# 53. Analysis of *CcDREB1D* promoter region from drought-tolerant and susceptible clones of *Coffea canephora* by homologous genetic transformation of *Coffea arabica*

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In several plant species, the *DREB* genes play a key role in responses to abiotic stress. Since the development of molecular markers is one of the major goals for accelerating breeding programs, a study was done to evaluate the sequence variability of the *DREB1D* gene in several *Coffea* genotypes. The promoter and coding regions of *DREB1D* gene were cloned and sequenced from 16 coffee plants (10 from *C. arabica* and 4 from *C. canephora*), most of them characterized by different phenotypes (tolerance *vs.* susceptibility) regarding to drought. This showed a high conservation of DREB1D proteins among the homologous sequences due to the low level of diversity and the high number of synonymous mutations and neutral changes which represents the majority of sequence variations. However, several nucleic polymorphisms ("single nucleotide polymorphism" and insertion/deletion [InDels]) were found in the coffee *DREB1D* promoters. A comparison of predicted *cis*-acting elements for all the promoter sequences signaled the loss of some regulatory DNA elements. The sequence variation and the loss of some regulatory DNA elements could explain the differences of *DREB1D* gene expression previously observed in leaves of drought tolerant (clone 14) and susceptible (clone 22) clones of *C. canephora*. In fact, both clones 14 and 22, have one same *CcDREB1D* allelic sequence (hp15), and diverge at a second allele. Thus, the *CcDREB1D* allele in the tolerant 14 (hp16) was considered to be the favorable/tolerant allele and the allele in 22 (hp17) was inferior/sensitive. The capacity of *CcDREB1D* promoter to control the expression of the *uidA* reporter gene is under evaluation in transgenic plants of *Coffea arabica* cv. caturra stably transformed by *Agrobacterium tumefaciens* mediated gene transfer procedure. Caturra transgenic embryos were placed on a clean bench and subjected to dehydration tests. Preliminary results of bioassays checking GUS (*β*-glucuronidase) activities indicate that the observed sequence variations have a direct role in the regulation of *CcDREB1D* expression. The proximal promoter of *CcDREB1D* for the three alleles tested (hp15, hp16 and hp17) equally induced the *uidA* gene expression, however, expression of *uidA* under control of the complete *CcDREB1D* promoter was significantly induced in the tolerant allele (hp16) in response to the osmotic stress, whereas, it was not significantly upregulated for the common (hp15) and sensitive alleles (hp17). These results also evidence that the sequence variation present at the first -700 bp of *CcDREB1D* promoter do not interfere the regulation activity of the promoter, probably due to the non-overlapping of SNPs and *cis*-regulatory elements. Though, the higher sequence variation and co-occurrence of SNPs and *cis*-regulatory elements observed between -700 and -1500 bp seems to affect the regulation of *CcDREB1D* promoter in response to drought stress.Support: CAPES COFECUB, INCT-Café, CNPq and Consórcio Pesquisa Café