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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-sintase (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

ABSTRACT

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 mantains 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.

References

ADASKAVEG, Jaclyn A.; SILVA, Christian J.; HUANG, Peng; BLANCO-ULATE, Barbara. Single and Double Mutations in Tomato Ripening Transcription Factors Have Distinct Effects on Fruit Development and Quality Traits. **Frontiers in Plant Science**, *[S. l.]*, v. 12, p. 672, 2021. DOI: 10.3389/FPLS.2021.647035/BIBTEX.

ALBA, Rob; PAYTON, Paxton; FEI, Zhanjun; MCQUINN, Ryan; DEBBIE, Paul; MARTIN, Gregory B.; TANKSLEY, Steven D.; GIOVANNONI, James J. Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. **The Plant cell**, *[S. l.]*, v. 17, n. 11, p. 2954–65, 2005. DOI: 10.1105/tpc.105.036053. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/16243903. Acesso em: 29 ago. 2019.

BARRY, Cornelius S.; LLOP-TOUS, M. Immaculada; GRIERSON, Donald. The Regulation of 1-Aminocyclopropane-1-Carboxylic Acid Synthase Gene Expression during the Transition from System-1 to System-2 Ethylene Synthesis in Tomato. **Plant Physiology**, *[S. l.]*, v. 123, n. 3, p. 979–986, 2000. DOI: 10.1104/pp.123.3.979.

BARTLEY, G. E.; SCOLNIK, P. A. cDNA cloning, expression during development, and genome mapping of PSY2, a second tomato gene encoding phytoene synthase. **The Journal of biological chemistry**, *[S. l.]*, v. 268, n. 34, p. 25718–21, 1993. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/8245008. Acesso em: 31 jul. 2019.

BARTLEY, G. E.; VIITANEN, P. V.; BACOT, K. O.; SCOLNIK, P. A. A tomato gene expressed during fruit ripening encodes an enzyme of the carotenoid biosynthesis pathway. **Journal of Biological Chemistry**, *[S. l.]*, v. 267, n. 8, p. 5036–5039, 1992. DOI: 10.1016/S0021-9258(18)42724-X.

BLEECKER, Anthony B.; ESCH, Jeffrey J.; HALL, Anne E.; RODRÍGUEZ, Fernando I.; BINDER, Brad M. The ethylene–receptor family from *Arabidopsis* : structure and function. **Philosophical Transactions of the Royal Society of London. Series B: Biological Sciences**, *[S. l.]*, v. 353, n. 1374, p. 1405–1412, 1998. DOI: 10.1098/rstb.1998.0295.

CHEN, Chong; ZHANG, Meng; ZHANG, Mingyue; YANG, Minmin; DAI, Shanshan; MENG, Qingwei; LV, Wei; ZHUANG, Kunyang. ETHYLENE-INSENSITIVE 3-LIKE 2 regulates β-carotene and ascorbic acid accumulation in tomatoes during ripening. **Plant Physiology**, *[S. l.]*, 2023. DOI: 10.1093/plphys/kiad151.

CHENG, Wan-Hsing; CHIANG, Ming-Hau; HWANG, San-Gwang; LIN, Pei-Chi. Antagonism between abscisic acid and ethylene in Arabidopsis acts in parallel with the reciprocal regulation of their metabolism and signaling pathways. **Plant Molecular Biology**, *[S. l.]*, v. 71, n. 1–2, p. 61–80, 2009. DOI: 10.1007/s11103-009-9509-7.

CLARK, Karen L.; LARSEN, Paul B.; WANG, Xiaoxia; CHANG, Caren. Association of the Arabidopsis CTR1 Raf-like kinase with the ETR1 and ERS ethylene receptors. **Proceedings of the National Academy of Sciences**, *[S. l.]*, v. 95, n. 9, p. 5401–5406, 1998. DOI: 10.1073/pnas.95.9.5401.

DIRETTO, Gianfranco et al. Manipulation of β-carotene levels in tomato fruits results in increased ABA content and extended shelf life. **Plant Biotechnology Journal**, *[S. l.]*, v. 18, n. 5, p. 1185–1199, 2020. DOI: 10.1111/pbi.13283. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.13283. Acesso em: 3 jun. 2021.

EFREMOV, Gleb I.; SLUGINA, Maria A.; SHCHENNIKOVA, Anna V.; KOCHIEVA, Elena Z. Differential Regulation of Phytoene Synthase PSY1 During Fruit Carotenogenesis in Cultivated and Wild Tomato Species (*Solanum* section *Lycopersicon*). **Plants**, *[S. l.]*, v. 9, n. 9, p. 1169, 2020. DOI: 10.3390/plants9091169. Disponível em: https://www.mdpi.com/2223-7747/9/9/1169. Acesso em: 15 jan. 2021.

