Beyond nutrition: modification of the carotenoid profile as a potential strategy to regulate tomato fruit ripening and shelf life

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RESUMO

Além de nutrição: modificação do perfil de carotenoides como uma estratégia potencial para regular o amadurecimento e a vida útil de frutos de tomateiro

Carotenoides são importantes pigmentos na definição do aroma, sabor, aparência e propriedades nutricionais de frutos. Além disso, eles também estão envolvidos na síntese de reguladores do desenvolvimento das plantas, como o ácido abscísico (ABA). Anteriormente, observamos que frutos de tomateiro com diferentes perfis de carotenoides exibem produção alterada de etileno (ET). Relatos recentes sugerem uma conexão entre enzimas sintetizadoras de carotenoides e a regulação do amadurecimento, uma vez que sua manipulação genética altera a produção de ABA e, por sua vez, os níveis de ET na etapa final do amadurecimento. Apesar desses relatos, a manipulação genética do perfil de carotenoides pela indústria frequentemente visa melhorar as propriedades nutricionais de frutos, e muito ainda precisa ser explorado sobre um papel adicional dos carotenoides como componentes da cascata hormonal que regula o amadurecimento. Aqui, reunimos dados fisiológicos sobre o amadurecimento pós-colheita de linhagens quase isogênicas de Micro Tom carregando alelos que alteram o acúmulo de carotenoides e mostramos que o avanço do amadurecimento pode ser afetado pelo perfil dos pigmentos. Dois mutantes de carotenoides, um com perda de função da enzima fitoeno-síntase (PSY1) e outro com ganho de função da enzima licopeno-β-ciclase, foram confrontados com o mutante sitiens deficiente em ABA e o mutante em amadurecimento ripening inhibitor. A partir dessa abordagem, observamos que mutações espontâneas de carotenoides alteram a interação entre ABA e ET e, com isso, o avanço do amadurecimento. Os resultados foram detalhados em três capítulos, onde discutimos (i) um possível envolvimento de PSY1 no feedback que regula a síntese auto catalítica de ET; (ii) o impacto do perfil de carotenoides na interação ABA-ET e na qualidade dos frutos, e (iii) os diferentes efeitos nos parâmetros de maturidade de frutos que foram amadurecidos na planta ou em armazenamento pós-colheita. Juntos, nossos resultados sugerem que a manipulação do perfil de carotenoides pode ser uma ferramenta estratégica para o armazenamento pós-colheita, visando frutos com propriedade nutricional superior e, adicionalmente, com maior vida útil.

Palavras-chave: Fitoeno sintase, Etileno, Ácido abscísico, Pós-colheita
Beyond nutrition: modification of the carotenoid profile as a potential strategy to regulate tomato fruit ripening and shelf-life

Carotenoids are important pigments in defining the aroma, flavor, appearance, and nutritional properties of fruits. In addition, they are also involved in the synthesis of regulators of plant development, such as abscisic acid (ABA). We previously observed that fruits from different carotenoid profile display altered ethylene (ETH) production. Recent reports suggest a link between carotenoid-synthetizing enzymes and the regulation of ripening, as their genetic manipulation alters ABA production and, in turn, ETH levels during late ripening. Despite these reports, the genetic manipulation of the carotenoid profile by the industry often aims at improved nutritional properties of fruits, and much is still to be explored about an additional role of carotenoids as components of the hormonal cascade that regulates ripening. Here, we gathered physiological data on the postharvest ripening of Micro Tom near-isogenic lines carrying alleles that alter the accumulation of carotenoids and showed that the ripening progress may be affected by the pigment profile. Two carotenoid mutants, one with impaired activity of Phytoene-synthase (PSY1), and another with gain-of-function of Lycopene-β-cyclase were confronted with the ABA-deficient mutant *sitiens* and the ripening mutant *ripening inhibitor*. From this approach, we observed that spontaneous carotenoid mutations alter the ABA and ETH crosstalk and with that, the advancement of ripening. Results were detailed throughout three chapters where we discuss (i) a possible involvement of PSY1 in the autocatalytic feedback loop that regulates ETH synthesis; (ii) the impact of the carotenoid profile in the ABA-ETH network and fruit quality, and (iii) the different outcomes on maturity parameters when fruits ripens attached to the plant or in postharvest storage. Together, our findings suggest that the manipulation of the carotenoid profile may be a strategic tool for postharvest storage, aiming at fruits with improved nutrition properties and additionally, with extended shelf life.

