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ANALYSIS OF THE EXPRESSION OF MANNOSE-6-PHOSPHATE REDUCTASE GENE IN ROOTS OF DIFFERENT CLONES OF *C. canephora* SUBMITTED TO WATER DEFICIT

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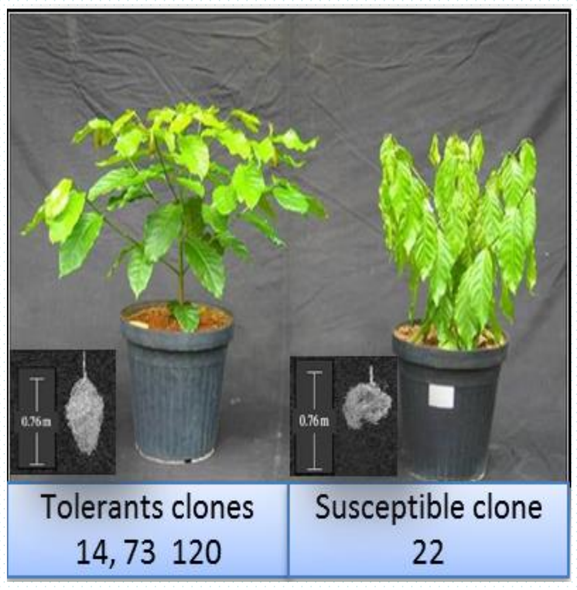
Abstract:

This work studied the expression of the *CcM6PR* gene coding the mannose-6-phosphate reductase in roots of drought-tolerant and drought-susceptible clones of *C. canephora* grown in greenhouse and submitted or not to water limitation. Highest expression levels were observed in roots of the drought-tolerant clone 14 suggesting a key role of this enzyme in response of coffee plants to drought.

Introduction

The mannose-6-phosphate reductase is responsible for the dephosphorylation of mannitol-phosphate giving mannitol (1, 2), an important osmoprotector of plant cells in response to oxidative and osmotic stresses occuring during drought (3). Previous studies already reported differential expression of the *M6PR* gene in leaves of *C. arabica* (4, 5) and *C. canephora* (6) under drought. This study aims to evaluate the expression of the *CcM6PR* gene in roots of drought-tolerant (D^T) and drought-susceptible (D^S) clones of *C. canephora* Conilon grown under controlled drought conditions.

Methods



D^T (14, 73 and 120) and D^S (22) clones of *C. canephora* were grown in greenhouse (UFV, Viçosa-Brazil) and submitted (NI: non-irrigated, Ψ_{pd}= -3.0 MPa) or not (I: irrigated, Ψ_{pd}= -0.2 MPa) to drought.

For each clone and water condition, total RNA was extracted from roots and transcriptome profiles (RNAseq.) were studied after 454 sequencing (Fig. 1). Expression of *CcM6PR* was checked by real-time quantitative PCR (RT-qPCR) using the primer pair 11142 and the *CcUBQ10* gene as reference (Table 1).