EGEA, Isabel; BIAN, Wanping; BARSAN, Cristina; JAUNEAU, Alain; PECH, Jean Claude; LATCHÉ, Alain; LI, Zhengguo; CHERVIN, Christian. Chloroplast to chromoplast transition in tomato fruit: Spectral confocal microscopy analyses of carotenoids and chlorophylls in isolated plastids and time-lapse recording on intact live tissue. **Annals of Botany**, *[S. l.]*, v. 108, n. 2, p. 291–297, 2011. DOI: 10.1093/aob/mcr140. Disponível em: /pmc/articles/PMC3143050/?report=abstract. Acesso em: 19 jan. 2021.

FRAY, Rupert G.; GRIERSON, Donald. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. **Plant Molecular Biology**, *[S. l.]*, v. 22, n. 4, p. 589–602, 1993. DOI: 10.1007/BF00047400.

GALPAZ, Navot; WANG, Qiang; MENDA, Naama; ZAMIR, Dani; HIRSCHBERG, Joseph. Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content. **Plant Journal**, *[S. l.]*, v. 53, n. 5, p. 717–730, 2008. DOI: 10.1111/j.1365-313X.2007.03362.x. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-313X.2007.03362.x. Acesso em: 27 maio. 2021.

GIOVANNONI, James J. Genetic regulation of fruit development and ripening. **The Plant cell**, *[S. l.]*, v. 16 Suppl, n. suppl 1, p. S170-80, 2004. DOI: 10.1105/tpc.019158. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/15010516. Acesso em: 1 ago. 2019.

GIULIANO, Giovanni; BARTLEY, Glenn E.; SCOLNIK, Pablo A. Regulation of Carotenoid Biosynthesis during Tomato Development. **The Plant Cell**, *[S. l.]*, v. 5, p. 379–387, 1993. Disponível em: https://academic.oup.com/plcell/article/5/4/379/5984490. Acesso em: 28 abr. 2022.

HOWITT, C. A.; POGSON, B. J. Carotenoid accumulation and function in seeds and non-green tissues. **Plant, Cell and Environment**, *[S. l.]*, v. 29, n. 3, p. 435–445, 2006. DOI: 10.1111/j.1365-3040.2005.01492.x.

JI, Kai et al. SlNCED1 and SlCYP707A2: key genes involved in ABA metabolism during tomato fruit ripening. **Journal of Experimental Botany**, *[S. l.]*, v. 65, n. 18, p. 5243–5255, 2014. DOI: 10.1093/JXB/ERU288. Disponível em: https://academic.oup.com/jxb/article/65/18/5243/2884963. Acesso em: 17 abr. 2023.

KIM, Joonyul; DELLAPENNA, Dean. Defining the primary route for lutein synthesis in plants: The role of Arabidopsis carotenoid β-ring hydroxylase CYP97A3. **Proceedings of the National Academy of Sciences**, *[S. l.]*, v. 103, n. 9, p. 3474–3479, 2006. DOI: 10.1073/pnas.0511207103.

KOVÁCS, Katalin; FRAY, Rupert G.; TIKUNOV, Yury; GRAHAM, Neil; BRADLEY, Glyn; SEYMOUR, Graham B.; BOVY, Arnaud G.; GRIERSON, Donald. Effect of tomato pleiotropic ripening mutations on flavour volatile biosynthesis. **Phytochemistry**, *[S. l.]*, v. 70, n. 8, p. 1003–1008, 2009. DOI: 10.1016/J.PHYTOCHEM.2009.05.014.

LI, Shan; ZHU, Benzhong; PIRRELLO, Julien; XU, Changjie; ZHANG, Bo; BOUZAYEN, Mondher; CHEN, Kunsong; GRIERSON, Donald. Roles of RIN and ethylene in tomato fruit ripening and ripening-associated traits. **New Phytologist**, *[S. l.]*, v. 226, n. 2, p. 460–475, 2020. DOI: 10.1111/nph.16362. Disponível em: /pmc/articles/PMC7154718/. Acesso em: 9 fev. 2021.

LIU, Mingchun et al. Comprehensive profiling of ethylene response factor expression identifies ripening-associated ERF genes and their link to key regulators of fruit ripening in tomato. **Plant Physiology**, *[S. l.]*, v. 170, n. 3, p. 1732–1744, 2016. DOI: 10.1104/pp.15.01859. Disponível em: /pmc/articles/PMC4775140/?report=abstract. Acesso em: 8 fev. 2021.

MARTY, I.; BUREAU, S.; SARKISSIAN, G.; GOUBLE, B.; AUDERGON, J. M.; ALBAGNAC, G. Ethylene regulation of carotenoid accumulation and carotenogenic gene expression in colour-contrasted apricot varieties (*Prunus armeniaca*). **Journal of Experimental Botany**, *[S. l.]*, v. 56, n. 417, p. 1877–1886, 2005. DOI: 10.1093/jxb/eri177. Disponível em: https://pubmed.ncbi.nlm.nih.gov/15911563/. Acesso em: 7 jan. 2021.

MCMURCHIE, E. J.; MCGLASSON, W. B.; EAKS, I. L. Treatment of Fruit with Propylene gives Information about the Biogenesis of Ethylene. **Nature**, *[S. l.]*, v. 237, n. 5352, p. 235–236, 1972. DOI: 10.1038/237235a0.

