54. Functional analysis of *CcDREB1D* promoter region from haplotypes of *Coffea canephora* through genetic transformation of *Nicotiana tabacum*

Sinara O. de Aquino[1], Fernanda A. Carneiro[1], Érica C. S. Rêgo[1], Gabriel S. C. Alves[3], Karoline E. Duarte[3], Pierre Marraccini[2], Alan C. Andrade[1].
[1]Embrapa Genetic Resources and Biotechnology, Brasília, DF, 70770-917, BR, [2]CIRAD UMR AGAP, Montpellier, FR, [3]University of Lavras, Lavras, MG, 37200-000, BR.
Email: alan.andrade@embrapa.br

Recent studies in coffee resulted in the identification of many candidate genes for drought tolerance characterized by differential expression profiles in leaves of drought-tolerant and susceptible clones of *Coffea canephora* Conilon. Among those are found the genes involved in the water stress response pathway, such as *CcDREB1D* (coding for DREB transcription factors) that showed higher expression under water stress conditions in the leaves of clone 14 (drought-tolerant) than in those of the clone 22 (drought-sensitive). After sequencing, nucleic polymorphisms were identified in the promoter regions of *CcDREB1D* gene of clones 14 and 22, indicating the presence of three haplotypes (15, 16 and 17) of this promoter. With the aim of studying the participation of these polymorphisms in the response to drought, several genetic constructions of the *CcDREB1D* promoter regions were made in the pBI101 binary vector and tested via genetic transformation of *Nicotiana tabacum* cv. SRI, by evaluating the capacity of such fragments in controlling the expression of the *β*-glucoronidase (*uidA*) reporter gene. For the constructions containing the longest versions (D) of the *pDREB1D*, a basal expression of *uidA* gene was observed in both leaves and roots of T0 plants grown without drought stress. To see if *CcDREB1D* haplotypes would respond to abiotic stresses, T0 tobacco plants were submitted to dehydration and elevated temperature assays, and subsequently analyzed for the expression of the *uidA* reporter gene by GUS histochemical tests and real-time quantitative PCR (RT-qPCR). A slight induction of the *uidA* gene was confirmed in the leaves of T0 plants transformed with pD22-hp17D. However, gene expression levels were much lower than those measured in plants transformed with the positive control (pBI121). No induction of the reporter gene was observed in plants transformed with the different constructions containing the other haplotypes (15 and 16) of the *CcDREB1D* promoter. Altogether, these results showed that (i) the *pDREB1D* promoters of *C. canephora* are weak in tobacco and that (ii) the haplotype 17 of this promoter, derived from *C. canephora* clone 22, was induced with abiotic stresses in the tobacco leaves. This indicates that the molecular mechanisms implicated in the regulation of the gene expression in response to drought are (at least partially) conserved between coffee and tobacco plants and that the functioning of the *pDREB1D* promoters from coffee clones 14 and 22 is different in tobacco, suggesting that the polymorphisms previously identified are important in regulating these promoters.

Support: CAPES, INCT-Café, CNPq and Consórcio Pesquisa Café