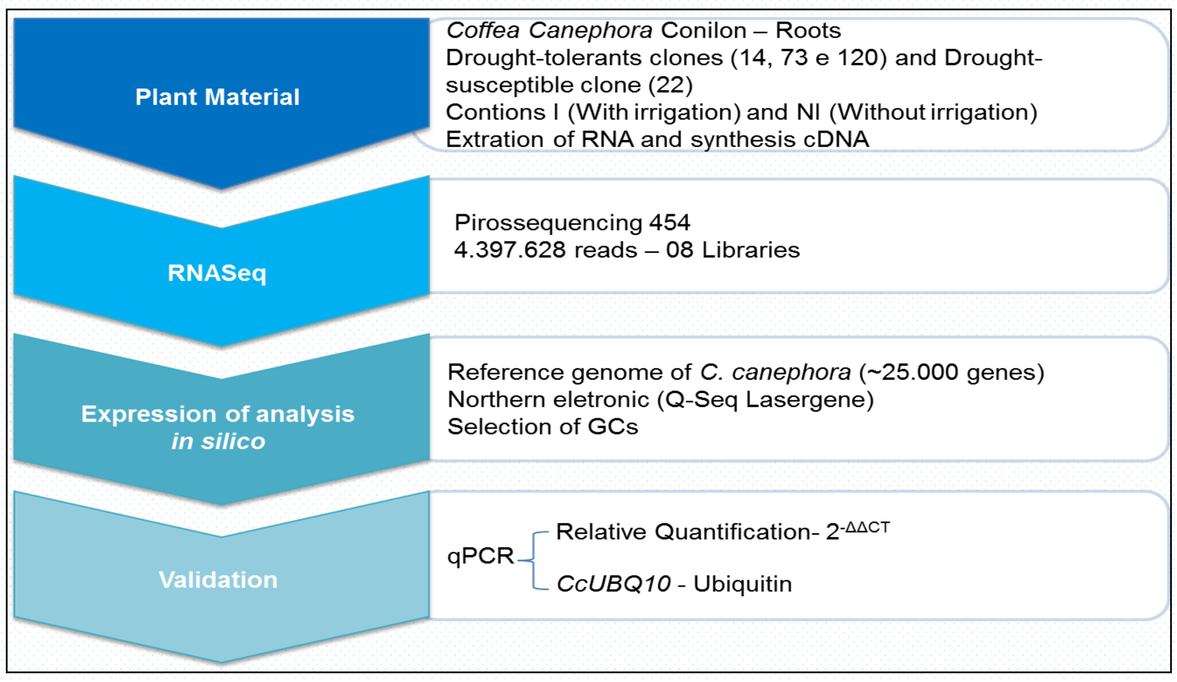


Figure 1. Flowchart of experiments realized during this work

Gene Name	GB numbers	Primer names	Primer sequences
CcM6PR	GT649707	11142-F	CGTTCTCGAGGCTTGCAAAG3
		11142-R	ATGCCTTGTGGGTACTGGAAAAT
CcUBQ10	GW488515	BUBI-F	AAGACAGCTTCAACAGAGTACAGCAT
		BUBI-R	GGCAGGACCTTGGCTGACTATA

Table 1. Primer pairs used for RT-qPCR assays. GB numbers corresponded to coffee ESTs available at NCBI (http://www.ncbi.nlm.nih.gov)

Results and Discussion

In silico analyses clearly highlighted up-regulated expression of *CcM6PR* gene under drought in roots of both D^T and D^T clones of *C. canephora* (Fig. 2A). These results were confirmed by qPCR assays, mainly in roots of the D^T clone 14, and to a lesser extent, in those of D^T (73 and 120) and D^S (22) clones of *C. canephora*.

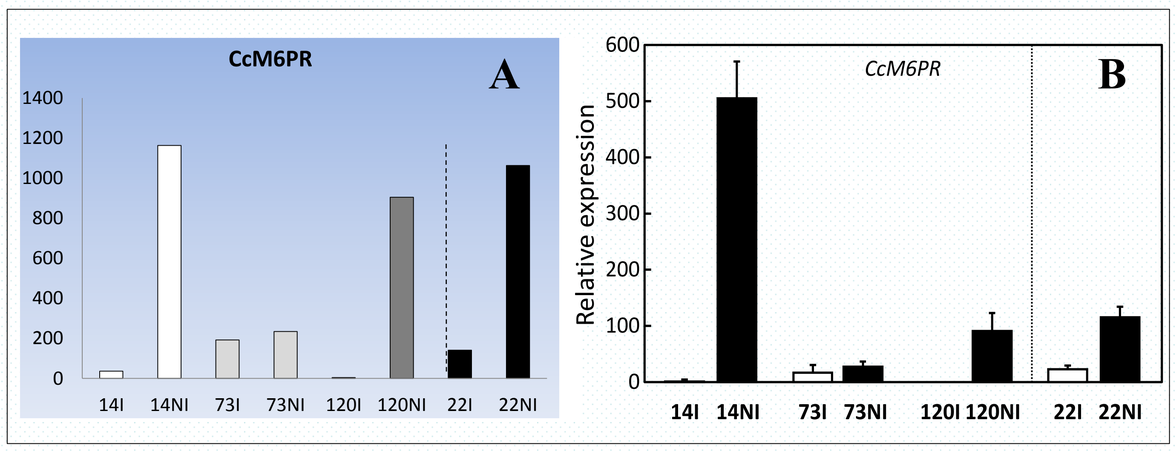


Figure 2. Gene expression profiles of *CcM6PR* in roots of D^T (14, 73 and 120) and D^S (22) clones of *C. canephora* grown with (I) or without (NI) irrigation, deduced from *in silico analyses* (A) and obtained by qPCR experiments (B). For qPCR, expression of the *CcUBQ10* gene was used as a reference and sample 14I as internal calibrator.

Conclusion:

Drought-induced expression of *CcM6PR* gene in roots of D^T clone 14 suggests that mannitol metabolic pathway plays important roles in protecting coffee roots against water limitation in this clone. This gene could be used as a molecular marker to assess the level of stress of the coffee plants subjected to drought.

References:

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