Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Research International 38 (2005) 29–36
FOOD RESEARCH INTERNATIONAL
www.elsevier.com/locate/foodres

On the induction of cold acclimation in carrots (*Daucus carota* L.) and its influence on storage performance

Federico Gómez Galindo [a,*], Luisa Elias [b], Vassilis Gekas [a,c], Werner B. Herppich [d], Maggie Smallwood [b], Marianne Sommarin [e], Dawn Worrall [b], Ingegerd Sjöholm [a]

[a] *Department of Food Engineering, Lund University, P.O. Box 124, SE – 22100, Lund, Sweden*
[b] *Department of Biology, Centre for Novel Agricultural Products, University of York, P.O. Box 373, York, UK*
[c] *Department of Environmental Engineering, Technical University of Crete, Chania, Greece*
[d] *Institute of Agricultural Engineering, Division of Horticultural Engineering, Max-Eyth-Allee 100, D-14469, Potsdam, Germany*
[e] *Department of Plant Biochemistry, Lund University, P.O. Box 124, SE – 22100, Lund, Sweden*

Received 26 April 2004; accepted 28 July 2004

Abstract

We investigated the role of cold acclimation in carrot plants with respect to its influence on the storage performance of the harvested taproots. The induction of cold acclimation was followed in plants cultivated in a growth chamber under strict climate control and in taproots harvested from two separate field cultivations where the plants had been exposed to the natural variations in climate. Under controlled growth conditions, levels of antifreeze protein (AFP) mRNA were used as a marker for cold acclimation in carrot taproot tissue. Expression of this gene was induced by cold in discs excised from harvested taproots and this induction was clearly affected by the growth temperature of the plants from which the taproots were taken. These in vitro data were consistent with those from field-grown plants. In the cell wall of taproots harvested in year 2000, where the intact plants had frequently been exposed to temperatures below 6 °C, a 36 kDa AFP accumulated to higher levels during storage than in the taproots harvested from plants grown in year 2001, where cultivation temperatures had rarely dropped below 6 °C. The taproots from 2001 exhibited poor storage performance as shown by an earlier increase in relative electrolyte leakage and decrease in dry matter compared to taproots harvested in 2000. The capacity of the AFP to accumulate during storage was consistent with a high storage performance.

Keywords: Carrots; Cold acclimation; Antifreeze protein; Storage performance

1. Introduction

Carrots (*Daucus carota* L.) are biennial plants, and survival of the underground storage root over winter is fundamental to initiate the re-growth of the plant to flower and set seed in the second year. Like other plants, carrots increase their freezing tolerance in response to a period of exposure to low, but non-freezing temperature, a process known as cold acclimation (Graham & Pattersson, 1982).

Cold acclimation is a very complex process involving anatomical (Rapacz, 2002) and histological adjustments (Strand et al., 1999) and, specially, numerous physiological alterations that are regulated at the gene expression level (Thomashow, 1999). The latter changes involve increased concentrations of sugars, soluble proteins, amino-acids, organic acids and alteration in membrane lipid composition (Danyluk et al., 1998; Strand et al., 1999; Svenning, Rosnes, & Junttila, 1997). Some of the low-temperature-responsive genes are predicted to encode

* Corresponding author. Tel.: +46 4622 29822; fax: +46 4622 24622.
E-mail address: federico.gomez@livstek.lth.se (F. Gómez Galindo).

0963-9969/$ - see front matter
doi:10.1016/j.foodres.2004.07.004

antifreeze proteins (AFPs; Thomashow, 1998). These apoplastic proteins have the ability to modify the growth of ice crystals, to inhibit the recrystallization of ice and to exhibit thermal-hysteresis activity (Smallwood et al., 1999; Worrall et al., 1998). Accumulation and activity of AFPs are always cold-induced and it has been suggested they could be used as a biological marker for crop improvement programs (Chun, Yu, & Griffith, 1998; Griffith, Ala, Yang, Hon, & Moffatt, 1992).