MENG, Chen; YANG, Dongyue; MA, Xiaocui; ZHAO, Weiyang; LIANG, Xiaoqing; MA, Nana; MENG, Qingwei. Suppression of tomato SlNAC1 transcription factor delays fruit ripening. **Journal of Plant Physiology**, *[S. l.]*, v. 193, p. 88–96, 2016. DOI: 10.1016/J.JPLPH.2016.01.014.

MIZRAHI, Yosef; DOSTAL, Herbert C.; MCGLASSON, William B.; CHERRY, Joe H. Effects of Abscisic Acid and Benzyladenine on Fruits of Normal and *rin* Mutant Tomatoes. **Plant Physiology**, *[S. l.]*, v. 56, n. 4, p. 544–546, 1975. DOI: 10.1104/PP.56.4.544. Disponível em: https://academic.oup.com/plphys/article/56/4/544/6074382. Acesso em: 18 abr. 2023.

MOU, Wangshu; LI, Dongdong; BU, Jianwen; JIANG, Yuanyuan; KHAN, Zia Ullah; LUO, Zisheng; MAO, Linchun; YING, Tiejin. Comprehensive Analysis of ABA Effects on Ethylene Biosynthesis and Signaling during Tomato Fruit Ripening. **PLOS ONE**, *[S. l.]*, v. 11, n. 4, p. e0154072, 2016. DOI: 10.1371/journal.pone.0154072. Disponível em: https://dx.plos.org/10.1371/journal.pone.0154072. Acesso em: 28 maio. 2020.

NITSCH, L. et al. ABA-deficiency results in reduced plant and fruit size in tomato. **Journal of Plant Physiology**, *[S. l.]*, v. 169, n. 9, p. 878–883, 2012. DOI: 10.1016/j.jplph.2012.02.004. Disponível em: https://pubmed.ncbi.nlm.nih.gov/22424572/. Acesso em: 28 maio. 2021.

PECKER, Iris; GABBAY, Rachel; CUNNINGHAM, Francis X.; HIRSCHBERG, Joseph. Cloning and characterization of the cDNA for lycopene fl-cyclase from tomato reveals decrease in its expression during fruit ripening. **Plant Molecular Biology**, *[S. l.]*, v. 30, p. 807–819, 1996.

POMA, Betsabé Antezana; MALUF, Wilson Roberto; GOUVEIA, Beatriz Tome; DE OLIVEIRA, Alisson Marcel Souza; FERREIRA, Rodolfo de Paula Duarte; CARVALHO, Regis de Castro. Fruit color and post-harvest shelf life in tomato affected by the *og, nor,* and *rin* allleles. **Pesquisa Agropecuária Brasileira**, *[S. l.]*, v. 52, n. 9, p. 743–750, 2017. DOI: 10.1590/S0100-204X2017000900006. Disponível em: http://www.scielo.br/j/pab/a/wjfWHHgpSdMJRGdMHpDYP8F/abstract/?lang=en. Acesso em: 22 abr. 2023.

RONEN, G.; CARMEL-GOREN, L.; ZAMIR, D.; HIRSCHBERG, J. An alternative pathway to beta -carotene formation in plant chromoplasts discovered by map-based cloning of *beta* and *old-gold* color mutations in tomato. **Proceedings of the National Academy of Sciences of the United States of America**, *[S. l.]*, v. 97, n. 20, p. 11102–7, 2000. DOI: 10.1073/pnas.190177497. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10995464. Acesso em: 31 jul. 2019.

RONEN, G.; COHEN, M.; ZAMIR, D.; HIRSCHBERG, J. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. **The Plant journal : for cell and molecular biology**, *[S. l.]*, v. 17, n. 4, p. 341–51, 1999. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10205893. Acesso em: 31 jul. 2019.

SATEKGE, Thabiso Kenneth; MAGWAZA, Lembe Samukelo. Postharvest Application of 1-Methylcyclopropene (1-MCP) on Climacteric Fruits: Factors Affecting Efficacy. **https://doi.org/10.1080/15538362.2022.2085231**, *[S. l.]*, v. 22, n. 1, p. 595–607, 2022. DOI: 10.1080/15538362.2022.2085231. Disponível em: https://www.tandfonline.com/doi/abs/10.1080/15538362.2022.2085231. Acesso em: 18 mar. 2023.

SOTO, Alvaro; RUIZ, Karina B.; RAVAGLIA, Daniela; COSTA, Guglielmo; TORRIGIANI, Patrizia. ABA may promote or delay peach fruit ripening through modulation of ripening- and hormone-related gene expression depending on the developmental stage. **Plant Physiology and Biochemistry**, *[S. l.]*, v. 64, p. 11–24, 2013. DOI: 10.1016/J.PLAPHY.2012.12.011.

