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The phosphoinositide dependent-phospholipase C pathway differentially controls the basal expression of *DREB1* and *DREB2* genes

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Abbreviations: C-repeat, CRT; CRT-binding factors, CBF; diacylglycerol-kinase, DGK; drought responsive elements, DRE; Phosphoinositide-dependent phospholipase C, PI-PLC

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We recently showed that—in *Arabidopsis thaliana* suspension cells—phosphoinositide dependent-phospholipase C (PI-PLC) and diacylglycerol kinase (DGK) negatively regulated the basal expression of most *DREB2* genes. *DREB2* genes encode transcription factors that bind to Drought Responsive Elements (DRE). Those elements are also bound by DREB1 factors. While DREB2 factors are mostly involved in drought and heat responses, DREB1s are induced in the response to chilling. We here show that the pharmacological inhibition of PI-PLC or DGK leads to the basal induction of *DREB1* genes. However, the induction is much less marked for the *DREB1* genes than that of *DREB2A*, a member of the DREB2 family. This illustrates that *DREB1* and *DREB2* genes, while having the same targets, are not submitted to the same transcription regulation, and that lipid signaling might in part explain these differences in the regulation of the *DREB* genes.

Plants are always submitted to changes in their environment. They can be submitted to abiotic stresses, such as drought, exposure to heat, or to low temperatures, changes of light intensity or quality. They can also be submitted to biotic stresses. Because plants are sessile organisms, they need, in order to survive, to be able to cope with these stresses. A major process of the resistance of plants to stresses is transcriptome remodelling. This occurs through the early induction of transcription factors. *DREB2A* and *DREB2B* transcription factors are highly induced by drought, NaCl, or heat, while poor induction is seen in response to cold or abscissic acid.[1,2] The encoded protein binds to drought responsive elements (DRE)/C-repeat (CRT) on the promoters of genes, thus inducting their transcription. Interestingly, the same DRE/CRT elements can be bound by DREB1 proteins.[3] These factors, also named CRT-binding factors (CBF), are induced by cold, but not by drought.[4] DREB1 and DREB2 proteins share identity in the DNA binding domain, but are very little identical in the rest of the protein.[5] Because the DREB1 and DREB2 proteins are key regulators of the response to major abiotic stress, it is of high importance to understand how they are regulated. The regulation of *DREB* gene expression occurs in response to a stress, but also in basal conditions. Among the signaling pathways active in control conditions are the ones that generate or consume bioactive phosphoglycerolipids. Phosphoinositide-dependent phospholipase C (PI-PLC) hydrolyses phosphatidylinositol-4,5-bisphosphate into inositol triphosphate and diacylglycerol. This lipid can be phosphorylated into phosphatidic acid by diacylglycerol-kinases (DGK).[6] The PI-PLC/DGK pathway is active in non-stimulated Arabidopsis cells or plants.[7,8] We recently showed that this basal activity negatively regulated the expression of *DREB2A*, *DREB2B*, *DREB2C*, *DREB2E*, and *DREB2H* genes. Indeed, when inhibiting the activity of PI-PLC by edelfosine or

