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Ripening related processes in strawberry, a nonclimacteric fruit: a short overview

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Summary: fruits are essential part of the human diet: they provide vitamins, minerals, antioxidants to the mankind. Physiologically they can be divided into two groups-climacteric and nonclimacteric - depending if they display any respiratory peak and dramatic increase in ethylene biosynthesis or do not. Ethylene is a gaseous hormone playing a very important role in several physiological processes in plants. While climacteric fruits, like apples, bananas, tomatoes, peaches, apricots show increased ethylene biosynthesis and dramatic respiratory peak during their ripening, nonclimacteric fruits, like strawberries, grapes, citrus do not.

The most widely used fruits for studying nonclimacteric ripening are strawberries: several papers are focusing on the identification and characterization of ripening related genes from this plant. Therefore here we attempt to summarize the most important advances in strawberry fruit development. and ripening.

Key words: strawberry, nonclimacteric fruit ripening

Introduction

Anatomically, fruits are mature, expanded ovaries (*Giovannoni*, 2004, *White*, 2002). Botanically – unlike other fruit species – strawberries are aggregate fruits, actually, they are modified receptacles with several achenes located on their surface (*Coombe*, 1976, *Perkins-Veazie*, 1995). Additionally, according to their ripening behaviour, they are classified into the group of nonclimacteric fruits. These features, and the valuable horticultural aspects make strawberry an important model system for fruit biology studies.

At the onset of ripening, several physiological changes can be detected in strawberries: the maturing is accompanied by changes in colour, sugar metabolism, texture, flavour, aroma and resistance against pathogen infection. Strawberries develop and ripen rapidly, approximately 30 days from bloom to ripeness, and berries can be rank into small green, large green, white, pink, and red ripe classes according to their developmental stage (*Culpepper* et al., 1935, *Huber*, 1984, *Perkins-Veazie*, 1995). The fruit ripening is a genetically regulated developmental process affected by different factors, like environmental signals, age, and endogenous hormones (*Civello* et al., 1999).

The role of ethylene and ethylene biosynthetic genes in strawberries

Strawberries are considered to be nonclimacteric fruits, since the ripening hormone ethylene has just a little or no effect on their ripening (*Perkins-Veazie*, 1995, *Castillejo* et al., 2004). Nevertheless, they produce endogenous ethylene in traces (*Knee* et al., 1977, *El-Kazazz* et al., 1983, *Perkins-Veazie* et al., 1988, *Abeles & Takeda*, 1990, *Perkins-Veazie*, 1995). The highest amount of ethylene was detected in young, small green fruits. Afterwards, a continuos decrease could be observed until the stage of white fruits. Then, during the ripening process, a slight, continuous increase was found until the fruits became red (*Abeles & Takeda*, 1990, *Perkins-Veazie* et al., 1996, *Trainotti* et al., 2005). In strawberry flowers, high rates of this hormone was detected, especially during petal abscission (*Knee* et al., 1977, *Trainotti* et al., 2005).

Trainotti et al. (2005) have already isolated and characterized ACC-oxidase (1-aminocycloprapene 1-carboxylate oxidase) from the ethylene biosynthetic genes (*FaACO1*, *FaACO2*) from strawberry. They proved that both genes displayed the highest expression level in flowers. Then a decrease could be detected in developing young fruits.

In the case of *FaACO1*, an expression increment was observed from the large green to the white stages, then a continuous decrease showed until a minimum level in red ripe fruits. The *FaACO2* gene displayed the minimum in the white stages, which was followed by a continuous slight increment during the ripening phase. Both a cDNA and genomic clone of *FaACO* were isolated by *Balogh* et al. (2006). In a semiquantitative RT-PCR the highest level of *FaACO* transcripts was detected in the red fruit. The same result was found by *Aharoni* et al. (2002) who reported that the mRNA level of *ACO* is 3-fold higher in the red then in the turning (pink) stage.

A full lengh cDNA of *FaACS* was isolated by RACE (Rapid Amplification of cDNA Ends) method (*Balogh* et al., 2006, *Kiss* et al., 2007). It was found that the highest transcript amount was present in green fruits, and the gene was wound-induced in young leaves.

Strawberry ethylene receptor genes (*FaETR1*, *FaERS1*, *FaETR2*) were cloned and characterized by *Trainotti* et al. (2005). They found, that the gene expression of *FaETR1* and *FaERS1* display a continuous increment, while *FaETR2* shows the maximum in white fruits, and remains high throughout ripening. Two of these receptors (FaETR1, FaERS1) belong to the type I, while the third (FaETR2) belongs to the type II receptors.

Continuous expression of the *FaCTR1* (CONSTITUTIVE TRIPLE RESPONSE 1),-which acts as a negative regulator in the ethylene signal transduction pathway-was observed both in receptacles and vegetative tissues throughout the ripening process (*Balogh* et al., 2006, *Kiss* et al., 2007).

