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Low temperature induce differential expression genes in banana fruits

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Abstract

Differential display was used to identify gene expression of banana fruit in response to low temperature stress. Banana fruits were kept at 10 °C for 8 h and 60 differential expressed fragments in pulp and peel were obtained. These fragments had an average size of 200 pb or greater and Dot blotting hybridization as well as Northern blot corroborated specific expression of these differential expressed fragments. Among the several differentially expressed genes, we found genes involved in response to pathogen attack, wounding and a ripening-associated gene. We consider these genes to belong to a general pathway which is activated upon general stress signaling.

Keywords: Bananas; cDNA; Low temperature injury; Differential display; *Musa acuminata*

1. Introduction

Environmental stresses include several factors that are well known to have an effect on plant growth and seed production (Shinozaki et al., 2003). In banana fruits low temperature causes alterations in the physiology which includes retarded development of the yellow skin color and failure of the fruit to become soft (Jiang et al., 2004). Banana fruits contain high levels of starch (15–39%) which undergo a drastic biochemical change during ripening (Lizada et al., 1990). The change includes a marked increase in soluble sugars and organic acids, chlorophyll breakdown and polymerization of phenolic compounds in the ripening fruits (Pawlowski et al., 1994). When tropical and subtropical fruits are exposed to low temperature injury, abnormal ripening, pitting, water-soaked areas and increased susceptibility to fungal infection occur (Sharom et al., 1994). Cellular membrane damage is a typical symptom that occurs during early low temperature injury (Marangoni et al., 1996). It has been reported that the browning (low temperature injury symptom) in banana peel is caused by the oxidation of polyphenols caused primarily by polyphenol oxidase (PPO) (Yang et al., 2000). Nguyen Thuy et al. (2003) report that when the banana fruits are exposed to low temperatures, an increase activity of both phenylalanine ammonia lyase (PAL) and PPO occurs (Nguyen Thuy et al., 2003). In low temperature sensitive plants oxidative stress is a major component of chilling stress and active oxygen species (AOS) such as hydrogen peroxide, superoxide radicals and hydroxyl radicals that can react very rapidly with DNA, lipids and proteins causing severe cellular damage (Van Breusegem et al., 1999).

Many genes induced by stress have been identified to be essential for stress tolerance, which included genes of lipid metabolism, chloroplast function, free radical detoxification, etc. (Provart et al., 2003). Many important crops and fruits are injured by exposure to low, nonfreezing temperatures in the range of 0–12 °C. The effects may be multigenic and the molecular mechanisms are not well understood. Low temperature injury also limits the extended life storage of many vegetables and fruits (Jiang et al., 2004; Ratule et al., 2006; Wang et al., 2006; Sanchez-Ballesta et al., 2003; Trakulnaleumsai et al., 2006).

In tropical and subtropical fruits the damage caused by low temperature stress as surface discoloration where the subepidermal tissues reveal dark-brown streaks and pulp browning

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are well documented among several physiological symptoms (Kader A., *com pers*; Jones, 2000); however, the genes involved during this type of stress are unknown in banana fruits. Therefore, in order to elucidate the molecular mechanisms underlying the low temperature response genes of banana fruits, we first used mRNA differential display (DD) to search for differentially expressed genes during the low temperatures process. This allows a first step in defining some of the players involved directly and indirectly in the response of the fruit to an adverse low temperature injury. The effect of low temperatures result in severe losses for the banana producers during winter cold spells in the tropics. Therefore this work is a first attempt to define the genes expressed under low temperatures.

2. Material and methods

2.1. Fruit material

Bananas fruits (*Musa acuminata* cv "*Grand Nain*") were obtained from banana plants grown in a set breeding ground. Plants were cultivated at 1500–2000 $\mu molm^{-2}S^{-1}$, 40% RH and average temperature of 37 °C. The fruits were harvested in the fourth stage of ripening (more yellow than green); according to stage proposed to Customers Services Department Chiquita Brands, Inc., Cincinnati, OH. (Clendennen and May, 1997). We harvested the first two hands of each cluster (four bananas) and took one hand of each cluster for treatment, hands of fruit randomized and allocated to treatment. For the low temperature untreated and treated, fruits were incubated at room temperature (23 °C) or at 10 °C during 8 h, respectively (Jones, 2000). Each treatment had two replicas. Four fruits of each hand were collected, pulp and peel were separated and frozen in liquid nitrogen.

