

Differential Expression of Native Potatoes Genes in Response to Drought Conditions

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Graphical Abstract

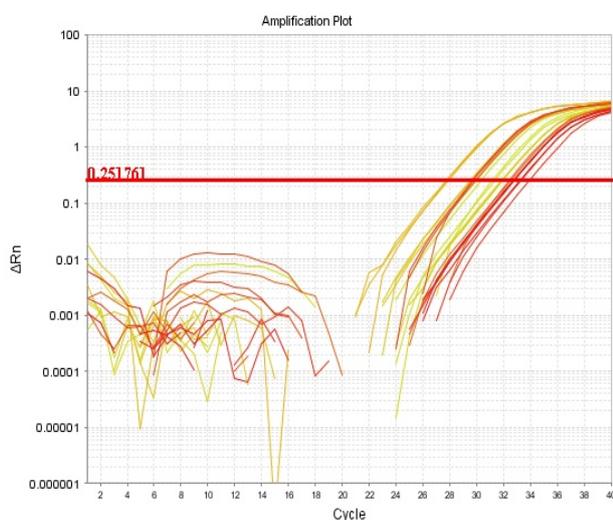


Figure 3. RT-PCR amplification plots representing gene expression for a fatty acid desaturase after drought treatments in native potato plants (drought-tolerant represented in red and drought-susceptible plants represented in yellow).

Abstract.

The exposure of plants to drought conditions increases production of toxic reactive oxygen species (ROS), and negatively affects photosynthesis and carbohydrate metabolism. Few studies of drought tolerance in potatoes have been based on native potato species. Because of their high genetic diversity, native potatoes from the Andean regions of Peru, Ecuador and Bolivia, are well adapted to the harsh environmental conditions that prevail in the Andes, including drought. This makes them ideal candidates for gene expression studies associated with drought tolerance. The identification of drought tolerance traits and genes for potato will facilitate breeding for high yield stability under drought conditions. A drought experiment with both tolerant and susceptible native potato species, using an aeroponics growth system was conducted. Selected drought-associated candidate genes from RNA sequence analysis were used in primer design and quantitative RT-PCR analysis. Differential gene expression in tolerant vs. susceptible cultivars was confirmed for chloroplastic heat shock protein 1, for triacyl glycerol lipase, and for a fatty acid desaturase.

Introduction

Potato, the fourth most important crop in the world, is generally considered to be drought-sensitive. Drought susceptibility in potato impacts all stages of the crop, from emergence to tuber initiation (Evers *et al.* 2010). Although there have been various studies of the global changes in gene expression profiles during drought conditions in potatoes, few studies have been based on native potato species. Because of their high genetic diversity, native potatoes from the Andean regions of Peru, Ecuador and Bolivia, cultivated at altitudes as high as 11,480 ft. (3500m) above sea level, are well adapted to the harsh environmental conditions that prevail in the Andes, including drought. This makes them ideal candidates for gene expression studies associated with drought tolerance. In a previous

phase of this study conducted at the Universidad Peruana Cayetano Heredia (UPCH), RNAseq of both a drought-tolerant- and a drought- resistant native potato species was used to identify a large set of drought-tolerant genes. The purpose of this study was to confirm differential gene expression in drought-associated candidate genes obtained from RNAseq data during drought response and recovery.

Materials and Methods

Plant Material.- *Solanum tuberosum* ssp. *andigena*, Negrita 703 671 (drought-tolerant) and Wila - HuakaLajra 703 248 (susceptible to drought) plants were used in the aeroponics drought experiment. Previously propagated in vitro, rooted and transferred to an aeroponics growing system at the INIA Experimental Station in Huancayo, Peru, they received misted water on the roots every 15 minutes, for 10 continuous minutes. After both varieties were adapted to the aeroponics system, a drought experiment was conducted. The four treatments consisted on, control, early, late response and recovery (Table 1). Following the treatments, three plants were randomly selected for each of the four groups in the two varieties. Three leaves were collected from each of the selected plants and immediately frozen in liquid nitrogen inside 50 mL tubes.