THOMPSON, Andrew J.; TOR, Mahmut; BARRY, Cornelius S.; VREBALOV, Julia; ORFILA, Caroline; JARVIS, Michael C.; GIOVANNONI, James J.; GRIERSON, Donald; SEYMOUR, Graham B. Molecular and Genetic Characterization of a Novel Pleiotropic Tomato-Ripening Mutant1. **Plant Physiology**, *[S. l.]*, v. 120, n. 2, p. 383–390, 1999. DOI: 10.1104/pp.120.2.383.

TIEMAN, Denise M.; TAYLOR, Mark G.; CIARDI, Joseph A.; KLEE, Harry J. The tomato ethylene receptors NR and LeETR4 are negative regulators of ethylene response and exhibit functional compensation within a multigene family. **Proceedings of the National Academy of Sciences**, *[S. l.]*, v. 97, n. 10, p. 5663–5668, 2000. DOI: 10.1073/pnas.090550597.

TOMES, M. L.; QUACKENBUSH, F. W.; O. E. NELSON, Jr.; NORTH, Betty. The Inheritance of Carotenoid Pigment Systems in the Tomato. **Genetics**, *[S. l.]*, v. 38, n. 2, p. 117, 1953. DOI: 10.1093/GENETICS/38.2.117. Disponível em: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1209592/. Acesso em: 22 abr. 2023.

WANG, Rufang; LAMMERS, Michiel; TIKUNOV, Yury; BOVY, Arnaud G.; ANGENENT, Gerco C.; DE MAAGD, Ruud A. The *rin, nor* and *Cnr* spontaneous mutations inhibit tomato fruit ripening in additive and epistatic manners. **Plant Science**, *[S. l.]*, v. 294, n. October 2019, p. 110436, 2020. DOI: 10.1016/j.plantsci.2020.110436. Disponível em: https://doi.org/10.1016/j.plantsci.2020.110436.

WU, Qiong; BAI, Jiawei; TAO, Xiaoya; MOU, Wangshu; LUO, Zisheng; MAO, Linchun; BAN, Zhaojun; YING, Tiejin; LI, Li. Synergistic effect of abscisic acid and ethylene on color development in tomato (Solanum lycopersicum L.) fruit. **Scientia Horticulturae**, *[S. l.]*, v. 235, p. 169–180, 2018. DOI: 10.1016/j.scienta.2018.02.078.

ZHANG, Mei; YUAN, Bing; LENG, Ping. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. **Journal of Experimental Botany**, *[S. l.]*, v. 60, n. 6, p. 1579–1588, 2009. DOI: 10.1093/JXB/ERP026. Disponível em: https://academic.oup.com/jxb/article/60/6/1579/512178. Acesso em: 28 abr. 2022.

ZHIJIN, Zhang; HAIWEN, Zhang; RUIDAN, Quan; XUE-CHEN, Wang; RONGFENG, Huang. Transcriptional regulation of the ethylene response factor leerf2 in the expression of ethylene biosynthesis genes controls ethylene production in tomato and tobacco. **Plant Physiology**, *[S. l.]*, v. 150, n. 1, p. 365–377, 2009. DOI: 10.1104/pp.109.135830. Disponível em: https://pubmed.ncbi.nlm.nih.gov/19261734/. Acesso em: 8 fev. 2021.

ZOU, Jian et al. Co-silencing of ABA receptors (SlRCAR) reveals interactions between ABA and ethylene signaling during tomato fruit ripening. **Horticulture Research**, *[S. l.]*, v. 9, 2022. DOI: 10.1093/HR/UHAC057. Disponível em: https://academic.oup.com/hr/article/doi/10.1093/hr/uhac057/6603453. Acesso em: 17 abr. 2023.

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 ripens, 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-β-cyclase (CYC-B) is downregulated, reducing the synthesis of β-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-esponse 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.

References

ADASKAVEG, Jaclyn A.; SILVA, Christian J.; HUANG, Peng; BLANCO-ULATE, Barbara. Single and Double Mutations in Tomato Ripening Transcription Factors Have Distinct Effects on Fruit Development and Quality Traits. **Frontiers in Plant Science**, *[S. l.]*, v. 12, p. 672, 2021. DOI: 10.3389/FPLS.2021.647035/BIBTEX.

ADATO, Avital et al. Fruit-Surface Flavonoid Accumulation in Tomato Is Controlled by a SlMYB12-Regulated Transcriptional Network. **PLoS Genetics**, *[S. l.]*, v. 5, n. 12, p. e1000777, 2009. DOI: 10.1371/journal.pgen.1000777.

ALBA, Rob; PAYTON, Paxton; FEI, Zhanjun; MCQUINN, Ryan; DEBBIE, Paul; MARTIN, Gregory B.; TANKSLEY, Steven D.; GIOVANNONI, James J. Transcriptome and selected metabolite analyses reveal multiple points of ethylene control during tomato fruit development. **The Plant cell**, *[S. l.]*, v. 17, n. 11, p. 2954–65, 2005. DOI: 10.1105/tpc.105.036053. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/16243903. Acesso em: 29 ago. 2019.

