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.

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