Keywords: Phytoene synthase, Ethylene, Abscisic acid, Postharvest
1. GENERAL INTRODUCTION

Tomato fruits undergo remarkable changes during ripening. A well-orchestrated coordination of regulatory steps in several catabolic pathways transforms the green, unpalatable fruits into fleshy red fruits, enriched in carotenoids and other bioactive compounds (reviewed by Giovannoni, 2004). While the climacteric nature of tomato is associated with dramatic changes that ultimately leads to fruit senescence, the ripening-related events are essential to improve the flavor and to give the fruits appealing visual appearance (Kovács et al., 2009).

Carotenoids are the primary pigments conferring the distinctive red color of ripe tomatoes, wherein they play a major role in the attraction of seed dispersers (reviewed by Howitt and Pogson, 2006). Because of their activity as radical scavengers, carotenoids are also important antioxidants and essential components of the photosynthetic machinery in green tissues, preventing photoinhibition (Kim and DellaPenna, 2006). During tomato ripening, striking modifications lead to the differentiation of chloroplasts into chromoplasts, in which the thylakoid components are transformed into membrane-shaped structures capable of storing high amounts of lycopene (Egea et al., 2011). This transformation is followed by alterations in the carotenoid profile, as they assume a new major role in fruit pigmentation. With that, several regulations at the transcriptional level reduce the synthesis of β-carotene and xanthophylls, which played a primordial role on photosynthesis, and favor the accumulation of the red carotene lycopene (Efremov et al., 2020; Kim and DellaPenna, 2006; Pecker et al., 1996; Ronen et al., 1999).

The definition of the carotenoid profile in ripe fruit relies on the activity of chromoplast-specific enzymes that assume the regulation of the carotenoid synthesis in the carotenogenesis pathway. In green tissues containing chloroplasts, the first committed step of carotenogenesis is catalyzed by the Phytoene synthase 2 (PSY2) enzyme (Bartley and Scolnik, 1993). During ripening, PSY2 is downregulated, and a new isoform called PSY1 becomes the key enzyme leading to the formation of the first carotene phytoene (Bartley et al., 1992). A series of unsaturation reactions in the phytoene backbone lead to the formation of trans-lycopene by action of enzymes such as Phytoene desaturase (PDS), Carotenoid-isomerase (CrtISO) and ζ-carotene desaturase (ZDS), which are upregulated during ripening (Giuliano et al., 1993). From this step, Lycopene ε-cyclase (LCY-E) and the chromoplast-specific Lycopene β-cyclase (CYC-B), synthetize α-carotene and β-carotene, respectively (Ronen et al., 2000, 1999).

Mutants available in tomato had key contribution to unravel this control. In tomato, the allele for red fruit color (R) is dominant (Tomes et al., 1953). Fruits of the yellow flesh mutant, carrying the r allele, display yellow coloration when ripe due to the loss-of-function of the PSY1
gene and the consequent lack of carotenoids in ripe fruits (Fray and Grierson, 1993). In the Beta carotene (B) mutant, a gain-of-function in the activity of CYC-B leads to fruits with orange color, due to the enhanced content of β-carotene at the expense of lycopene (Ronen et al., 2000).

Alongside with the ripening-related events that culminate with altered texture and flavor in ripe fruit, the accumulation of carotenoids is also under control of the major ripening regulator ethylene (ETH) (Alba et al., 2005). In climacteric fruit such as tomato, ETH regulates its own synthesis through two distinct signaling pathways. In system 1, an autoinhibitory negative feedback loop maintains low levels of the hormone, whereas system 2 operates by an autocatalytic positive feedback loop that maintains high levels of the hormone. Once fruits switch from system 1 to system 2 the ripening events drive intense irreversible modifications in the tissues (Barry et al., 2000; Mcmurchie et al., 1972).

