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AP2/EREBP Transcription Factors in Rice

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Plants have the ability to defend themselves against pathogens by activating a series of defense responses. SA is known to be a signal molecule in plant defense responses. Nevertheles, SA is not the only one signal mediating defense responses. In addition to SA, ethylene and jasmonic acid have also been known to mediate plant defense responses against pathogens. The activation of a series of plant defense responses is known to be through varieties of transcription factors. Specially AP2/EREBP transcription factors are involved in ethylene mediated defense signaling. In this review, recent progress on AP2/EREBP transcription factors in arabidopsis, tomato and tobacco and a few of AP2/EREBP transcription factors in rice related to biotic stresses will be discussed.

Ethylene-responsive cis-regulatory elements

The biosynthesis of ethylene is increased during natural processes such as fruit ripening and plant senescence and in response to abiotic stresses and biotic stresses such pathogen infection (Deikman 1997). Among the different classes of ethylene-responsive genes, the most studied are defense genes whose expression is activated by ethylene in response to pathogen infection. Ethylene induces class I basic chitinase, class I β-1,3-glucanase and other basic-type pathogenesis-related (PR) proteins. The conserved sequence was identified by the deletion analysis of promoters in ethylene responsive genes induced against pathogen infection and named as the GCC box (AGCCGCC). The GCC box is also known as the ethylene -responsive element (ERE) and is known to be sufficient for the ethylene responsiveness (Ohme-Takagi and Shinshi 1995; Shinshi et al., 1995). However, the GCC box has not been found in the promoter of genes involved in fruit ripening such as the tomato E4 gene (Montgomery et al., 1993; Coupe and Deikman 1997). Therefore two different type of the cis element is involved in the ethylene mediated gene expression

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General feature of AP2/EREBP transcription factors

The GCC box (AGCCGCC) sequence is commonly found in the 5' upstream regions of ethylene-inducible defense genes (Ohme-Takagi and Shinshi 1990, Ohme-Takagi and Shinshi 1995; Shinshi et al., 1995). Four different ethylene responsive element binding proteins (EREBP) also known as ethylene responsive factors (ERF) that interact the GCC box were initially reported from tobacco plants. Recently Park et al. (2001) reported Tsi gene in tobacco plants. The DNA-binding domain of ERFs has been found in a variety of plant regulatory proteins. In arabidopsis, these proteins include TINY (Wilson et al., 1996), AtEBP (Buttner and Singh, 1997), ERF1 (Solano et al., 1998), ABI4 (Finelstein et al., 1998), Apetala2 (AP2; Jofuku et al., 1994) and Aintegumenta (ANT; Elliot et al., 1996). Glossy 15 in maize (Moose and Sisco 1996) and Pti4/5/6 in tomato also contain the ERF DNA binding domain (Zhou *et al.*, 1997) ERFs contain a highly conserved DNA binding domain consisting of 58 or 59 amino acid residues that designated as ERF domain (Ohme-Takagi and Shinshi 1995; Hao et al., 1998) (Fig. 1). The solution structure of ERF domain with DNAs revealed that the ERF domain consists of a three-stranded antiparallel β-sheet and an α-helix (Allen et al., 1998). Arabidopsis genome sequencing project revealed that ERFs consist of a large multigene family with many members in both dicots and monocots. Genes encoding ERF proteins have been found only in higher plants and not in other fungi and animals. The domain within ERFs shows >60% amino acid sequence identity but with AP2 domain

