UNCOVERING GENES INVOLVED IN CASSAVA STARCH BIOSYNTHESIS THROUGH SUPPRESSION SUBTRACTIVE HYBRIDIZATION

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ABSTRACT

In an effort to understand the genetic control of starch biosynthesis in cassava, the efficient functional genomic tool, Suppression Subtractive Hybridization, was chosen to investigate the cassava transcriptome. Based on the fact that the initial water supply is documented as one of the main factors affecting the starch quality and yield, the experiment was carried out by subtracting the good starch-producing crop represented by the 6 months-old cassava storage roots grown in the wet season from the poor starch-producing one represented by those grown in the dry season. Two subtracted cDNA libraries were constructed and approximately a thousand clones were subjected to PCR-based differential screening. Hundreds of clones gave positive differential screening results. A number of them were selected to determine the sequence. Several functional proteins, unknown proteins, hits with low significant similarity, and no homology hits were found to be up-regulated in both subtracted libraries. The functional proteins were involved in several physiological processes such as transcription factors, signal transduction proteins, hormone responding proteins, defense responding proteins, cell wall-associated proteins, transporters, and chaperones. The unknown function proteins, hits with low significance, and no hits were good candidates for cassava specific or novel functional proteins. Some of the identified transcripts were subjected to expression profile analysis by semi-quantitative RT-PCR technique with fibrous roots and storage roots (at 3, 6, 9 and 12 months old) grown in wet and dry crops as experimental samples. The genes from the wet subtracted library: AtbZIP60, CCR4-NOT complex, neuronal nitric oxide synthase protein inhibitor, RAV-like DNA binding protein and regulator of gene silencing-calmodulin-like protein were up-regulated in all ages of the wet crop samples while low expression levels were detected or absent in the dry crop samples. Interestingly, these 5 genes and NAP57 were highly expressed in the fibrous roots and could potentially play roles in storage root growth and development. In the dry library, genes coding for ARP1, aquaporin, D28H4, and low molecular weight heat shock protein were proved to be differentially expressed in drought condition and are therefore good candidates for future exploitation in drought improvement. The knowledge gained from this study provides more insights into the starch biosynthesis process and water stress response of the cassava plant.

KEY WORDS: CASSAVA/ TRANSCRIPTOMIC STUDY / SUPPRESSION SUBTRACTIVE HYBRIDIZATION/ STARCH BIOSYNTHESIS/ DROUGHT STRESS

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