The signaling cascades that regulate the synthesis of ETH and other ripening-relates genes controled by the hormone, depends on the binding of ETH to its receptors (Tieman et al., 2000). In the most accepted model, the receptors negatively regulate the response pathway, while in the presence of the hormone, the negative regulation is released (Bleecker et al., 1998; Clark et al., 1998). The binding of ETH to its receptors activate the expression of ethylene response factors (ERF), which regulate downstream responses of the hormone by interacting with the promoter region of ripening-related genes, including PSY1 and genes that encode enzymes involved in ETH synthesis, such as 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO) and 1-Aminocyclopropane-1-Carboxylic Acid Synthase (ACS) (Liu et al., 2016; Zhijin et al., 2009). Although the synthesis of carotenoids is also dependent on other ripening regulators, such as RIPENING INHIBITOR (RIN), NON-RIPENING (NOR) and COLORLESS NON-RIPENING (CNR) transcription factors (Adaskaveg et al., 2021; Poma et al., 2017; Thompson et al., 1999; Wang et al., 2020), the ETH-dependent accumulation of lycopene was evidenced by several reports using exogenous treatments with ETH inductors/inhibitors, or with mutant lines with altered synthesis/sensitivity of the hormone (Li et al., 2020; Marty et al., 2005; Satekge and Magwaza, 2022).

While ETH is long-term considered the major regulator of the ripening process in climacteric fruit, the hormone abscisic acid (ABA) has shown a limiting role at the onset of ripening, even prior to ETH action (Ji et al., 2014). Different reports suggest that ABA signaling is located upstream of ETH in the hormonal cascade that controls the climacteric ripening, implying that ABA may be a regulator of its synthesis (Zhang et al., 2009; Zou et al., 2022). Additionally, the positive regulation of ABA on ripening has been extensively demonstrated with
exogenous application of ABA inductors, which often drives ETH production and accelerates ripening (Mizrahi et al., 1975; Mou et al., 2016; Zhang et al., 2009).

The ETH-ABA network has been broadly documented in different organs. In other tissues, both hormones regulate plant growth and development processes, such as seed germination and seedling development, via an antagonistic interaction (Cheng et al., 2009). Differently, in tissues of unripe fruits, they operate synergistically to accelerate the ripening process, by inducing the activity of cell wall hydrolases and the synthesis of carotenoids (Wu et al., 2018). Concomitant treatment of fruit with 1-MCP and ABA showed that ETH may be essential for the induction of ABA signaling at the onset of ripening (Mou et al., 2016). Moreover, elements that act upstream both hormones, such as transcription factors from the NAC family, regulate ripening-related events via ABA- and ETH-dependent manners (Meng et al., 2016). Despite the well-documented positive role of ABA on the induction of ETH and ripening advancement, its effects seem to occur in a time-dependent manner in climacteric fruits, either inducing or delaying ripening. This was observed with exogenous application of ABA, which results in different outcomes in the progression of ripening depending on the developmental stage when the treatment was applied (Soto et al., 2013). Supporting this, it has been suggested that ABA and ETH interact via an antagonistic interaction during late ripening (Diretto et al., 2020). Indeed, antagonistic interactions between both hormones were also observed in fruits of the mutant high pigment3 which displays reduced content of ABA and higher levels of ETH (Galpaz et al., 2008), and other ABA-deficient mutants such as notabilis and flacca, which display higher ETH evolution rates at the onset of ripening, although the hormone was not quantified in late stages (Nitsch et al., 2012).

Interestingly, considering their close relationship with the ABA hormone, which is a carotene-derivate, it has also been suggested that carotenoids may integrate the ABA-ETH crosstalk during late ripening. In this model, a feedback loop promoted by ETH boosts its own synthesis by decreasing the activity of CYC-B and, in turn, reducing the levels of β-carotene and ABA (Diretto et al., 2020). Supporting this hypothesis, it was recently shown that the levels of β-carotene are abnormally enhanced during late ripening in mutant fruits that are insensitive to ETH (Chen et al., 2023). Besides that, the altered ABA-ETH network observed in the ABA-deficient mutant sit seems to also integrate the carotenogenesis pathway, considering that the lycopene levels are significantly enhanced in fruits due to the reduced flux of precursors for ABA synthesis (Galpaz et al., 2008).