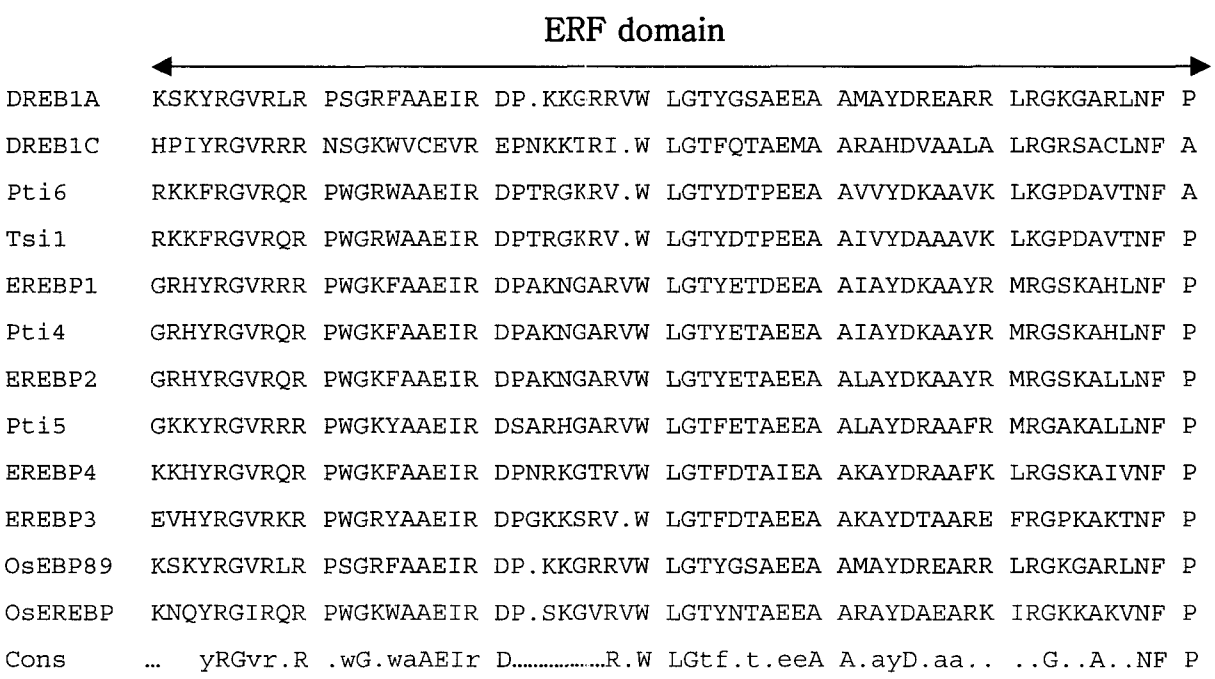

Fig. 1. Alignment of the ERF domain from various ERF proteins in arabidopsis, tomato, tobacco, and rice. Yellow boxed area indicates amino acid identity at the position.

shows only <30% amino acid identity (Ohme-Takagi, M. et al., 2000). The AP2 domain ERF domain is closely related to the AP2 domain. However, they are quite distinct in terms of the cis element that they bind (Ohme-Takagi, M. et al., 2000). The ERF is likely to have a different DNA recognition as well as different target DNAs.

NtERF2, 3 and 4 are localized to the nucleus (Ohta M., et al., 2000). Ohta et al. (2000) reported that NtERF2 and 4 transactivate the reporter gene containing the GCC box in their promoter in yeast as well as in tobacco protoplasts but NtERF3 reduced the expression of the reporter gene in tobacco protoplasts. Fujimoto et al. (2000) also reported that AtERF1, AtERF2, and AtERF5 transactivate the reporter gene but AtERF3 and 4 reduced the expression of the reporter gene. Therefore ERFs act as transcriptional activators or repressors depending on the domain outside the ERF DNA binding domain. Ohta, M. et al. (2001) identified an essential motif, EAR motif (L/FDLNL/F (×) P) from class II ERFs for the active repression. This motif is essential and sufficient for the active repression.

ERFs involved in elicitor-responsive expression of defense genes

The GCC box was originally found in promoters of several defenses related genes such as β-1,3-glucanase, chitinase, and PR1 genes. However, the GCC box was not found in the promoter of ethylene responsive genes involved in fruit ripening such as E4 gene. Thus the GCC box is the unique cis element to regulate the plant-pathogen interaction. The GCC box is involved in ethylene mediated gene expression. The plant-pathogen interaction is known to produce ethylene. Therefore it has been suggested that the plant-pathogen interaction transcriptionally regulates defense genes through the GCC box in their promoters by ethylene. However there are several recent reports that the GCC box-mediated transcription of genes does not always requires ethylene. Yamamoto et al. (1999) reported that a fungal elicitor, xylanase activate the GCC box-mediated transcription in ethylene independent manner. Fujimoto et al. (2000) reported that AtERF1, 2,5 transactivated the reporter gene containing the GCC box in the promoter in ethylene biosynthesis mutant, ein2. The direct link between ERFs and the plant-pathogen interaction came from the discovery of Pti4 and Pti5, tomato homologs of the tobacco ERF2 which interact directly with a protein kinase encoded by the Pto gene, which confers resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (Zou et al., 1997). Tara et al. (1999) reported that *Pseudomanas syringae* pv. *tomato* induces the Pti4 and Pti5 but not through SA, ethylene, and jasmonate-signaling pathway. Their result showed that Pti4 and Pti5 induced in tomato plants carrying the nahG transgene, the Nr, the def1 mutant.

