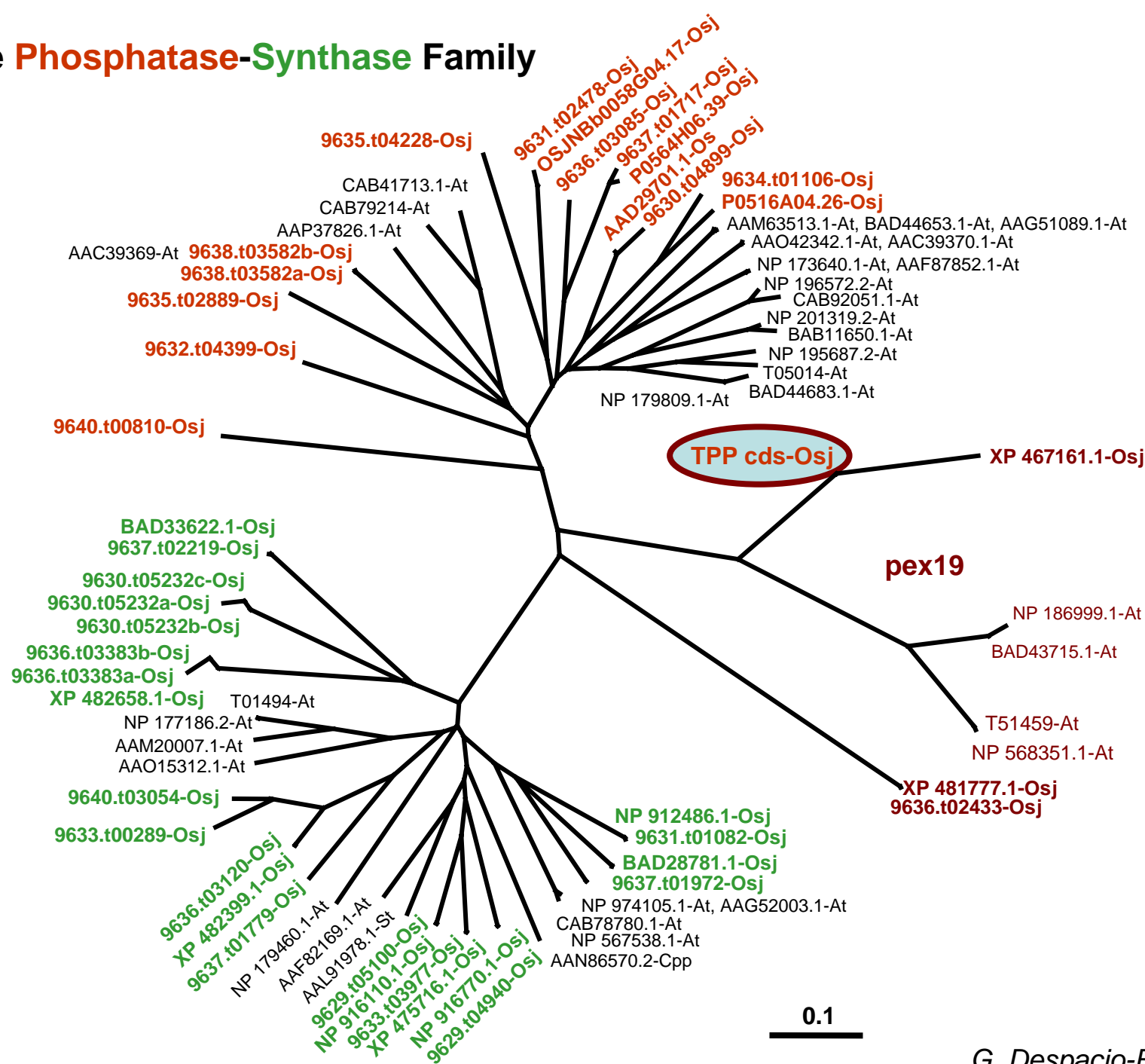
14-3-3 Family



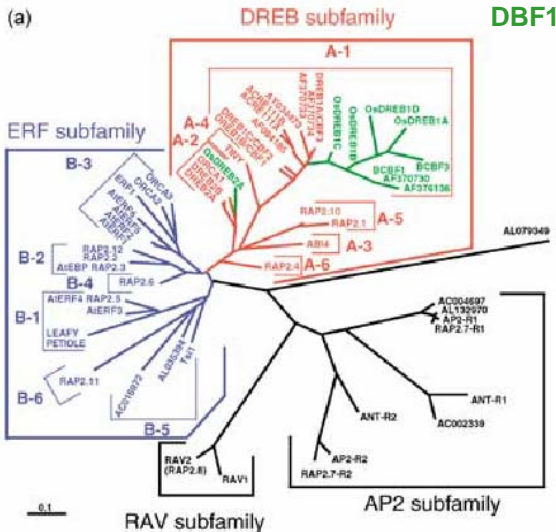