Despite the close relationship between carotenoids and ABA, the modification of the carotenoid profile by the industry often aims at improved nutritional quality and visual
appearance, and much is still to be explored about an additional role of carotenoids as components of the hormonal cascade that regulates ripening. This has particular significance for postharvest as an alternative for breeding of fruits with improved color and nutritional parameters, as well as extended shelf life. Given that tomato is a major source of lycopene, and it is also a climacteric species characterized by dramatic changes that driven fruit to senescence, it provides an excellent model for addressing the dual role of carotenoids on color parameters and fruit shelf life. Additionally, spontaneous allelic variations that affect the activity of enzymes from the carotenogenesis are available for tomato and are a valuable opportunity to explore the significance of carotenoids in the ripening process (Fray and Grierson, 1993; Ronen et al., 2000). To isolate the contribution of the carotenoid profile and to avoid the effects of different genetic backgrounds, we used near-isogenic lines carrying the $r$ and $B$ allelic variations as contrasting comparisons against the lycopene-accumulating Micro Tom (MT). By comparing the carotenoid mutants with the classical ripening mutant $ripening inhibitor$ and the ABA-deficient $sit$, we gathered physiological data about the impact of the carotenoid profile on the ABA-ETH network and maturity parameters of fruit that ripened in shelf conditions or attached to the plant. The findings of this work gather relevant information for postharvest of tomato and explore a more significant role of carotenoids in the ripening advancement, in addition to their well-known contribution in fruit pigmentation.

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2. ETHYLENE SYNTHESIS AND SENSITIVITY IS ALTERED IN CAROTENOID-DEFICIENT TOMATO FRUITS

Abstract

Carotenoids are the main pigments found in tomato fruits. Recent reports have suggested that carotenoids integrate a negative feedback mechanism during late ripening by which ethylene (ETH) regulates its own synthesis. Here, we monitored the postharvest ripening of a near-isogenic line carrying the \( r \) allele, which impairs the activity of phytoene synthase (PSY1), the enzyme that catalyzes the first and rate limiting step of carotenogenesis. From this approach we observed that the system 2 of ETH production is anticipated and amplified in \( r \) fruits, which is coordinated with higher activity of 1-Aminocyclopropane-1-carboxylic acid oxidase (ACO). Exogenous treatments with ETH and an inhibitor of its action suggested that \( r \) fruits are more perceptive to the hormone. The enhancement in ETH production is followed by higher levels of soluble solids, accelerated softening and color transition, which was suppressed by the presence of the ripening inhibitor \( (rin) \) allele in a double mutant line \( (rin/r) \). While the \( rin \) mutation repressed the pleiotropic effects on ripening caused by the \( r \) allele, \( rin/r \) fruits still developed a yellow-greenish color when ripe. The accelerated degreening of \( r \) was not associated with faster chlorophyll degradation but is likely due to higher levels of flavonoids. The findings of this work, suggest that PSY1 also impacts the feedback loop that regulates ETH synthesis and suggest that the manipulation of carotenoids may be a tool for breeding of fruits with improved color parameters and extended shelf life.

Keywords: Phytoene synthase; Ripening inhibitor; Lycopene

2.1. Introduction

Carotenoids are the main pigments accumulated in tomato fruits. In chloroplast-containing tissues such as leaves and green fruits, carotenoids play a role in photosynthesis by preventing photoinhibition (Kim and DellaPenna, 2006). As fruits ripen, chloroplasts are differentiated into chromoplasts, allowing greater accumulation of carotenoids, which in turn assume a new major role in fruits pigmentation (Egea et al., 2011). During this transition, the profile of carotenoids is regulated at the transcriptional level, where upregulation of the chromoplast-specific lycopene-\( \beta \)-cyclase (CYC-B) is downregulated, reducing the synthesis of \( \beta \)-carotene that played a primordial role on photosynthesis (Kim and DellaPenna, 2006; Pecker et al., 1996; Ronen et al., 1999). In coordination, the enzyme that catalyzes the first step of carotenogenesis and drives the carbon flux to carotenoid synthesis, phytoene synthase (PSY1), is upregulated, favoring the accumulation of the red carotene lycopene (Efremov et al., 2020; Fray and Grierson, 1993).
This intricate regulation is partially under control of one of the major drivers of ripening, the hormone ethylene (ETH) (Alba et al., 2005), which was previously associated to both the downregulation of CYC-B and the upregulation of PSY1 (Chen et al., 2023; Liu et al., 2014). Two opposing systems operate in ETH production. While the self-inhibitory system 1 is found in vegetative tissues and immature fruits, the autocatalytic system 2 is activated in ripening fruits and marks the beginning of the climacteric phase and acquisition of ripening competency (Barry et al., 2000; Mcmurchie et al., 1972). In both systems, ETH levels are under regulation of a feedback loop that involves positive and negative regulators acting at the transcription level to control the expression of genes associated with ETH biosynthesis, signaling and perception (Karlova et al., 2011; Kevany et al., 2007; Zhang et al., 2009).