BARRY, Cornelius S.; LLOP-TOUS, M. Immaculada; GRIERSON, Donald. The Regulation of 1-Aminocyclopropane-1-Carboxylic Acid Synthase Gene Expression during the Transition from System-1 to System-2 Ethylene Synthesis in Tomato. **Plant Physiology**, *[S. l.]*, v. 123, n. 3, p. 979–986, 2000. DOI: 10.1104/pp.123.3.979.

CHATTOPADHYAY, Tirthartha; HAZRA, Pranab; AKHTAR, Shirin; MAURYA, Deepak; MUKHERJEE, Arnab; ROY, Sheuli. Skin colour, carotenogenesis and chlorophyll degradation mutant alleles: genetic orchestration behind the fruit colour variation in tomato. **Plant Cell Reports**, *[S. l.]*, v. 40, n. 5, p. 767–782, 2021. DOI: 10.1007/s00299-020-02650-9.

CHEN, Chong; ZHANG, Meng; ZHANG, Mingyue; YANG, Minmin; DAI, Shanshan; MENG, Qingwei; LV, Wei; ZHUANG, Kunyang. ETHYLENE-INSENSITIVE 3-LIKE 2 regulates β-carotene and ascorbic acid accumulation in tomatoes during ripening. **Plant Physiology**, *[S. l.]*, 2023. DOI: 10.1093/plphys/kiad151.

CHEN, Yi-Feng; SHAKEEL, Samina N.; BOWERS, Julie; ZHAO, Xue-Chu; ETHERIDGE, Naomi; SCHALLER, G. Eric. Ligand-induced Degradation of the Ethylene Receptor ETR2 through a Proteasome-dependent Pathway in Arabidopsis. **Journal of Biological Chemistry**, *[S. l.]*, v. 282, n. 34, p. 24752–24758, 2007. DOI: 10.1074/jbc.M704419200.

CHUNG, Mi-Young; VREBALOV, Julia; ALBA, Rob; LEE, JeMin; MCQUINN, Ryan; CHUNG, Jae-Dong; KLEIN, Patricia; GIOVANNONI, James. A tomato (*Solanum lycopersicum*) APETALA2/ERF gene, SlAP2a, is a negative regulator of fruit ripening. **The Plant Journal**, *[S. l.]*, v. 64, n. 6, p. 936–947, 2010. DOI: 10.1111/j.1365-313X.2010.04384.x.

DENG, Heng; PIRRELLO, Julien; CHEN, Yao; LI, Nan; ZHU, Sihua; CHIRINOS, Ximena; BOUZAYEN, Mondher; LIU, Yongsheng; LIU, Mingchun. A novel tomato F-box protein, SlEBF3, is involved in tuning ethylene signaling during plant development and climacteric fruit ripening. **The Plant Journal**, *[S. l.]*, v. 95, n. 4, p. 648–658, 2018. DOI: 10.1111/tpj.13976.

DIRETTO, Gianfranco et al. Manipulation of β-carotene levels in tomato fruits results in increased ABA content and extended shelf life. **Plant Biotechnology Journal**, *[S. l.]*, v. 18, n. 5, p. 1185–1199, 2020. DOI: 10.1111/pbi.13283. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.13283. Acesso em: 3 jun. 2021.

DONO, Gabriella; RAMBLA, Jose Luis; FRUSCIANTE, Sarah; GRANELL, Antonio; DIRETTO, Gianfranco; MAZZUCATO, Andrea. Color Mutations Alter the Biochemical Composition in the San Marzano Tomato Fruit. **Metabolites**, *[S. l.]*, v. 10, n. 3, p. 110, 2020. DOI: 10.3390/metabo10030110.

EFREMOV, Gleb I.; SLUGINA, Maria A.; SHCHENNIKOVA, Anna V.; KOCHIEVA, Elena Z. Differential Regulation of Phytoene Synthase PSY1 During Fruit Carotenogenesis in Cultivated and Wild Tomato Species (*Solanum* section *Lycopersicon*). **Plants**, *[S. l.]*, v. 9, n. 9, p. 1169, 2020. DOI: 10.3390/plants9091169. Disponível em: https://www.mdpi.com/2223-7747/9/9/1169. Acesso em: 15 jan. 2021.

EGEA, Isabel; BIAN, Wanping; BARSAN, Cristina; JAUNEAU, Alain; PECH, Jean Claude; LATCHÉ, Alain; LI, Zhengguo; CHERVIN, Christian. Chloroplast to chromoplast transition in tomato fruit: Spectral confocal microscopy analyses of carotenoids and chlorophylls in isolated plastids and time-lapse recording on intact live tissue. **Annals of Botany**, *[S. l.]*, v. 108, n. 2, p. 291–297, 2011. DOI: 10.1093/aob/mcr140. Disponível em: /pmc/articles/PMC3143050/?report=abstract. Acesso em: 19 jan. 2021.