Overexpression of Pti4/5/6 enhanced disease resistance in arabidopsis (Gu et al., 2001). Tobacco Tsi gene enhances resistance against pathogen and osmotic stress when it is

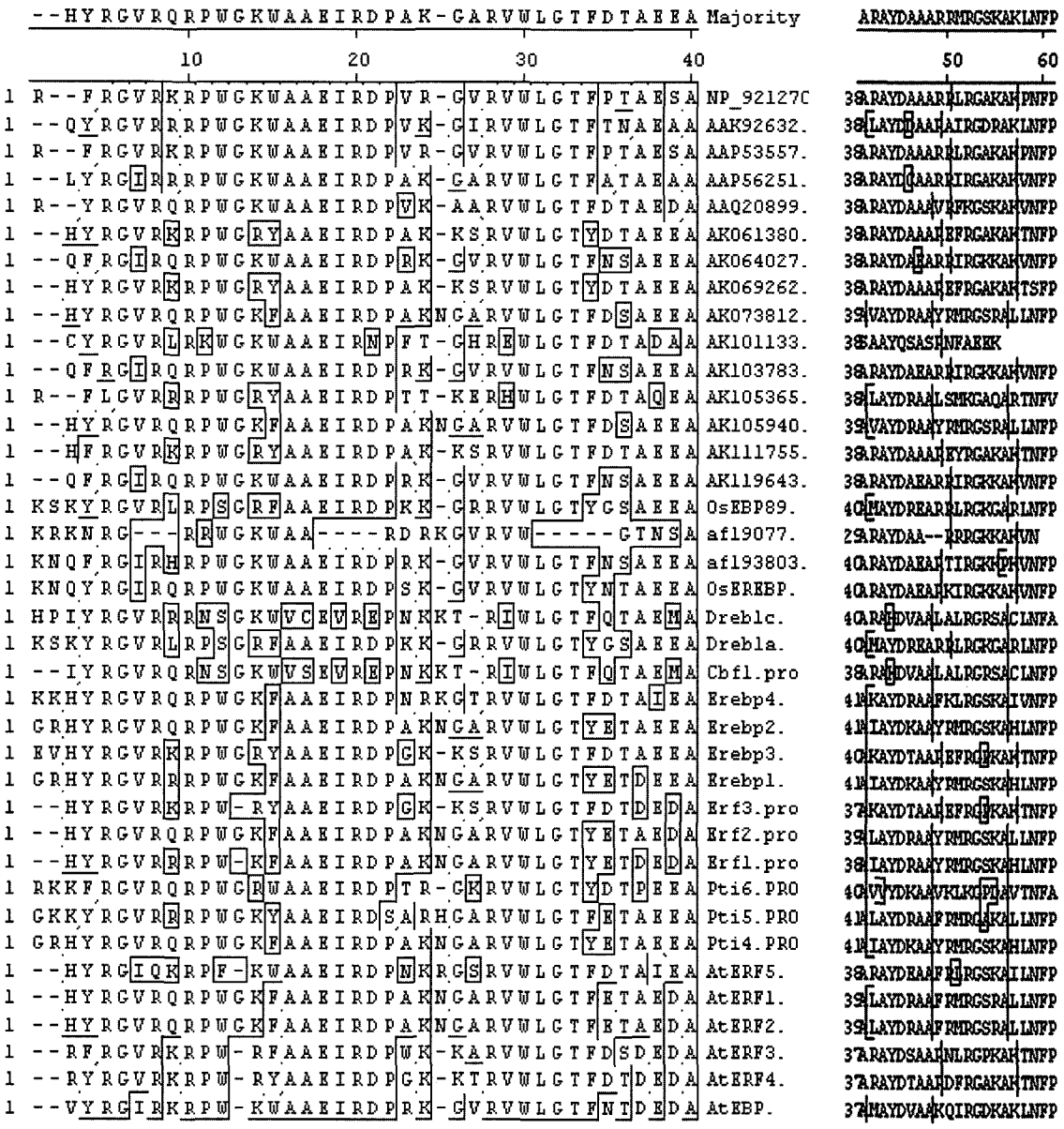

Fig. 2. Alignment of the ERF domain from various ERF proteins in arabidopsis, tomato, tobacco, and rice. OsERFs are indicated as the gene bank number. Yellow boxed area indicates amino acid identity at the position.

overexpressed (Park, J. M. et al., 2001). Berrocal-Lobo, M. et al. (2002) reported that overexpression of AtERF1 enhances resistance to several necrotrophic fungi.

ERFs in rice

Compared to Arabidopsis, not much is known about ERFs in rice. However, the nucleotide sequence of ERFs had been reported due to the completion of rice genome sequencing project. Goff et al. (2002) reported the rice draft sequence last year. The 143 AP2/ERF/RAV transcription factors have been found in the rice draft sequence (Goff et al., 2002). In this study, 20 OsERF genes were extracted from the NCBI annotated database. The phylogeny tree was made from the 20 OsERF genes compared to the AtERFs and the other ERFs known so far (Fig. 2). According to the phylogeny tree in Fig. 3, OsERFs can be classified into 8 different subfamilies. Among OsERFs, two OsERFs has been studied in detail. Cheong et al. (2003) reported that BWMK1, a rice MAPK phosphorylates OsEREBP1 (AF193803), thereby enhancing the transactivation activity of the OsEREBP1. The other well-known OsERF, OsEBP-89 is temporally expressed in developing endosperm and intercalary meristem (Yang et al., 2002).