Recent studies have shown that changes in the activity of carotenoid-synthesizing enzymes lead to altered ETH production in tomato fruits (Chen et al., 2023; Diretto et al., 2020; McQuinn et al., 2020; Mi et al., 2022; Orsi, 2018). Recent molecular approaches placed CYC-B as a component of a negative feedback mechanism that operates during late ripening wherein its transcription is downregulated by ETH through interaction with Ethylene-response factors (ERFs), which ultimately results in increased levels of the hormone (Chen et al., 2023; Diretto et al., 2020). These findings are of particular importance for postharvest, as they uncover an additional role of carotenoids in the feedback loop that orchestrates ETH synthesis, besides the one played in fruit pigmentation.

Ripening mutants such as ripening inhibitor (rin), non-ripening (nor) and Colorless non-ripening (Cnr) were instrumental to untangle the significant contribution of ETH on carotenoid accumulation (Robinson and Tomes, 1968; Thompson et al., 1999; Tigchelaar et al., 1973). Because of their wide-ranging effect in other ripening-related processes, these mutations are also tools to breed for extended fruits shelf life (Osei et al., 2017; Wang et al., 2020). In a similar manner, carotenoid mutants provide an opportunity to explore the significance of these pigments on ripening. Tomato has a great variety of spontaneous mutations that alter the activity of carotenogenic enzymes (Fray and Grierson, 1993; Isaacson et al., 2002; Ronen et al., 2000, 1999), however, the diversity of the genetic background makes it harder to isolate the contribution of the carotenoid profile on fruit shelf life. To overcome this issue, we used Micro Tom (MT) near-isogenic lines carrying the yellow flesh (r) mutation, which impairs PSY1 synthesis, as a tool to explore the significance of the lack of carotenoids in the postharvest ripening. From this approach, we gathered physiological data from both ETH-treated and untreated fruits, suggesting that PSY1 may also be a component in the feedback loop regulating system 2 of ETH production. By confronting r fruits with the rin mutant, and a double mutant line rin/r, we
observed that the lack of carotenoids also impacts fruit maturity/quality parameters and has a slight effect on shelf life.

2.2. Conclusion

Fruits of a near isogenic line carrying the \( r \) allele, which disturbs PSY1 activity, display anticipated and amplified ETH climacteric production, which was coordinated with enhanced activity of ACO. Exogenous treatments with ETH and 1-MCP suggest that \( r \) fruits are more perceptive to the hormone. Assessment of maturity-related traits revealed that \( r \) fruits have enhanced content of soluble solids, accelerated softening and color transition, resembling a typical phenotype of an advanced-ripening mutant. Such effects are repressed by the presence of the \( rin \) mutation; however, fruits still manage to develop a yellow-greenish color when ripe. The faster degreening of \( r \) was not associated to accelerated chlorophyll degradation but is probably a result of enriched levels of flavonoids.

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Abstract

The accumulation of carotenoids is one of the main modifications during fruit ripening and is under the control of an intricate hormonal crosstalk, including abscisic acid (ABA) and ethylene (ETH). Because carotenoids are ABA precursors, we assessed the impact of allelic variations that alter the carotenoid profile on the hormone network, and quality parameters of fruits during postharvest storage. Carotenoid-deficient fruits carrying the r allele showed reduced content of ABA and enhanced ETH production, whereas enriched-β-carotene fruits, carrying the B allele showed the opposite phenotype, suggesting an antagonistic interaction between two important regulators of ripening. This was further observed in double mutant lines carrying the sit allele, which disturbs ABA synthesis. Fruits of sit/B showed a greater extend in ABA enhancement, and a more pronounced reduction in ETH. Similarly, ETH production in sit/r was and anticipated in comparison to r and the control Micro Tom (MT). In contrast, exogenous application of ABA triggered ETH production and led to earlier ripening, which reinforces previous contrasting outcomes between exogenous and endogenous modification of ABA. Assessment of maturity/quality parameters revealed that carotenoid-deficient fruits have reduced firmness and, along with B fruits, have improved content of soluble sugars and organic acids. Taken together, we gathered evidence that the fruit-specific carotenogenesis could be a strategy to regulate ABA levels and, consequently, ETH production and fruit quality.