EL-KEREAMY, Ashraf et al. Exogenous ethylene stimulates the long-term expression of genes related to anthocyanin biosynthesis in grape berries. **Physiologia Plantarum**, *[S. l.]*, v. 119, n. 2, p. 175–182, 2003. DOI: 10.1034/j.1399-3054.2003.00165.x.

FRAY, Rupert G.; GRIERSON, Donald. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. **Plant Molecular Biology**, *[S. l.]*, v. 22, n. 4, p. 589–602, 1993. DOI: 10.1007/BF00047400.

GIVEN, N. K.; VENIS, M. A.; GRIERSON, D. Phenylalanine Ammonia-Lyase Activity and Anthocyanin Synthesis in Ripening Strawberry Fruit. **Journal of Plant Physiology**, *[S. l.]*, v. 133, n. 1, p. 25–30, 1988. DOI: 10.1016/S0176-1617(88)80079-8.

HOUBEN, Maarten; VAN DE POEL, Bram. 1-Aminocyclopropane-1-Carboxylic Acid Oxidase (ACO): The Enzyme That Makes the Plant Hormone Ethylene. **Frontiers in Plant Science**, *[S. l.]*, v. 10, 2019. DOI: 10.3389/fpls.2019.00695.

ISAACSON, Tal; RONEN, Gil; ZAMIR, Dani; HIRSCHBERG, Joseph. Cloning of tangerine from tomato reveals a carotenoid isomerase essential for the production of beta-carotene and xanthophylls in plants. **The Plant cell**, *[S. l.]*, v. 14, n. 2, p. 333–42, 2002. DOI: 10.1105/tpc.010303. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/11884678. Acesso em: 31 jul. 2019.

ITO, Yasuhiro; NISHIZAWA-YOKOI, Ayako; ENDO, Masaki; MIKAMI, Masafumi; SHIMA, Yoko; NAKAMURA, Nobutaka; KOTAKE-NARA, Eiichi; KAWASAKI, Susumu; TOKI, Seiichi. Re-evaluation of the rin mutation and the role of RIN in the induction of tomato ripening. **Nature Plants**, *[S. l.]*, v. 3, n. 11, p. 866–874, 2017. DOI: 10.1038/s41477-017-0041-5. Disponível em: https://doi.org/10.1038/s41477-017-0041-5. Acesso em: 28 maio. 2021.

KARLOVA, Rumyana et al. Transcriptome and Metabolite Profiling Show That APETALA2a Is a Major Regulator of Tomato Fruit Ripening . **The Plant Cell**, *[S. l.]*, v. 23, n. 3, p. 923–941, 2011. DOI: 10.1105/tpc.110.081273.

KENDRICK, Mandy D.; CHANG, Caren. Ethylene signaling: new levels of complexity and regulation. **Current Opinion in Plant Biology**, *[S. l.]*, v. 11, n. 5, p. 479–485, 2008. DOI: 10.1016/j.pbi.2008.06.011.

KEVANY, Brian M.; TIEMAN, Denise M.; TAYLOR, Mark G.; CIN, Valeriano Dal; KLEE, Harry J. Ethylene receptor degradation controls the timing of ripening in tomato fruit. **The Plant Journal**, *[S. l.]*, v. 51, n. 3, p. 458–467, 2007. DOI: 10.1111/j.1365-313X.2007.03170.x.

KIM, Joonyul; DELLAPENNA, Dean. Defining the primary route for lutein synthesis in plants: The role of Arabidopsis carotenoid β-ring hydroxylase CYP97A3. **Proceedings of the National Academy of Sciences**, *[S. l.]*, v. 103, n. 9, p. 3474–3479, 2006. DOI: 10.1073/pnas.0511207103.

KLEE, Harry J.; GIOVANNONI, James J. Genetics and Control of Tomato Fruit Ripening and Quality Attributes. **Annual Review of Genetics**, *[S. l.]*, v. 45, n. 1, p. 41–59, 2011. DOI: 10.1146/annurev-genet-110410-132507.

KOVÁCS, Katalin; FRAY, Rupert G.; TIKUNOV, Yury; GRAHAM, Neil; BRADLEY, Glyn; SEYMOUR, Graham B.; BOVY, Arnaud G.; GRIERSON, Donald. Effect of tomato pleiotropic ripening mutations on flavour volatile biosynthesis. **Phytochemistry**, *[S. l.]*, v. 70, n. 8, p. 1003–1008, 2009. DOI: 10.1016/J.PHYTOCHEM.2009.05.014.

LEE, Je Min; JOUNG, Je-Gun; MCQUINN, Ryan; CHUNG, Mi-Young; FEI, Zhangjun; TIEMAN, Denise; KLEE, Harry; GIOVANNONI, James. Combined transcriptome, genetic diversity and metabolite profiling in tomato fruit reveals that the ethylene response factor SlERF6 plays an important role in ripening and carotenoid accumulation. **The Plant Journal**, *[S. l.]*, v. 70, n. 2, p. 191–204, 2012. DOI: 10.1111/j.1365-313X.2011.04863.x. Disponível em: http://doi.wiley.com/10.1111/j.1365-313X.2011.04863.x. Acesso em: 29 ago. 2019.