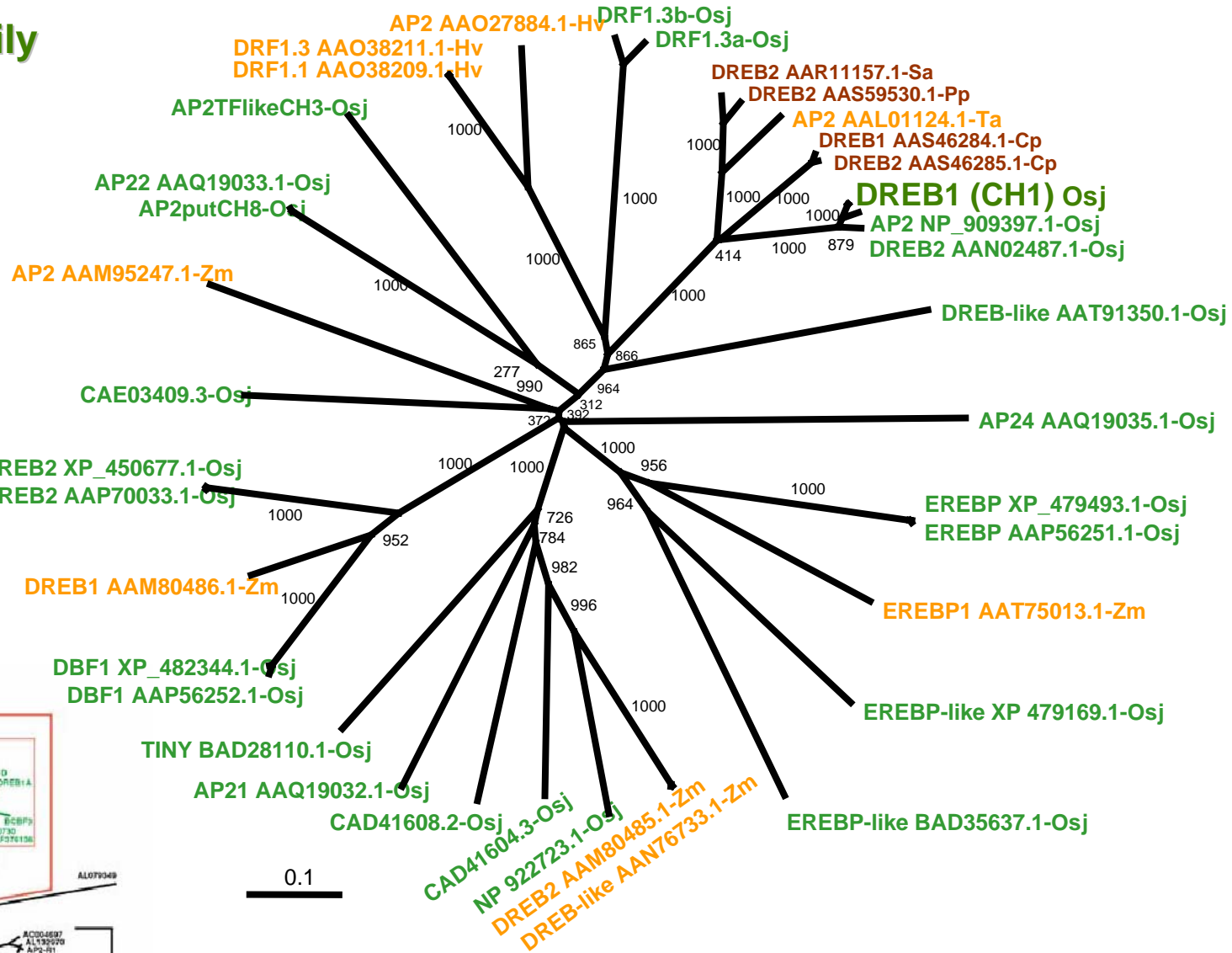
Ferl 1996 *Annu. Rev. Plant Phys. Plant Mol. Biol.* 47:49-73