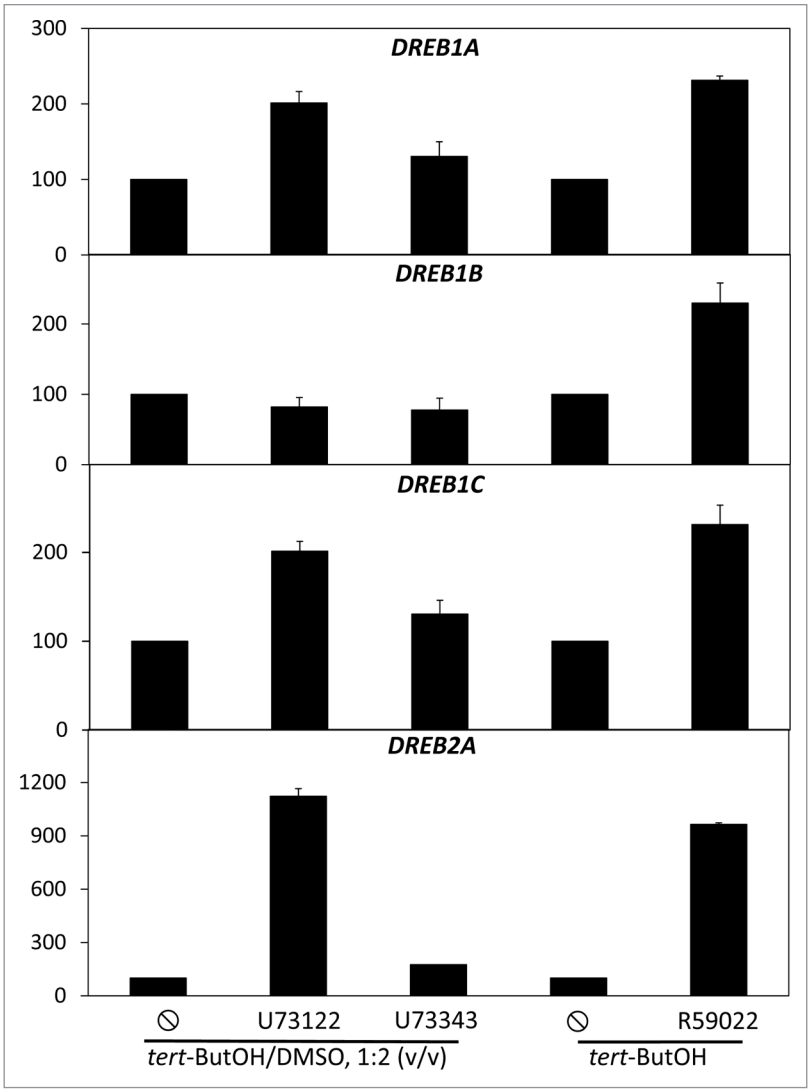

Figure 1. Effects of inhibitors of PI-PLC or DGK on the expression levels of *DREB2A* and *DREB1* genes. U73122 and U73343 were used at final concentration 60 µM. R59022 was used at final concentration 100 µM. Transcript levels were quantified by qPCR, and expressed as % of solvent-treated cells. Cells were treated with inhibitors for 4 h, at 22 °C, before harvesting. *tert*-ButOH is for tertiary butanol.

U73122, or when inhibiting the activity of DGK by R59022 or R59949, the expression of these genes was upregulated in Arabidopsis suspension cells.[7] We wanted to know if this was also the case for the *DREB1* genes. Cells were treated by U73122 or its inactive analog U73343. Cells were also separately inhibited with R59022. Four hours later, cells were harvested and transcripts isolated. The level of *DREB1A-C* were quantified by real-time PCR and compared with that of *DREB2A*. Inhibiting basal PI-PLC or DGK led to the induction of *DREB1* genes. However, the induction is much less marked than that of *DREB2A*. U73122 led to a 10-fold increase of *DREB2A* expression when compared with the effect of U7343; on the contrary, U73122 had no effect on the expression of *DREB1B*, and only slight inducing effects on that of *DREB1A* or *DREB1C*. R59022 led to a 10-fold increase of *DREB2A* expression, while it led only to a 2.3-fold increase of that of *DREB1A-C* genes (**Fig. 1**). This illustrates that *DREB1* and *DREB2* genes are not submitted to the same transcription regulation. This is true in response to stresses, since they are not induced by the same stresses. But it also true in basal conditions. The way those genes are regulated is still poorly understood. It was shown that ABRE-BINDING PROTEIN 1, ABRE-BINDING PROTEIN 2, and ABRE-BINDING FACTOR 3 transcription factors can bind to and activate the *DREB2A* promoter in an ABRE-dependent manner. Concerning the DREB1 genes, *ICE1*—that encodes a MYC-like transcriptional activator—binds specifically to the MYC recognition sequences in the *DREB1A* promoter. *ICE1* overexpression in wild-type plants enhances the expression of the *CBF* regulon in the cold.[9] The CALMODULIN BINDING TRANSCRIPTION ACTIVATOR 3 is a positive regulator of *DREB1C* expression.[10] However, to what extent those transcription factors and *cis*-elements are important for the control of basal expression is not known. A challenging task would be now to identify which part of the *DREB2A* promoter is responsible for the regulation by basal PI-PLC/DGK activity. Finally, it has to be reminded that not all *DREB2* genes are dependent on the PI-PLC/DGK for their basal regulation. *DREB2D* expression is not stimulated by the inhibitors of these enzymes, while *DREB2F* and *DREB2G* transcripts could never be detected in any of the conditions tested.[7]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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