LI, Ling; ZHU, Benzhong; YANG, Pengyue; FU, Daqi; ZHU, Yi; LUO, Yunbo. The regulation mode of RIN transcription factor involved in ethylene biosynthesis in tomato fruit. **Journal of the Science of Food and Agriculture**, *[S. l.]*, v. 91, n. 10, p. 1822–1828, 2011. DOI: 10.1002/jsfa.4390.

LI, Shan et al. The RIN-MC Fusion of MADS-Box Transcription Factors Has Transcriptional Activity and Modulates Expression of Many Ripening Genes. **Plant Physiology**, *[S. l.]*, v. 176, n. 1, p. 891–909, 2018. DOI: 10.1104/pp.17.01449.

LI, Shan; ZHU, Benzhong; PIRRELLO, Julien; XU, Changjie; ZHANG, Bo; BOUZAYEN, Mondher; CHEN, Kunsong; GRIERSON, Donald. Roles of RIN and ethylene in tomato fruit ripening and ripening-associated traits. **New Phytologist**, *[S. l.]*, v. 226, n. 2, p. 460–475, 2020. DOI: 10.1111/nph.16362. Disponível em: /pmc/articles/PMC7154718/. Acesso em: 9 fev. 2021.

LIU, Mingchun et al. Comprehensive profiling of ethylene response factor expression identifies ripening-associated ERF genes and their link to key regulators of fruit ripening in tomato. **Plant Physiology**, *[S. l.]*, v. 170, n. 3, p. 1732–1744, 2016. DOI: 10.1104/pp.15.01859. Disponível em: /pmc/articles/PMC4775140/?report=abstract. Acesso em: 8 fev. 2021.

LIU, Mingchun; DIRETTO, Gianfranco; PIRRELLO, Julien; ROUSTAN, Jean-Paul; LI, Zhengguo; GIULIANO, Giovanni; REGAD, Farid; BOUZAYEN, Mondher. The chimeric repressor version of an *Ethylene Response Factor* (*ERF*) family member, *Sl-ERF.B3*, shows contrasting effects on tomato fruit ripening. **New Phytologist**, *[S. l.]*, v. 203, n. 1, p. 206–218, 2014. DOI: 10.1111/nph.12771.

LIU, Mingchun; PIRRELLO, Julien; CHERVIN, Christian; ROUSTAN, Jean Paul; BOUZAYEN, Mondher. **Ethylene control of fruit ripening: Revisiting the complex network of transcriptional regulation**. **Plant Physiology**American Society of Plant Biologists, , 2015. DOI: 10.1104/pp.15.01361.

MATA, Clara I.; FABRE, Bertrand; PARSONS, Harriet T.; HERTOG, Maarten L. A. T. M.; VAN RAEMDONCK, Geert; BAGGERMAN, Geert; VAN DE POEL, Bram; LILLEY, Kathryn S.; NICOLAÏ, Bart M. Ethylene Receptors, CTRs and EIN2 Target Protein Identification and Quantification Through Parallel Reaction Monitoring During Tomato Fruit Ripening. **Frontiers in Plant Science**, *[S. l.]*, v. 9, 2018. DOI: 10.3389/fpls.2018.01626.

MCMURCHIE, E. J.; MCGLASSON, W. B.; EAKS, I. L. Treatment of Fruit with Propylene gives Information about the Biogenesis of Ethylene. **Nature**, *[S. l.]*, v. 237, n. 5352, p. 235–236, 1972. DOI: 10.1038/237235a0.

MCQUINN, Ryan P.; GAPPER, Nigel E.; GRAY, Amanda G.; ZHONG, Silin; TOHGE, Takayuki; FEI, Zhangjun; FERNIE, Alisdair R.; GIOVANNONI, James J. Manipulation of ZDS in tomato exposes carotenoid- and ABA-specific effects on fruit development and ripening. **Plant Biotechnology Journal**, *[S. l.]*, v. 18, n. 11, p. 2210–2224, 2020. DOI: 10.1111/pbi.13377. Disponível em: www.arabidopsis.org; Acesso em: 28 maio. 2021.

MI, Jianing et al. A manipulation of carotenoid metabolism influence biomass partitioning and fitness in tomato. **Metabolic Engineering**, *[S. l.]*, v. 70, p. 166–180, 2022. DOI: 10.1016/J.YMBEN.2022.01.004.

MINOGGIO, M. et al. Polyphenol Pattern and Antioxidant Activity of Different Tomato Lines and Cultivars. **Annals of Nutrition and Metabolism**, *[S. l.]*, v. 47, n. 2, p. 64–69, 2003. DOI: 10.1159/000069277.

