Postharvest Biology and Technology 53 (2009) 51–57

Contents lists available at ScienceDirect

Postharvest Biology and Technology

journal homepage: www.elsevier.com/locate/postharvbio

Expression of five expansin genes during softening of *Fragaria chiloensis* fruit: Effect of auxin treatment

Carlos R. Figueroa[a], Paula Pimentel[a], Marcela C. Dotto[b], Pedro M. Civello[b,c], Gustavo A. Martínez[b,c], Raúl Herrera[a], María Alejandra Moya-León[a,*]

[a] *Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, Casilla 747, Talca, Chile*
[b] *IIB-INTECH (Instituto de Investigaciones Biotecnológicas-Instituto Tecnológico de Chascomús) (CONICET-UNSAM), Camino de Circunvalación Laguna, Km 6, (B7130IWA) Chascomús, Pcia, Buenos Aires, Argentina*
[c] *Facultad de Ciencias Exactas, Universidad Nacional de La Plata (UNLP), 47 y 115, (1900) La Plata, Argentina*

ARTICLE INFO

Article history:
Received 15 December 2008
Accepted 21 February 2009

Keywords:
Chilean strawberry
Fruit softening
Expansin
Auxin

ABSTRACT

The Chilean strawberry fruit (*Fragaria chiloensis*) has potential as a new exotic berry. The rate of softening differs between *F. chiloensis* and its related species *Fragaria* × *ananassa*. The expression profiles of five expansin genes isolated from *F.* × *ananassa* were analyzed during softening of *F. chiloensis* fruit and the regulatory effect of auxins on them observed. The rapid decrease in fruit firmness observed between the large green and the turning stages of *F. chiloensis* correlated with the large increase in transcript accumulation of *FaEXP2* and *FaEXP5*. *FaEXP4* and *FaEXP6* had lower expression levels in *F. chiloensis* than in *F.* × *ananassa*, and expression profiles were not related to fruit softening. Auxins strongly repressed the expression of *FaEXP1* and *FaEXP2*, and had a minor repressive effect on *FaEXP4* and *FaEXP5*. In addition, tissue-specific expression was probed in different *F. chiloensis* tissues: *FaEXP2* and *FaEXP5* transcripts were found only in fruit tissues, while *FaEXP4* and *FaEXP6* transcripts were also found in runners, roots, leaves and flowers. In conclusion, most of the strawberry-expansin genes are expressed in *F. chiloensis* and some family members are closely related to fruit softening, especially *FaEXP2* and *FaEXP5*. This study reveals the repressive effect of auxins on the expression of those expansin genes related to softening in *F. chiloensis* fruit.

1. Introduction

The Chilean strawberry (*Fragaria chiloensis* L. (Duch.)) fruit is one of the wild parents of the common strawberry *Fragaria* × *ananassa* Duch. (Hancock, 1999). It is noted for its good fruit quality characters, having the potential to become a new berry fruit that Chile can export (Retamales et al., 2005). In addition, *F. chiloensis* is emerging as a new fruit model to help in understanding several ripening-associated processes in strawberry, such as anthocyanin biosynthesis (Cheel et al., 2005) and cell wall degradation (Nishizawa et al., 2002). Studies on fruit softening are important as this influences the postharvest life of highly perishable fruit (Koh and Melton, 2002).

The ripening-associated softening of fleshy fruit has been largely seen as a direct consequence of enzyme-mediated cell wall degradation. Events such as depolymerization and solubilization of hemicelluloses and pectins within the cell wall often occurs in many fleshy fruit (Rose et al., 2003). As *F.* × *ananassa* and *F. chiloensis* are related species, and previous reports have shown that Chilean strawberry fruit has a faster softening rate than *F.* × *ananassa* cv. Chandler fruit (Figueroa et al., 2008), a comparative analysis of the cell wall degradation process and associated genes might help to clarify the differences found in softening rates. Using this approach, the higher softening rate of *F. chiloensis* fruit has been associated with higher expression levels of the polygalacturonase gene at the beginning of ripening (Figueroa et al., 2008). Polygalacturonase and other genes such as pectate lyase, pectin methylesterase, endoglucanase, β-galactosidase and expansins increased their expression during strawberry ripening and their participation on cell wall degradation is well documented (Harpster et al., 1998; Trainotti et al., 2001; Castillejo et al., 2004; Dotto et al., 2006; Villarreal et al., 2008; Santiago-Doménech et al., 2008). Several reports indicate that pectin metabolism has a significant impact on strawberry fruit firmness (Rosli et al., 2004; Santiago-Doménech et al., 2008) rather than hemicellulose or cellulose catabolism (Koh et al., 1997; Woolley et al., 2001). Nevertheless, hemicellulose modification and its impact on strawberry softening cannot be discarded.