Key words: Carotenoids; Phytoene synthase; β-carotene; Shelf life.

3.1. Introduction

Tomato fruits undergo remarkable changes during ripening. One of the most striking transformations involves the conversion of chloroplasts into chromoplasts, with the parallel chlorophyll breakdown and marked accumulation of carotenoids, that ultimately lead to intense red color at full ripeness (Egea et al., 2011; Ernesto Bianchetti et al., 2018). Alongside, changes in texture and accumulation of sugars and organic acids improve organoleptic features and make the fruits more attractive (reviewed by Klee and Giovannoni, 2011). These, and other ripening-related processes, are under an intricate hormonal control of master ripening regulators, such as ethylene (ETH) and abscisic acid (ABA) (Alexander and Grierson, 2002; Kou et al., 2021; Yang et al., 2021). Despite the long-standing role of ETH in the regulation of climacteric ripening, ABA has also been identified as a key player in this process. Different evidence suggests that ABA signaling may act upstream of ETH in the signal cascade that regulates ripening, but the
complexity of this crosstalk is highlighted by different outcomes of their interaction. While some studies have placed ABA a positive regulator of ETH synthesis and ripening advancement (Gupta et al., 2022; Kai et al., 2019; Weng et al., 2015), other reports observed an antagonistic interaction between them, mainly in the late ripening (Galpaz et al., 2008; Martín Rodriguez et al., 2010; Nitsch et al., 2012). The contribution of both hormones to the transition of green to red color in tomato fruits was broadly demonstrated using exogenous treatment with inductors or inhibitors of their synthesis, which confirmed their positive role on carotenoid accumulation and improvement of tomato appearance (Barickman et al., 2017; Tao et al., 2020; Wu et al., 2018), in a dose- and time-dependent manner (Barickman et al., 2014; Soto et al., 2013).

Each step of the carotenogenesis pathway leads to the formation of a different carotenoid, by a series of unsaturation and cyclization reactions (Giuliano et al., 1993; Ronen et al., 2000, 1999). In this way, the final carotenoid profile of fruits is highly dependent on the level of activity of each enzyme in the pathway, which is under extensive control at molecular level. During the progress of tomato ripening, this control comprises upregulation of genes encoding enzymes downstream lycopene, such as phytene synthase 1 (PSY1), phytoene desaturase (PDS), carotenoid-isomerase (CrtISO) and ζ-carotene desaturase (ZDS) (Corona et al., 1996; Fraser et al., 1999; Pecker et al., 1992). In contrast, the activity of cyclase enzymes such as lycopene ε-cyclase (LCY-E) and lycopene β-cyclase (LCY-B), which were active in chloroplasts, is dramatically down-regulated (Pecker et al., 1996; Ronen et al., 1999), favoring the accumulation of the linear carotene lycopene.

Besides their role in pigmentation, carotenoids are also precursors of plant hormones. After cyclization, carotenes are oxygenated to generate xanthophylls (Sun et al., 1996), and cleaved by 9-cis-expoxycarotenoid dioxygenases (NCEDs) to generate ABA (reviewed by Liu et al., 2015). Precisely because of their close relationship with ABA, the manipulation of carotenoids at genetic level leads to different pleiotropic effects such as dwarfism, viviparity, and altered root growth (Avendaño-Vázquez et al., 2014; Conti et al., 2004; Fray et al., 1995; Yin et al., 2015). The occurrence of such detrimental effects reinforces the relationship between carotenoids and ABA, but also suggest an indirect role of carotenoids in developmental processes, besides their contribution to photosynthesis and fruits pigmentation. Despite the numerous studies on the modified carotenoid profile, via natural variations or genetic engineering, the focus has primarily been on the impact on fruit color and nutritional quality, and many gaps still exist regarding a possible contribution of carotenoids to the ripening process, even though they are closely related to the ripening-regulator ABA.
Previously, we had shown that near-isogenic lines of Micro Tom (MT) carrying allelic variations in the carotenoid pathway had divergent climacteric production of ETH and speed of ripening, thereby, altering fruits shelf life (Orsi, 2018; Orsi et al., 2021). Similarly, LYC-B-overexpressing lines had enhanced content of β-carotene, which in turn enhanced ABA production and reduced ETH levels, delaying ripening (Diretto et al., 2020), whereas fruits with impaired ZDS activity displayed reduced ABA content and enhanced ETH (McQuinn et al., 2020), further confirming that the modification of the carotenoid profile has significant impact on ripening-related events.

