Flesh softening in melting flesh, non-melting flesh and stony hard peaches: endopolygalacturonase expression and phosphorylation of soluble polypeptides in relation to ethylene production


Dipartimento di Produzione Vegetale, University of Milan, via Celoria 2, 20133 Milan, Italy, Phone: +39-03714662474; Fax: +39-0250316521 (*Corresponding author: alessandra.ghiani@unimi.it)

1. Introduction

Ethylene plays a role in many developmental processes among which fruit ripening has practical importance to the human diet. Peach is a climacteric fruit whose ripening, characterized by increased ethylene production, is accompanied by dramatic changes in colour, flavour, aroma and flesh texture. The latter is an important quality parameter affecting fruit shelf-life. Peaches can be classified as non-melting flesh (NMF, preferred for canning), melting flesh (MF, appreciated for the fresh market, Brovelli et al., 1998) and stony hard (HD, maintaining high flesh firmness also when ripe and with a long shelf-life, Goffreda, 1992). Slow ripening mutants (SR) with altered ripening phenotype (impaired fruit development and ethylene production) also there exist, representing an interesting model for studies on ripening (Brecht et al., 1984). The differences in the peach fruit softening pattern depend, among other factors, on presence and activity of cell wall hydrolytic enzymes, with particular regard to endopolygalacturonase (endoPG). In peach, the melting flesh (M) locus encodes endoPG and has been proposed to control fruit firmness. Our previous data showed that softening of MF fruits was characterized by increased levels of endoPG which was also present, although in very limited amounts, in NMF fruits. The differences in endoPG levels seemed due to different transcription of an endoPG gene (Morgutti et al., 2005; Morgutti et al., 2006). These peach fruit phenotypes are characterized also by different ethylene production. MF and NMF fruits do produce ethylene, even if in different amounts, while HD fruits do not produce ethylene, even if they can soften when treated with the hormone. Genetic
analysis indicated that *stony hard (hd)* is a recessive locus, different from *M/m*, and is believed to result from a mutation in ethylene production (Haji et al., 2005). Different expression of the MF/NMF/HD traits may result from the ability of the tissues to respond to ethylene across a broad concentration range due to some form of signal modulation. Kinase-mediated protein phosphorylation is a common means of signal modulation (Chen et al., 2005). Our previous results indicated that the Ca\(^{2+}\)-dependent phosphorylation of a soluble polypeptide (Mr approx. 52 kDa) decreased with ripening in NMF and remained constant in MF fruits. In SR phenotypes, which neither softened nor produced endoPG phosphorylation of this polypeptide was not detectable, suggesting that phosphorylation of the 52 kDa polypeptide may be involved in the modulation of ethylene signalling and flesh softening (Morgutti et al., 2005).

This study has dealt with the evaluation of ethylene evolution, protein phosphorylation patterns and endoPG expression in fruits from a HD and a SR phenotype, whose fruits do not produce ethylene and remain small, green and very firm. The results have been compared with those obtained in NMF and MF fruits and discussed in relation to possible differences in ethylene perception.

**2. Materials and Methods**

Peach (*Prunus persica* L. Batsch.) fruits of NMF ‘Oro A’, MF ‘Bolero’, HD ‘Ghiaccio’ and of one nectarine (*Prunus persica* L. Batsch. var. *nectarina*) SR phenotype (‘BO 95021043’) were used. Fruits were harvested at one time (beginning of physiological ripening) and divided into classes, representing distinct maturity categories, based on epicarp ground colour. Ethylene evolution from fruits was determined with a Dani 3800 gas chromatograph immediately after harvest or after ethylene treatment (SR phenotype, 100 ppm for 5 days). Mesocarp samples from single fruits of known firmness (Effegi hand penetrometer) were frozen in liquid N\(_2\), stored at –80°C, and used for *in vitro* analyses. EndoPGs were extracted from the cell walls and probed by Western analysis as previously described (Morgutti et al., 2006). Soluble protein extraction, *in vitro* protein phosphorylation, SDS-PAGE and phosphorylation pattern detection were conducted as previously described (Negrini et al., 2000; Morgutti et al., 2001). Northern analysis was performed by hybridizing the RNA extracted from fruits with \(^{35}\)P[dATP]-labelled cDNA of Bolero *endoPG* (Gene Bank DQ340809) as a probe (Morgutti et al., 2006).