Expansins are proteins that probably disrupt the hydrogen bonds between cellulose microfibrils and the polysaccharides of the cell wall matrix, and thereby increase the accessibility to

* Corresponding author at: Laboratorio de Fisiología Vegetal y Genética Molecular, Instituto de Biología Vegetal y Biotecnología, Universidad de Talca, 2 Norte 685, Talca, Chile. Tel.: +56 71 200 280; fax: +56 71 200 276.
E-mail address: alemoya@utalca.cl (M.A. Moya-León).

0925-5214/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.postharvbio.2009.02.005

various cell wall hydrolases (McQueen-Mason and Cosgrove, 1995; Rose and Bennett, 1999). These proteins were originally considered to play a role on elongation growth, and high levels of expansin mRNAs have been detected in various growing tissues, including hypocotyls, roots (Rt), leaves (L) and young fruit (Shcherban et al., 1995; Catalá et al., 2000). In addition to growing tissues, expansin proteins also have been detected in a range of ripe fruit, such as tomato (Rose et al., 1997), strawberry (Civello et al., 1999), peach (Hayama et al., 2003), pear (Hiwasa et al., 2003), banana (Asha et al., 2007), grape (Ishimaru et al., 2007) and apple (Goulao et al., 2008). On the other hand, the constitutive suppression of *LeEXP1* in tomato rendered firmer fruit while overexpression of the gene caused higher fruit softening (Brummell et al., 1999), suggesting that expansins play a major role in fruit softening. In strawberry fruit, seven expansin genes have been identified and the expression profile of each of them characterized in developing fruit and in leaves, roots, runners (Ru) and flowers (F) (Civello et al., 1999; Harrison et al., 2001; Dotto et al., 2006). The expression of the strawberry-expansin genes *FaEXP1*, *FaEXP2* and *FaEXP5* has been correlated with fruit firmness reduction, and the latter two genes are fruit specific (Harrison et al., 2001; Dotto et al., 2006). In the case of expansins *FaEXP3* and *FaEXP7,* their expression level is low in the fruit, with the highest expression of *FaEXP7* in runners (Harrison et al., 2001). The expression pattern of expansins *FaEXP4* and *FaEXP6* is not correlated with fruit softening (Dotto et al., 2006) and both genes are expressed in fruit, runners, roots and leaves (Harrison et al., 2001).

Hormonal regulation could play a role in expansin gene expression. In strawberry fruit it has been shown that auxins delay ripening by altering the expression of many ripening-associated genes (Given et al., 1988). Most of strawberry ripening-related genes are negatively regulated by auxins, although a few auxin up-regulated genes have also been described (Manning, 1998; Aharoni et al., 2002). Genes encoding pectate lyase and endoglucanase are activated at the onset of ripening of strawberry fruit, while their expression is reduced by the application of exogenous auxins (Medina-Escobar et al., 1997; Harpster et al., 1998). On the other hand, expression of *FaEXP2* was not affected by auxin treatment (Civello et al., 1999). In order to assess the role of expansin genes on softening of the Chilean strawberry fruit, we analyzed the expression pattern of five expansins in four developmental and ripening stages of both *Fragaria* species, *F. chiloensis* and *F.* × *ananassa.* In addition, a hormonal assay was set up in *F. chiloensis* fruit in order to evaluate the regulatory effect of auxins on the expression of each expansin gene.

