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Abstract Book

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S8/O1: THE FAMILY OF DOF TRANSCRIPTION FACTORS IN *BRACHYPODIUM DISTACHYON*: BDDOF24 IN GERMINATING SEEDS

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The DOF (DNA binding with One Finger) transcription factor (TF) family is characterized by a binding domain of 52 amino acid residues that is structured as a Cys₂/Cys₂ Zn²⁺ finger that recognizes the common core 5'-T/AAAAG-3' in the promoter regions of their target genes. DOF TFs have been associated with biological processes exclusive to higher plants and their close ancestors (algae, mosses and ferns). In the genome of the model species *Brachypodium distachyon* we have annotated 27 Dof proteins, and to explore the evolutionary relationship among the *Brachypodium* DOF proteins, and their orthologs in *Oryza sativa* (30 genes) and *Hordeum vulgare* (26 genes), a combined phylogenetic tree has been constructed (PhyML method). According to this analysis, DOF proteins can be classified into four Major Clusters of Orthologous Genes (MCOGs). An expression profile study of the annotated BdDof genes across four different organs (leaves, roots, spikes and seeds) has been performed using RT-qPCR. For those Dof genes highly or preferentially expressed in seeds (maturation, dry stage and germination) a more detailed expression analysis has been done(1).

During cereal seed germination, Gibberelic Acid (GA), which is synthesized in the embryo, induces the synthesis of hydrolytic enzymes in the aleurone layer. Functional analysis of the promoters of hydrolase genes induced upon germination, such are those encoding α -amylases and thiol-proteases (Cathepsin B; *CathB* gene), has led to the identification of a conserved cis-element required for GA dependent expression, termed the GA Responsive Complex (GARC), that includes(2): a pyrimidine box 5'-CCTTTT-3' (recognized by DOF TFs) and the GA Response Element (GARE) 5'-TAACAAA-3' (recognized by MYBR2R3 TFs).

The most abundant Dof transcript in germinating seeds is *BdDof24*. We have characterized at the molecular level this gene and explored its putative role as a transcriptional regulator of the GA-induced *BdCathB* gene. The interaction of the BdDOF24 protein with BdGAMYB, has been validated by molecular Yeast-2-Hybrid assays and by Bimolecular-Fluorescence-Complementation in planta. The functional relevance of this interaction has been further validated by transient expression assays and by reverse genetics using T-DNA insertion mutants and over-expression transgenic lines for those TFs.

(1) Hernando-Amado*, Gonzalez-Calle* et al. (2012). BMC Plant Biol. 12: 202.

(2) Mena et al. (2002). Plant Physiol. 130: 111-119.

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