When cultivated at low temperature, carrot storage roots accumulate large amounts of AFPs in their cell walls (Smallwood et al., 1999; Worrall et al., 1998). Harvested carrots increase their overall content of osmotically active substances and adjust the elastic properties of their root tissue during storage (Herppich, Mempel, & Geyer, 2001). All these changes are thought to contribute to the increased resistance to freezing-induced damage and dehydration associated with cold-acclimation (Pearce, 2001; Thomashow, 1999).

Cold stress and the cold acclimation status of carrots, that develops during growth in the field, has been suggested to play an important role in the maintenance of yield quality and high sensory attributes during storage (Rosenfeld, Baardseth, & Skrede, 1997a; Rosenfeld, Riskvik, Samuelsen, & Rödbotten, 1997b; Suojala, 2000). Thus a deeper knowledge of the cold acclimation process, including its environmental regulation in both growing and harvested carrot roots is necessary to understand the metabolic changes that can be related to postharvest properties of the vegetable. It is possible that characterisation of the cold acclimation process during the growth period could be used as a method to forecast storability in the marketable taproots.

The purpose of this investigation was to study the induction of cold acclimation in both growing and harvested carrots to understand the influence of this process on the storage potential of the harvested roots. Cold acclimation was studied in carrot plants grown in a controlled growth chamber and in carrots grown in the field during two years which differed in climatic conditions. The temperature at which carrot plants grow in the field and the levels of AFP, the biological marker for cold acclimation that we have used, are useful criteria to know how late in the year carrots should be harvested and to forecast storage performance.

2. Materials and methods

2.1. Carrot plants grown under controlled conditions: Experiment 1 – Detection of AFP transcript in carrot tissue of intact carrot plants

Carrot plants (*D. carota* L. cv. Autumn King) were cultivated and cold treated as described by Smallwood et al. (1999). Carrot plants were sown in pots and cultivated for 12 weeks in a greenhouse. Cold acclimation was performed by transferring the plants to a Conviron growth chamber set at a cycle of 6 °C for 8 h in the light and 2 °C for 16 h in the dark for 1–21 days. Taproots were harvested and total RNA immediately isolated.

2.2. Carrot plants grown under controlled conditions: Experiment 2 – Detection of AFP transcript in incubated tissue discs from harvested taproots

Induction of *AFP* message was followed in taproot tissue slices obtained from carrot plants cultivated under green house conditions and from plants cold-acclimated for 21 days under the controlled conditions described above. In both cases, the taproots were harvested and immediately sliced into cross-sections approximately 5 mm thick and placed on water saturated 3 MM filter paper (Whatman) in plastic Petri dishes. This procedure was carried out at room temperature. The slices were incubated in the dark either at 21 °C (room temperature) or at 4 °C for 1–7 days; two slices were harvested and used for preparation of RNA.

2.3. RNA gel-blot analyses

Total RNA was isolated using the method of Verwoerd, Dekker, and Hoekema (1989). RNA (10 μg) was denatured in 1 × TBE buffer (100 mM Tris base/90 mM boric acid/2 mM EDTA, pH 8.0) containig 25% formamide and heated to 65 °C for 5 min before separation by agarose-gel electrophoresis. The RNA was then blotted on to Zetaprobe membrane (Bio-Rad) by capillary transfer. The blot was hybridized with the radiolabelled *AFP* probe, derived from carrot cDNA (Smallwood et al., 1999), at 65 °C using the buffer system of Church and Gilbert (1984) described in the Zetaprobe manual (BioRad).

2.4. Carrot plants grown under natural conditions: field data and preharvest climate conditions

Carrot plants (*D. carota* L., cv. Nerac) sown the second week of May by growers in southern Sweden were used in this study in two successive years. Climate data covering the growth area obtained during 86 days, corresponding to August, September and October (the last three months of the growth period) each year, was given to us as a courtesy of the Swedish Meteorological and Hydrological Institute, Norrköping, Sweden.

2.5. Harvested taproots, handling and storage

Taproots were harvested the last week of October of each year from the same location in southern Sweden. The carrots, 400 kg, were washed, hydrocooled