ASIC2016 CHINA•KUNMING
分享咖啡科技进步
Sharing Advance in Coffee Science & Technology

PB231

FUNCTIONAL ANALYSIS OF DIFFERENT PROMOTER HAPLOTYPES OF THE COFFEE (*COFFEA CANEPHORA*) *CcDREB1D* GENE THROUGH GENETIC TRANSFORMATION OF *NICOTIANA TABACUM*

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Abstract:

The regulation of HP15, HP16 and HP17 promoter haplotypes of *CcDREB1D* gene from *Coffea canephora* was studied by performing GUS staining in plants of transgenic *Nicotiana tabacum* subjected to dehydration, heat-shock and cold stress. The increase of GUS staining observed in T1 plantlets transformed by these promoters showed that all of them were upregulated to all tested abiotic stress confirming the importance of these sequences in controlling the expression of *CcDREB1D*.

Introduction

The sequencing of *CcDREB1D* (coding for a Dehydration Responsive Element Binding Protein) promoter regions revealed the presence of three haplotypes (HP15, HP16 and HP17) diverging by single nucleotide polymorphisms and insertions/deletions in the drought-tolerant clone 14 (D^T: HP15/HP16) and drought-susceptible clone 22 (D^S: HP15/HP17) of *C. canephora* Conilon (1). The full-length sequences of these promoters were cloned in the binary vector pBI101 upstream of the *uidA* (coding the *β*-glucuronidase, GUS) (Fig. 1) and transferred using by *A. tumefaciens* mediated transformation in tobacco to study their regulation by performing GUS staining in T1 transgenic plants subjected to different dehydration (DH, Fig. 2A), heat-shock (HS, Fig. 2B) and cold stress (CS, Fig. 3).

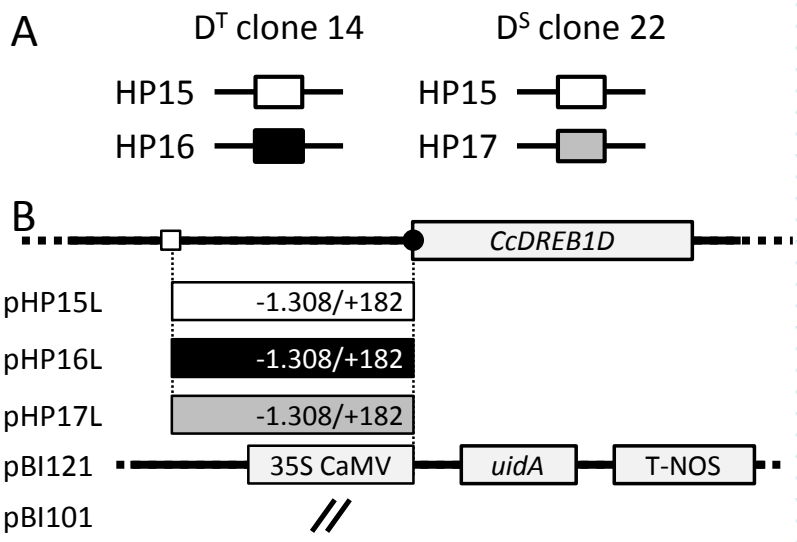

Figure 1. A: *CcDREB1D* haplotypes found in D^T clone 14 and D^S clone 22 of *C. canephora*. Color code for haplotypes: HP15 (white), HP16 (black) and HP17 (gray). **B:** Schematic representation of constructions analyzed in transgenic plants of *N. tabacum*. In all assays, pBI121 (p35S:*uidA*) and pBI101 (*uidA* promoter-less gene) were used as positive and negative controls, respectively.

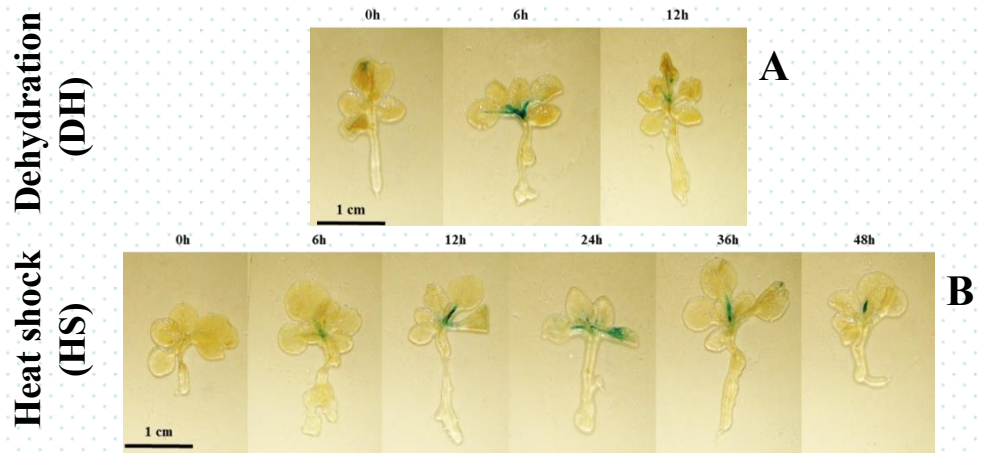

Figure 2. Histochemical localization of GUS activity in T1 transgenic tobacco plantlets transformed with pHP17L and subjected (A) to 6 and 12h of dehydration (DH) and (B) to 6, 12, 24, 36 and 48h of heat-shock (HS).

