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Functional Analysis of Different Promoter Haplotypes of the Coffee (*Coffea canephora*) CcDREB1D Gene Through Genetic Transformation of *Nicotiana tabacum*.

- Aquino, Sinara O.*,**, Carneiro, Fernanda A.*,**, Rêgo, Érica C. S.**, Alves, Gabriel S. C.*, **, Marraccini, Pierre**,***, Andrade, Alan C.**,****
- *Federal University of Lavras, MG, Lavras, BRAZIL.
- **Embrapa Genetic Resources and Biotechnology, Brasilia, DF, BRAZIL.
- ***CIRAD, UMR AGAP, Montpellier, FRANCE.
- ****EMBRAPA Coffee-INOVACAFÉ, Lavras, BRAZIL.

In many higher plants, *DREB* genes have been shown to be involved in abiotic stress transduction pathways. Previous results showed that *CcDREB1D* gene expression increased under drought stress in leaves of the drought-tolerant clone 14 of *Coffea canephora*Conilon but not in those of the drought-susceptible clone 22.

Rationale

By sequencing the *CcDREB1D* promoter regions of these clones, three haplotypes (hp15, hp16 and hp17) containingseveral single nucleotide polymorphisms and insertions/deletions were found. These haplotypes were cloned independently into the binary vector pBI101 in order to analyze their ability to control the expression of the *uidA* reporter gene in transgenic tobacco plants under different stress conditions.

Methods

The three haplotypes of *CcDREB1D* promoter sequences fused to the *uidA* reporter gene were transferred in *N.tabacum* cv. SRI via *A.tumefaciens* mediated transformation.Kanamycin-resistant plants were subjected to dehydration (DH), heat shock (HS) and cold (CS) stresses andfurther analyzed by histochemical GUS assays and expression of *uidA*by qPCR experiments.

Results

Under DH and HSconditions,GUS activity was detected in petioles and youngest leavesof pD22-hp17L-transformed tobaccoafter 6h of stress.Under CS conditions, GUS staining was detected after 36h and 48h of stress, mainly in leaf lamina, petioles and vascular tissues, but not in roots of pD14-hp15L-transformed tobacco. In pD22-hp17L-transformed plantlets, GUS activity was detected after 12h, 24h, 36h and 48h of CS treatment, mainly in leaf petioles and midribs.In pD14-hp16L-transformed plantlets, CS treatment alsoinduced*uidA* gene expression that did not present significant variations over stress duration. For all these constructions, *uidA* gene expression was not detected in unstressed conditions and recovered tobacco plantlets.

Conclusions &Perspectives

Our results clearly suggestthat (1) the hp17 haplotype of the *CcDREB1D* promoter functioned in a different manner in the DH/HS and CS treatments in transformed-tobacco plants and (2) the three*CcDREB1D*promoter haplotypes of*CcDREB1D*gene were inducible by abiotic stress in tobacco by with a weaker strengthcompared to that of the constitutive CaMV35S promoter. These results clearly support theidea that the molecular mechanisms implicated in the transcriptionalcontrol of *DREB* gene expression by abiotic stress are probably highly conservedbetween tobacco and coffee plants.