Auxin in strawberry ripening

In many fruits, ripening process is accompanied by a decline in auxin or an increment of ethylene level (*Perkins-Veazie*, 1995). In strawberries, auxin is synthetized in the achenes, and it may be the primary hormone in strawberry fruit ripening (*Given* et al., 1988, *Manning*, 1994, *Perkins-Veazie*, 1995, *Castillejo* et al., 2004). It is proven, that auxin is a negative regulator of strawberry ripening, since it is triggered by a decrease in the concentration of the hormone (*Perkins-Veazie*, 1995). Moreover, almost all of the identified strawberry ripening-related genes are negatively regulated by auxin (*Civello* et al., 1999).

Respiration

Strawberries do not show any respiratory peak during the ripening process, and the application of exogenous ethylene or ethylene analogs has not initiated or accelerated the respiration like in the climacteric fruits (*Perkins-Veazie*, 1995). The respiratory response to ethylene is also very low in wounded fruits (*Resen & Kader*, 1989). On the other hand, the respiration can be decreased by controlled storage conditions (*Dayawon & Shutak*, 1967, *Perkins Veazie*, 1995).

Remarkable advances in strawberry ripening associated research

Combining cDNA-AFLP and RACE methods, a set of novel strawberry derived genes expressed during ripening were identified (*Balogh* et al., 2005). By hierarchical clustering three groups could have been distinguished from the achene-, green and red receptacle-associated transcripts.

Using a cDNA microarray, Aharoni & O'Connell managed to display gene expression at different stages during the ripening phase and compared gene expression in achene and receptacle tissues (*White*, 2002, *Aharoni & O'Connell*, 2002). They found significant differences of 441 transcripts between the achenes and receptacle tissues. Furthermore, putative ethylene-response element binding factors (EREB) and ethylene-responsive genes were observed to be upregulated in achenes. It was found that proteins related to primary metabolism, pigmentation, cell wall modification are mainly expressed in receptacles. These results can contribute to the identification of relevant transcriptional factors playing important role in the ethylene-independent regulatory cascades. For instance, it has been proven that MADS-domain proteins are important transcriptional factors playing essential roles in many biological processes of plants (*Leseberg* et al., 2008). *Vrebalov* et al. (2002) has revealed that a MADS-box gene is necessary for fruit ripening at the tomato ripening-inhibitor (RIN) locus. Since homologues of *LeMADS-RIN* were also isolated from other fruits, like strawberry, it might suggest common regulatory mechanism in the ripening of both climacteric and nonclimacteric fruits (*White*, 2002, *Giovannoni*, 2004).

As an important factor of ripening, fruit softening mechanisms were also examined by different groups. Strawberries are developing a soft melting texture during the growth and ripening processes, which mean a plenty of modifications, like increase in pectin solubilization, xyloglucan depolymerization, and cell wall swelling (*Redgwell* et al., 1997, *Koh & Melton*, 2002, *Santiago-Doménech* et al., 2008). Pectins are macromolecules in the cell wall, and during softening they are solubilizing and depolymerizing. These processes are considered to promote cell wall loosening and disintegration (*Fischer & Bennett*, 1991, *Castillejo* et al., 2004). Numerous pectin-modifying genes were isolated from strawberries such as β-galactosidases, endopolygalacturonases, or pectate lyases so far.

Pectate lyase is an enzyme which catalyses the cleveage of unesterified galacturonosyl linkages (*Marín-Rodriguez* et al., 2002, *Santiago-Doménech* et al., 2008). *Jimenez-Bermúdez* et al. (2002), revealed that the inhibition in the expression of a strawberry pectate lyase gene resulted extended post-harvest life and firmer fruits.

Pectin esterases are also important enzymes involved in fruit softening. They catalyse the demethylation of pectin. *Castillejo* et al. 2004, cloned four pectin esterase genes (*FaPE1*, *FaPE2*, *FaPE3*, *FaPE4*) from strawberries. By expression analysis of *FaPE1*, they found that the gene is induced by auxin at the onset of ripening, and down-regulated by ethylene during senescence. From these data it was concluded that the repression of *FaPE1* could be an important factor determining strawberry fruit post-harvest decay.

Galactoses are sugar residues and they are abundant in pectins (*Brett & Waldron*, 1996, *Trainotti* et al., 2001). Early studies displayed that a loss of galactose (*Knee* et al. 1977,

Redgwell et al., 1997) occurs during strawberry fruit ripening, indicating that galactosidase enzyme activity could play a role in fruit softening. For this purpose, *Trainotti* et al. (2001), examined β-galactosidase activity from different stages of strawberry development and ripening. The activity was detected in large green, white, pink, and red fruits. During the ripeninig process, a decrease could be observed from the white stage to the red.