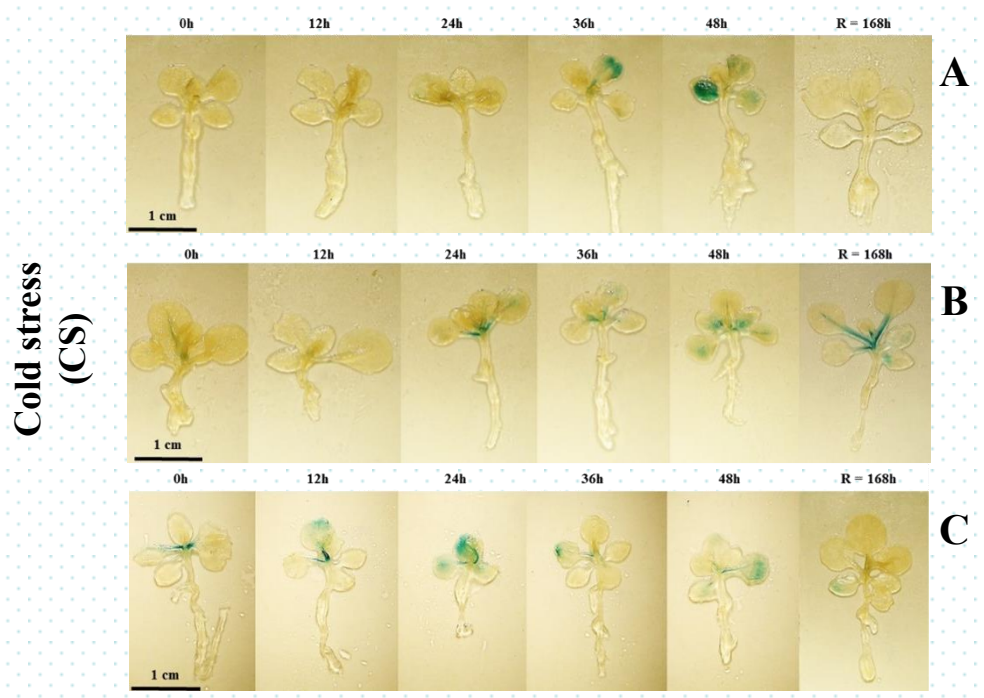

Figure 3. Histochemical localization of GUS activity in T1 transgenic tobacco plantlets transformed with the pHP15L (A), pHP16L (B) and pHP17L (C) and subjected to 12, 24, 36 and 48 of cold stress (CS). R: recovered plants grown without CS for 7 days.

Results and Discussion

- Under DH and HS conditions, GUS activity was detected at 6h in petioles and young leaves of pHP17L-transformed tobacco (Fig. 2A and B).
- Under CS, GUS staining was detected mainly in leaf lamina, petioles and vascular tissues after 36h, 24h and 12h in tobacco plantlets transformed by pHP15L (Fig. 3A), pHP16L (Fig. 3B) and pHP17L (Fig. 3C), respectively.
- For all these experiments, no GUS staining was detected in roots.

Conclusion

- The three haplotypes of the *CcDREB1D* promoter of *C. canephora* were inducible in tobacco by dehydration (DH), heat-shock (HS) and cold stress (CS), indicating that the molecular mechanisms implicated in the transcriptional control of *DREB* gene expression by abiotic stress are highly conserved between tobacco and coffee plants.
- For these three haplotypes, GUS staining was detected only in aerials tissues but not in roots, suggesting a function of *CcDREB1D* gene mainly in coffee leaves.
- GUS staining was always detected earlier in HP17-transformed tobacco plantlets than in those transformed by HP15 and HP16 coffee haplotypes, suggesting a better activation of HP17 by abiotic stress than compared to HP15 and HP16 haplotypes.
- Expression analyses by qPCR of *uidA* reporter gene are on-going to confirm the GUS staining presented here.

References:

1- Alves G.S.C., *et al. Coffea canephora DREB1D* promoter regulates gene expression in mesophyll and stomatal guard cells under drought stress, submitted for publication to *Plant Physiology*.

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