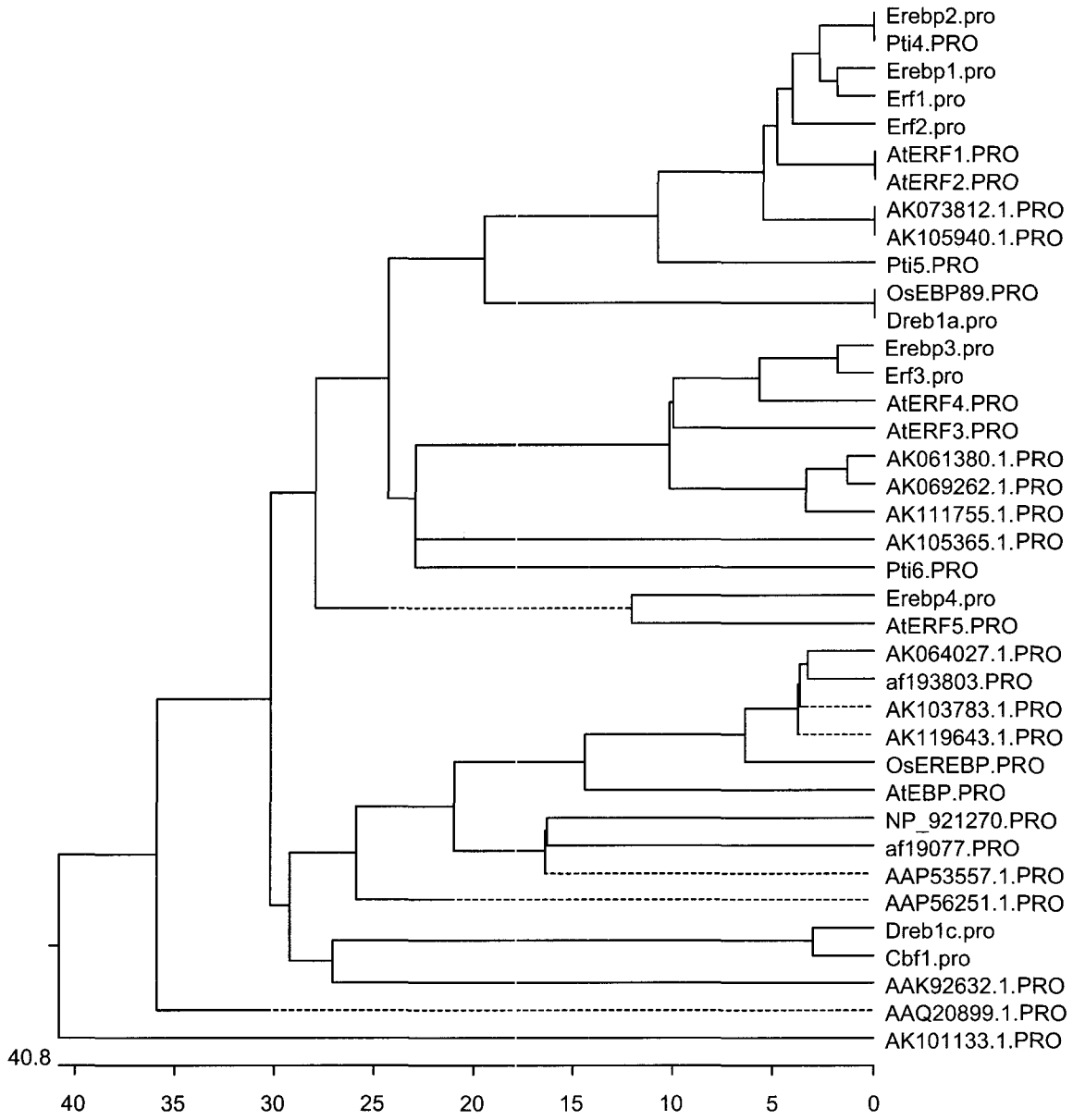

Fig. 3. Phylogenetic tree of ERF-domain proteins. A phylogenetic tree of the ERF proteins based on their ERF domains was generated by Megalign program of DNASTAR package.

Prospects

To understand the mechanism how AP2/EREBP transcription factors acts transcriptional activation or repression in detail, cofactors have to be extensively investigated by using the yeast two-hybrid and general proteomics tools. In the near future, protein interaction networks will be established for the AP2/EREBP transcription factors.

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