Our previous observations motivated us to assess the impact of natural allelic variations in the carotenogenesis and ABA pathway on the progress of ripening during postharvest storage. By monitoring of single and double mutants we observed that the modification of the carotenoid profile impacts ABA and ETH network and, in turn, important quality/maturity parameters.

3.2. Conclusion

Allelic variations that affect carotenogenesis can alter the balance between ABA and ETH during postharvest ripening. Carotenoid-deficient fruits display reduced content of ABA and enhanced/anticipated ETH production, whereas enriched-β-carotene fruits have higher levels of ABA and reduced ETH content. The presence of the *sit* allele in *sit/r* and *sit/B* showed addictive effects in ABA reduction or enhancement, respectively, and promoted accumulation of downstream carotenes in MT and mutant fruits, reinforcing the interdependence of both pathways. While the exogenous treatment with the ABA inhibitor fluridone reduced the amount of ethylene and the advancement of ripening, the impaired-ABA mutants displayed increased ethylene and faster fruit color change. Taken together, our results show that the fruits-specific carotenogenesis can also be a strategy to regulate ABA levels and, consequently, ETH production and fruit quality.

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4. ON AND OFF-VINE RIPENING OF TOMATO FRUITS FROM ALTERED CAROTENOID PROFILE

Abstract

The ripening condition, on-vine or in postharvest, has different impact on the flavor and visual appearance of tomato. It has been reported that the manipulation of carotenoids affects other horticultural traits beyond fruit color, however, the extend at which carotenoids contribute to the different outcomes associated to the harvest condition is yet to be explored. Here, we used two mutant lines, yellow flesh ($r$) and Beta-carotene ($B$), with different carotenoid profile as contrasting comparisons against the lycopene-accumulating Micro Tom (MT). Fruits were left to ripen on-vine or were detached earlier and ripened off-vine. Flavor-related parameters of mutant fruits were more affected by the early detachment, but fruits still displayed superior ratio of soluble sugars to organic acids than MT. In contrast, early harvest had a greater impact in the visual appearance of the lycopene-accumulating fruits. Fruits of the $B$ mutant, which has superior content of β-carotene, had increased content of abscisic acid (ABA) regardless the time of ripening. On the opposite, in $r$ fruits, characterized by the lack of carotenoids, we found reduced ABA content and enhanced ethylene production in both on- and off-vine ripened fruits, which was also coordinated with accelerated transition among ripening stages. The impairment in carotenoid accumulation in $r$ improved the accumulation of hydrophilic bioactive compounds and contributed to enhanced antioxidant capacity. Clustering analysis revealed that the carotenoid profile had a greater impact on the variation of fruit quality than the timing of harvest. Considering that the early harvesting affected the carotenoid mutants across a wider range of traits, our findings suggest that the modification of the carotenoid profile may be a strategic tool for postharvest to obtain fruits with superior flavor and visual appearance even when harvested earlier.

Keywords: Phytoene synthase; β-carotene; Ethylene, Abscisic acid, Postharvest.

4.1. Introduction

Tomato fruits exhibits climacteric behavior. During climacteric ripening, fruits undergo marked changes in color, texture, flavor, and nutritional quality, which are largely driven by the metabolism of sugars, pigments and cell wall disassembly, in coordination with phytohormones (reviewed by Klee and Giovannoni, 2011). Ethylene (ETH) is a major ripening regulator of the ripening process through two distinct signaling pathways. In system 1, an autoinhibitory negative feedback loop maintains low levels of the hormone, whereas system 2 operates by an autocatalytic positive feedback loop that enhances ETH production (Barry et al., 2000; Mcmurchie et al., 1972). The hormone abscisic acid (ABA) also plays a crucial role in regulating the onset of ripening, controlling the expression of key ripening-related genes (Gupta et al., 2022; Kai et al., 2019; Weng et al., 2015). When fruits reach the mature green stage and the competence
to ripen, a transient peak in ABA production is observed and immediately followed by a switch in ETH production from system 1 to system 2. After this, dramatic changes culminate with the advancement of ripening until senescence (Seymour et al., 2013).