2.2. RNA extraction

Total RNA was isolated as previously publish (Medina-Escobar et al., 1997) with the following modifications. All the steps were developed in ice and the pellets were obtained by centrifugation at 16060 × *g* in a Beckman rotor JA20 (Beckman). Two grams of fruit tissue (peel or pulp) were frozen in liquid nitrogen and ground to a fine powder. The ground material was homogenized in 10 ml of RNA extraction buffer (100 mM Tris–HCl pH 8, 50 mM EDTA pH 8 and 500 mM NaCl); 1.4% β-Mercaptoethanol and 2% SDS and incubated for 15 min at 65 °C. After of this incubation 1.5 M potassium acetate was added and the samples were incubated for 10 min at −20 °C. The supernatant was obtained by centrifugation 16060 × *g* for 30 min at 4 °C. The aqueous phase was recovered, and the total RNA was precipitate by addition of one volume of isopropanol; the samples were incubated for 1 h at −20 °C and centrifuged for 30 min at 4 °C. The pellets were resuspended with 100 μl of TE (100 mM Tris–HCl pH 8, 50 mM EDTA pH 8), and 300 mM acetate sodium and 50% isopropanol was added to the mixture; the samples were incubated overnight at −20 °C and centrifuged; the pellets washed with 70% EtOH, centrifuged and resuspended with nuclease free-water. LiCl (4 M) was added to the mixture to a final concentration of 4 M and the samples were incubated for overnight, centrifuged for 45 min as indicated above at 4 °C. The pellets washed with 70% EtOH, and resuspended with nuclease free-water.

2.3. mRNA differential display

mRNA differential display was carried out as is described in the handbook of GeneHunter Corporation (1997). Total RNA of four fruits were quantified and pooled, to realize DD. Total RNA was treated with Dnase I (Invitrogen) and used for reverse transcription. For each sample, three reverse transcription reactions were set up, containing one of the three different HT11M primer (where M can be A, C or G) anchored to the beginning of the poly(A+) tail. SuperScript II reverse transcriptase (Invitrogen) was added after pre-incubation for 5 min at 65 °C and for 2 min at 42 °C. Reverse transcription was performed by incubation at 42 °C for 50 min, then was stopped by inactivating the SuperScript II reverse transcriptase for 15 min at 70 °C. Polymerase chain reaction (PCR) amplification of each reverse transcription products was carried out in combination with one of eigth arbitrary primers. PCR was carried out in a thermal cycler (Techne TC-412) under the following conditions: 1 cycle of 94 °C for 30 s; 35 cycles of 94 °C for 30 s; 40 °C for 1 min; 72 °C for 30 s and a final extension at 72 °C for 15 min. The amplified cDNAs were separated by denaturing polyacrylamide electrophoresis using Bio-Rad Sequi-Gen® GT Nucleic Acid Electrophoresis Cell (Bio-Rad). 10 μl of each PCR sample was incubated with 3 μl of loading dye at 95 °C for 5 min immediately, cooled on ice and loaded in gel of polyacrylamide. Amplified fragments were visualized by a silver stain.

2.4. Silver staining

Silver Staining basically performed as describe previously by Bassam et al., 1991. With the following modifications; the gel was fixed with 10% acetic acid (v/v) and stained with 3% silver nitrate activated with 0.15% formaldehyde for 30 min and development with 3% sodium carbonate, 2% sodium thiosulfate and 0.15% formaldehyde. The reaction was stopped with 10% acetic acid (v/v).

2.5. Cloning and sequencing

Differentially expressed cDNA fragments were recovered as indicated (Wang et al., 1998). The bands of differentially expressed cDNA fragments were cut from the gel and transferred to an eppendorf tube with 50 μl of nuclease free water and incubated for 2 h at 40 °C. The gel debris was spin down and the supernatant containing the DNA was collected. This DNA was used as template to reamplify the specific fragment using the same set of primers. PCR was carried out in a thermal cycler under the following conditions: 35 cycles of 94 °C for 30 s; 40 °C for 1 min; 72 °C for 30 s and a final extension at 72 °C for 15 min. The samples were separated in