Expansins are cell wall proteins which disrupt hydrogen bonds between cellulose and hemicellulose microfibrils (*Civello* et al., 1999). By examination of *FaExp2*, an expansin gene it was found that the expression of the gene is ethylene-insensitive, and unaffected by auxin (*Civello* et al., 1999). These data suggest that endogenous signals other than ethylene and auxin may act on gene expression regulation in ripening strawberry (*Civello* et al., 1999).

Since fruit pigmentation is a crucial quality parameter, flavonoid biosynthesis was largely investigated in strawberry fruits. Flavonoids are secondary metabolites playing important roles in many processes (*Moyano* et al., 1998). Anthocyanins are a group of flavonoid pigments and they give the attractive colour to the fruits. Several strawberry pigments were characterized so far, but the anthocyanin biosynthesis pathway remain still poorly understood. In the hope that clear elucidation will be carry out concerning the biosynthesis of anthocyanins, *Moyano* et al. (1998), performed an expression analysis of a putative dihydroflavonol 4-reductase gene from strawberry where they found that the gene was expressed differentially during ripening. This gene was previously reported to have an important role in anthocyanin biosynthesis in numerous higher plants.

From the same motivation, *Griesser* et al. 2008., aimed to elucidate the in planta function of *FaGT*1, a putative glycosyltransferase gene. This enzyme catalyses the formation of the first stable intermediate in the anthocyanin pathway (*Griesser* et al., 2008). In a clever assay a newly developed transient gene-silencing approach was applied. This method was based on RNAi (RNA interference), in order to down-regulate *FaGT1* expression in strawberry fruits (*Hoffmann* et al., 2006, *Griesser* et al., 2008). As a result, it was demonstrated that *FaGT1* acts on anthocyanidins, and *FaGT1*-silenced fruits displayed a less intense red pigmentation with a different hue (*Griesser* et al., 2008).

Beside the essential vitamin, mineral, and nutrient content, strawberries have other attractive characteristcs, like aroma and taste. The production of flavour compounds is a concomitant phenomenon of ripening. Strawberry flavour components have been intensively studied, and more than 360 volatiles have been identified so far (*Nijssen*, 1996, *Lunkenbein* et al., 2006). From these just around 15–20 are considered to be key compounds (*Lunkenbein* et al., 2006). FaOMT (*Fragaria x ananassa* O-methyltransferase) is an enzyme which catalyses the methylation of HDMF (4-hydroxy-2,5-dimethyl-3(2H)-furanone) to DMMF (2,5-dimethyl-4-methoxy-3(2H)-furanone) during ripening. Moreover, HDMF is believed as the most important flavour compound of strawberry. By using transgenic approach, *Lunkenbein* et al. (2006) carried out successfully modifications in the ratio of odorous furanons, and they found that repression of *FaOMT* appeared also in the level of feruloyl 1-O-β-D-gucose and caffeoyl 1-O-β-D-glucose, indicating that *FaOMT* has dual function in strawberries.

The isolation and characterization of tissue-specific promoters are desirable in order to help the development of nutritional value and quality in strawberries. Transient expression assays are widely used techniques for studying promoter activity. By using this method the promoters of *FaEG1* and *FaEG3* (endo-β-1,4-glucanase, EGase) genes were analyzed (*Spolaroe* et al., 2003). EGases are cellulases carrying important economical role in fruit softening. It was shown that the two promoters of *FaEG1*, and *FaEG3*, displayed different behavior. Furthermore, they are including important regulatory elements, and the 3'region of *FaEG3* has a down-regulating effect which might be responsible for the lower amount of *FaEG3* mRNA in ripe fruits.

Using a biolistic transient gene expression assay the promoter of the *GalUR* gene was analyzed by *Agius* et al. (2005). This gene encodes an enzyme, named D-galacturonic acid reductase, which plays a role in the biosynthesis of vitamin C in strawberries (*Agius* et al., 2003). The promoter region of *GalUR* is highly interesting, because the gene is fruit-specific, and its expression increases during the ripening process. It was revealed that light is required for the activity of the GalUR promoter, and the level of its expression was similar to that of the CaMV 35S constitutive promoter. The minimum sequence which showed promoter activity is 397 bp, and contains a G-box-like element.

MADS box gene from strawberry have been characterized, and designated STAG1. Analysis of the expression of a GUS reporter gene driven by the STAG1 promoter showed that STAG1 was active in stamens, the base of the receptacle and the petals, and in the central pith and vascular tissue during floral development. During the ripening stage of fruit development, STAG1 activity was detected in achenes, pith cells, and cortical cells (*Rosin* et al., 2003).

Conclusions

Despite the advances of fruit biology research, many questions linking to the nonclimacteric ripening remained undefinied. Although expressive large-scale researches resulted important progresses which may improve fruit quality and nutritional value, moreover strawberries became the model plants for the studies of nonclimacteric fruits, several steps involved in their ripening processes are still unsolved. By the using of forward and reverse genetic approaches, predictably more and more interesting movements will be elucidated in the future.

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