Table 1. Drought treatments on native potato plants. Plants were grown on an aeroponics system in which they were normally watered by misting the roots with water every 15 min for 10 continuous minutes.

| Drought Treatment: | Abbrev. | Water Deprivation: |
|--------------------|---------|---|
| Control | T0 | None |
| Early Response | T1 | 40 min |
| Late Response | T2 | 120 min |
| Recovery | T3 | 120 min followed by 20 min of mist in the roots |

RNA Extraction.- After RNA was extracted from native potato varieties (tolerant and susceptible) using the TRIzol modified protocol, they were DNased using the DNA free kit (Ambion).

Primers for Drought-Associated Candidate Genes.- Genes were selected from the RNA sequencing data showing differential expression in drought-tolerant vs. susceptible varieties. Primer Express 3.0 software (Applied Biosystems) was used to design the primers. Glyceraldehyde 3-phosphate dehydrogenase (GADPH) gene were included as the internal control for gene expression experiments.

Quantitative reverse transcriptase-polymerase chain reaction (qRT-PCR).- For gene expression analysis, two-step RT-PCR was used. RNA was first reverse transcribed using the ThermoScript RT-PCR System (Invitrogen). The Fast 2X SYBR Green Master Mix (Applied Biosystems) together with the Step-One Plus Fast Real-Time PCR System (Applied Biosystems) was used for qPCR analysis. Primer efficiency was determined with the software included with the instrument. The gene expression analysis was done using the Pfaffl method.

Results and Discussion

Chloroplastic small heat shock protein was affected by the drought treatments in both susceptible and resistant varieties, with a drop in transcript abundance during late drought as well as after recovery (Table 2). This drop was more pronounced in the susceptible variety, perhaps indicating that the resistant variety was more capable of expressing this gene at normal levels even under the stressful drought conditions. Heat shock proteins are chaperone molecules that stabilize structures of proteins by preventing denaturation during stress (Evers *et al.*, 2010). Triacylglycerol lipase is almost unchanged in transcript level in the susceptible variety but shows a decrease during the drought recovery period in the resistant variety. Exposure to environmental stress leads to the generation or

reactive oxygen species (ROS). ROS cause lipid peroxidation with the subsequent destruction of membrane lipids affecting cell viability. Triacylglycerol lipase has a role in mobilization of triacylglycerol, lipid signaling and membrane degradation (Eastmond, 2006). While the susceptible variety showed a drop in transcript levels during late drought for the fatty acid desaturase gene, the resistant variety showed up to a two-fold increase during drought late response. This generated a 3.2 fold difference between both varieties pointing to a potential association of this gene to drought tolerance. Fatty acid desaturases regulate the overall level of fatty acids in plants and have been found associated with defense signaling pathways (Kachroo *et al.* 2001).

Table 2. Gene Expression Analysis of selected candidate genes. The Pflaff method was used to estimate fold difference between samples following qRT-PCR analysis. Data was normalized using GAPDH as housekeeping gene. Left: Chloroplastic heat shock protein 1; Center: Triacylglycerol lipase; Right: Fatty acid desaturase.

| Treatment | Susceptible | Resistant | Resistant vs. Susceptible | Treatment | Susceptible | Resistant | Resistant vs. Susceptible | Treatment | Susceptible | Resistant | Resistant vs. Susceptible |
|-----------|-------------|-----------|---------------------------|-----------|-------------|-----------|---------------------------|-----------|-------------|-----------|---------------------------|
| Time 0 | 1.0 | 1.0 | - | Time 0 | 1.0 | 1.0 | - | Time 0 | 1.0 | 1.0 | - |
| Time 1 | 1.1 | 1.0 | - | Time 1 | 1.3 | 1.1 | 0.8 | Time 1 | 1.1 | 1.4 | 1.3 |
| Time 2 | 0.4 | 0.7 | 1.8 | Time 2 | 1.0 | 0.9 | 0.9 | Time 2 | 0.6 | 2.0 | 3.2 |
| Time 3 | 0.2 | 0.5 | 2.5 | Time 3 | 1.0 | 0.6 | 0.6 | Time 3 | 1.0 | 1.7 | 1.7 |

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