The autocatalytic burst in ETH production has a pivotal biological role and significant commercial importance as it enables fruits to ripen after detachment from the plant. Because of this climacteric nature, tomato fruits can be picked prior to full ripeness, allowing better management of distribution within the industry (Chervin, 2020). Despite the associated advantages to the supply chain of tomato, the early harvest has substantial implications for fruit quality, often resulting in inferior fruits flavor and unappealing visual appearance (Min et al., 2022; Zhang et al., 2020; Zhou et al., 2021). The visual quality of fruits is highly dependent on the accumulation of carotenoids, the major pigments found in tomato (Giuliano et al., 1993; Yang et al., 2023; Yoo et al., 2017). Different reports showed the contribution of ETH and ABA in the regulation of carotenoid synthesis, wherein their exogenous application leads to enhanced pigmentation and accelerated color shift (Barickman et al., 2017; Su et al., 2015; Tao et al., 2020; Wu et al., 2018). The application of ETH in fruits harvested at early ripening stages is a common practice in the industry for inducing degreening and the expressive synthesis of lycopene (Bapat et al., 2010). However, accumulation of carotenoids also relies on environmental cues such as light and temperature, and because of this, fruits that ripen during postharvest in shelf conditions often display a less intense red color (Cruz et al., 2018; Gonzalez et al., 2015; Yoo et al., 2019; Zhou et al., 2021).

Despite their role in fruits pigmentation, recent reports have suggested an additional role of carotenoids as components of a feedback loop that acts during late ripening and affects ETH synthesis. For this dual role, fruits over accumulating β-carotene levels have reduced softness and improved shelf life as a result of altered crosstalk between ETH and ABA (Chen et al., 2023; Diretto et al., 2020). Similarly, fruits with reduced lycopene content have reduced content of ABA and enhanced ETH production (McQuinn et al., 2020). Likely because of their involvement in the hormone network, the manipulation of the carotenoid profile was also reported to impact important horticultural traits that determine the flavor of fruits (Kurina et al., 2021; Orsi et al., 2021; Vogel et al., 2010), suggesting that impact of carotenoids to fruit quality extends beyond fruit color.

While the impact of early harvest on fruit quality has been extensively investigated, most studies have been carried out with red tomato cultivars, and the extend at which carotenoids contribute to the different outcomes associated with the harvest time is yet to be explored. Given that tomato is a major source of lycopene, it provides an excellent model for addressing these
gaps. Additionally, spontaneous allelic variations that affect the activity of enzymes from the carotenogenesis are available for tomato and are a valuable opportunity to explore the significance of carotenoids in on-vine and off-vine ripening. Here, we used near-isogenic lines carrying the yellow flesh (r) mutation, which impairs carotenoid accumulation, and the Beta-carotene (B) mutation, which enhances the content of β-carotene in fruits, as contrasting comparisons to investigate the contribution of carotenoids to the quality of tomato ripened on-vine or in postharvest.

4.2. Conclusion

Flavor-related parameters of fruits with different carotenoid profile are more affected by the early detachment, but fruits still display superior TSS/TA ratio than fruits accumulating predominantly lycopene. In contrast, early harvest had a greater impact in the visual appearance of the lycopene-accumulating fruits. Fruits with enhanced content of β-carotene have increased content of ABA regardless the time of ripening. On the opposite, the lack of carotenoids in fruits carrying the r mutation leads to reduced ABA content and enhanced ETH production in both on- and off-vine ripening, which is coordinated with accelerated transition among ripening stages. The impairment in carotenoid accumulation in r improved the accumulation of bioactive compounds from the hydrophilic fraction and contributed to enhanced antioxidant capacity even in early detached fruits. Clustering analysis revealed that the carotenoid profile has a greater impact on the variation of fruit quality than the timing of harvest.

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