Trehalose Phosphatase-Synthase Family



DREB/AP2 family



Aquaporin (intrinsic membrane protein) family

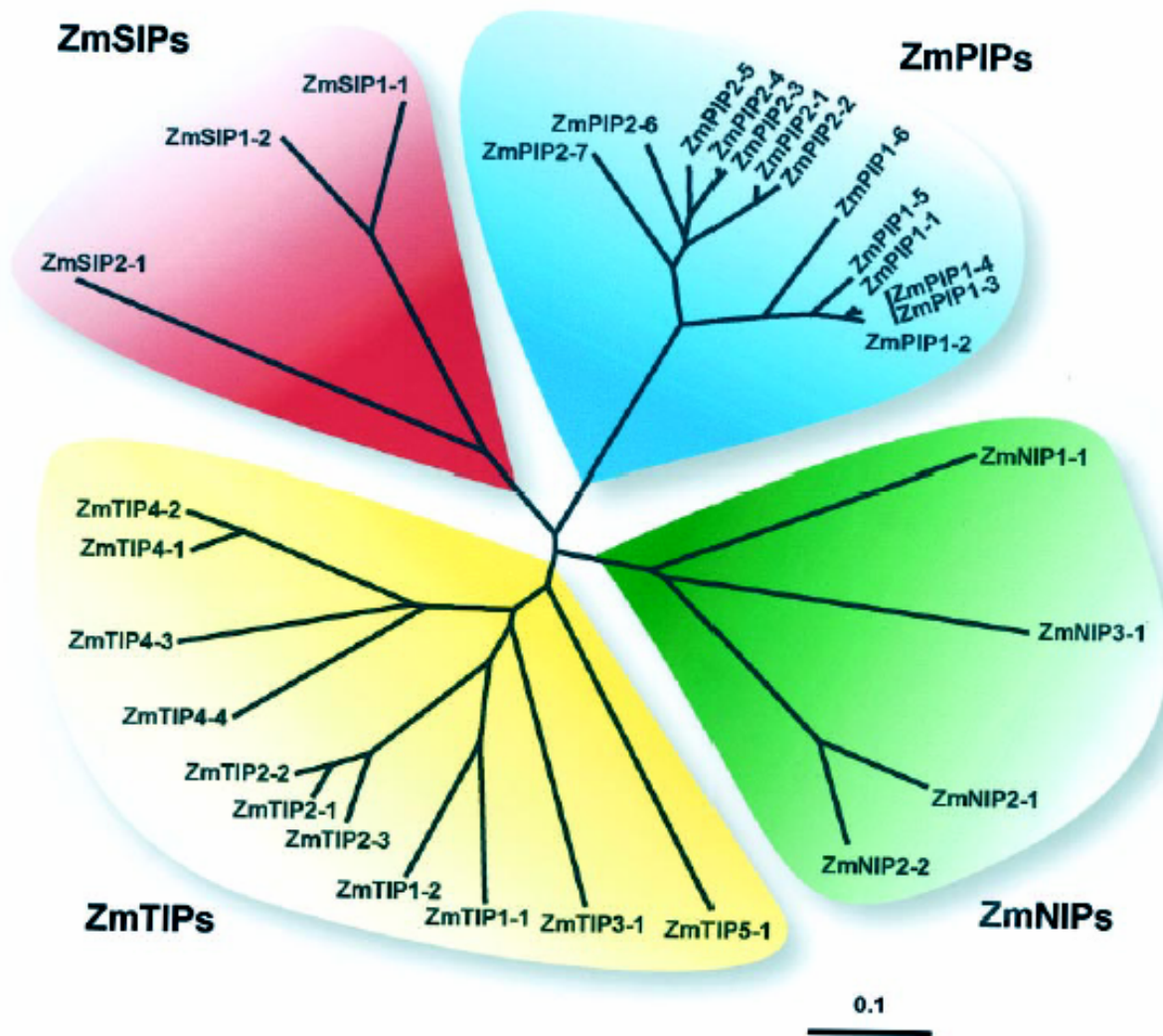
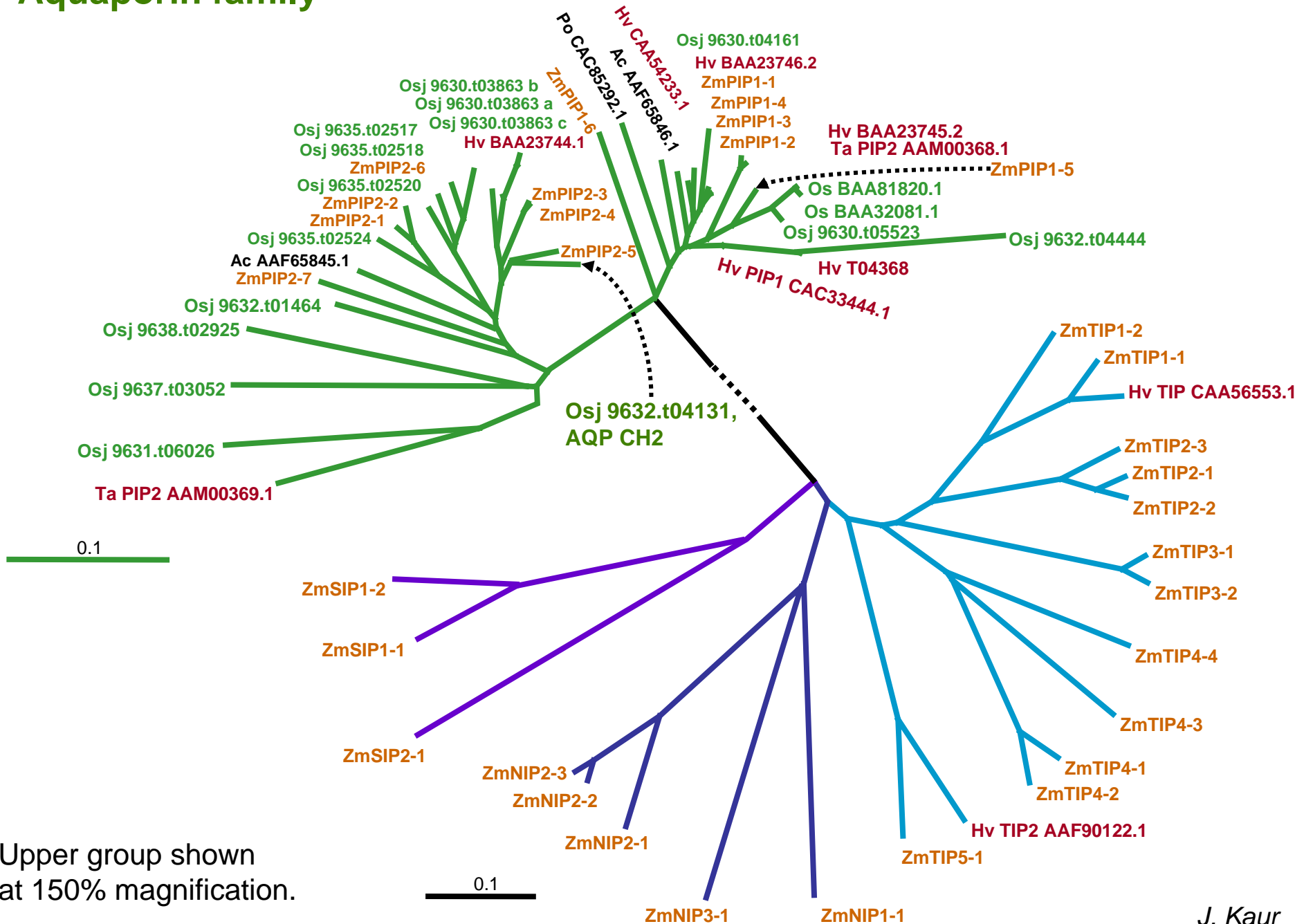


Figure 2. Phylogenetic analysis of 31 maize AQP proteins. The distance scale represents the evolutionary distance, expressed in the number of substitutions per amino acid. National Center for Biotechnology Information accession numbers are shown in Table I.

Aquaporin family



Conclusions

- Need to examine the whole family in order to define ortholog/paralog
- First pass analysis may not identify all of the relationships