2. Materials and methods

2.1. Plant material

Fruit of both strawberry species (*F.* × *ananassa* cv. Chandler and *F. chiloensis*) of different sizes and stages of ripeness were harvested from plants grown in the same commercial field at Contulmo, Biobío Region, Chile (latitude 38°04′8.6″ S; longitude 73°14′2.96″ W). Fruit were classified into four different developmental stages as previously reported by Figueroa et al. (2008), according to weight and color of the receptacle: small green fruit (SG); large green fruit (LG); turning fruit (T); and ripe fruit (R).

2.2. Firmness measurement

Firmness was measured using a digital force gauge pressure tester (model AD-4932-50N, ADTaiwan) fitted with a 5 mm cylinder tip. Two measurements were performed on opposite equatorial sides of each berry; 30 berries per each developmental stage were analyzed. The mean was recorded and expressed as Newtons (N) ± standard deviation (S.D.). After measurement of firmness, the peduncle and calyx of each fruit were removed, and the fruit cut longitudinally into two halves, frozen in liquid nitrogen and stored at −80 °C until use.

2.3. Auxin treatment

In order to test the role of auxins, entire *F. chiloensis* fruit at the LG stage were divided into four lots of 24 fruit each. Intact fruit from the first lot was treated with the synthetic auxin (naphthalene acetic acid, NAA). For that, each fruit was dipped for 3 min in a solution containing 1 mM NAA, 0.06 M citric acid, 0.074 M Na_2HPO_4, 5 mM DTT and 2% (v/v) DMSO (pH 4.5) (+/+ treatment). Intact fruit from the second lot were dipped in the same buffer solution without NAA for 3 min (+/− treatment). Achenes were carefully removed from fruit belonging to the third and fourth lots, using sharp tweezers. Fruit from the third lot were treated with 1 mM NAA solution (−/+ treatment) while fruit from the last lot was dipped in a solution without NAA (−/− treatment). To avoid dehydration, the peduncle of each fruit was immersed in a microcentrifuge tube containing distilled water. Auxin and buffer immersions were repeated every 12 h during the observation period. Fruit were maintained at 20 °C, samples were collected after 24 h of treatment and immediately frozen in liquid nitrogen and stored at −80 °C until use.

2.4. Preparation of probes

Probes for northern blot hybridization for each species were prepared by PCR amplification of a cDNA sample corresponding to ripe fruit using specific primers for each expansin gene. The sequences of primers and amplification conditions used were the same as in Dotto et al. (2006).

2.5. RNA isolation and northern blot analysis

Total RNA was isolated by using the CTAB methodology (Chang et al., 1993) from a pool of frozen tissue (8 g) for each fruit stage. Concentration of RNA was measured using a spectrophotometer (Thermo Scientific NanoDrop™, USA). Fifteen micrograms of total RNA sample was separated by electrophoresis in a 1.5% (w/v) denaturing agarose gel containing 17.6% (v/v) formaldehyde. Samples were then transferred to Hybond-N membranes (Amersham Bioscienes, UK). Membranes containing total RNA from each developmental stage and each auxin treatment were prehybridized for 4 h at 42 °C in a solution containing 50% deionized formamide, 1% SDS, 5× SSCE, 5× Denhart's solution and 100 μg/mL denatured salmon sperm DNA. The hybridization step was carried out overnight at 42 °C with denatured ^{32}P-labelled probe with gentle agitation. Washings were performed with SSC solutions all of them containing 0.1% (w/v) SDS; 2× SSC for 30 min at 42 °C and three times for 30 min at 50 °C with 1× SSC. The blots were exposed and autoradiograms were scanned in a densitometer (FLA-5100 Imaging System, Fujifilm, Japan). In the case of samples from auxin treatment, blots were exposed to X-ray films (X-OMAT AR-205, Kodak) at −80 °C for 4 d, and films were developed according to the manufacturers' recommendation. In both cases, blots were stripped of hybridizing probes and hybridized at 42 °C to a probe for strawberry 18S rRNA (Dotto et al., 2006), and then washed for 30 min with 0.1% (w/v) SDS and 1× SSC once at 42 °C and three times at 55 °C. All blots were normalized against the density of 18S rRNA using the MultiGauge program (Fujifilm). The expression of each expansin gene was assayed twice in each species analyzed.