MUBAROK, Syariful; OKABE, Yoshihiro; FUKUDA, Naoya; ARIIZUMI, Tohru; EZURA, Hiroshi. Potential Use of a Weak Ethylene Receptor Mutant, Sletr1-2, as Breeding Material To Extend Fruit Shelf Life of Tomato. **Journal of Agricultural and Food Chemistry**, *[S. l.]*, v. 63, n. 36, p. 7995–8007, 2015. DOI: 10.1021/ACS.JAFC.5B02742/ASSET/IMAGES/LARGE/JF-2015-027428_0011.JPEG. Disponível em: https://pubs.acs.org/doi/full/10.1021/acs.jafc.5b02742. Acesso em: 23 abr. 2023.

NAKATSUKA, Akira; MURACHI, Shiho; OKUNISHI, Hironori; SHIOMI, Shinjiro; NAKANO, Ryohei; KUBO, Yasutaka; INABA, Akitsugu. Differential expression and internal feedback regulation of 1-aminocyclopropane-1-carboxylate synthase, l-aminocyclopropane-1-carboxylate oxidase, and ethylene receptor genes in tomato fruit during development and ripening. **Plant Physiology**, *[S. l.]*, v. 118, n. 4, p. 1295–1305, 1998. DOI: 10.1104/pp.118.4.1295. Disponível em: https://plantphysiol.org. Acesso em: 2 fev. 2021.

ORSI, Bruna. **Variações alélicas que afetam a carotenogênese em tomateiro alteram o amadurecimento e a suscetibilidade dos frutos ao fungo Botrytis cinerea**. 2018. Biblioteca Digital de Teses e Dissertações da Universidade de São Paulo, Piracicaba, 2018. DOI: 10.11606/D.11.2018.TDE-08102018-171720. Disponível em: http://www.teses.usp.br/teses/disponiveis/11/11144/tde-08102018-171720/. Acesso em: 16 abr. 2023.

ORSI, Bruna; SESTARI, Ivan; PRECZENHAK, Ana Paula; TESSMER, Magda Andréia; DA SILVA SOUZA, Mayara Adja; HASSIMOTTO, Neuza Mariko Aymoto; KLUGE, Ricardo Alfredo. Allelic variations in the tomato carotenoid pathway lead to pleiotropic effects on fruit ripening and nutritional quality. **Postharvest Biology and Technology**, *[S. l.]*, v. 181, p. 111632, 2021. DOI: 10.1016/J.POSTHARVBIO.2021.111632.

OSEI, M. K.; DANQUAH, A.; BLAY, E. T.; DANQUAH, E.; ADU-DAPAAH, H. An overview of tomato fruit-ripening mutants and their use in increasing shelf life of tomato fruits. **African Journal of Agricultural Research**, *[S. l.]*, v. 12, n. 51, p. 3520–3528, 2017. DOI: 10.5897/AJAR2017.12756. Disponível em: https://academicjournals.org/journal/AJAR/article-abstract/0714CAF67131. Acesso em: 9 abr. 2023.

PECKER, Iris; GABBAY, Rachel; CUNNINGHAM, Francis X.; HIRSCHBERG, Joseph. Cloning and characterization of the cDNA for lycopene fl-cyclase from tomato reveals decrease in its expression during fruit ripening. **Plant Molecular Biology**, *[S. l.]*, v. 30, p. 807–819, 1996.

ROBINSON, R. W.; TOMES, M. L. Ripening inhibitor: a gene with múltiple effects on ripening. **Tomato Genet. Coop.**, *[S. l.]*, v. 18, p. 36–37, 1968.

RONEN, G.; CARMEL-GOREN, L.; ZAMIR, D.; HIRSCHBERG, J. An alternative pathway to beta -carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. **Proceedings of the National Academy of Sciences of the United States of America**, *[S. l.]*, v. 97, n. 20, p. 11102–7, 2000. DOI: 10.1073/pnas.190177497. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10995464. Acesso em: 31 jul. 2019.

RONEN, G.; COHEN, M.; ZAMIR, D.; HIRSCHBERG, J. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. **The Plant journal : for cell and molecular biology**, *[S. l.]*, v. 17, n. 4, p. 341–51, 1999. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10205893. Acesso em: 31 jul. 2019.

THOMPSON, Andrew J.; TOR, Mahmut; BARRY, Cornelius S.; VREBALOV, Julia; ORFILA, Caroline; JARVIS, Michael C.; GIOVANNONI, James J.; GRIERSON, Donald; SEYMOUR, Graham B. Molecular and Genetic Characterization of a Novel Pleiotropic Tomato-Ripening Mutant1. **Plant Physiology**, *[S. l.]*, v. 120, n. 2, p. 383–390, 1999. DOI: 10.1104/pp.120.2.383.

TIGCHELAAR, E. C.; MC GLASSON, W. B.; BUESCHER, R. W. Genetic Regulation of Tomato Fruit Ripening. **HortScience**, *[S. l.]*, v. 13, n. 5, p. 508–513, 1978.

TIGCHELAAR, EC; TOMES, M.; KERR, EA; BARMAN, RJ. A new fruit ripening mutant, non-ripening (nor). **Rep. Tomato Genet. Coop**, *[S. l.]*, v. 23, p. 33–34, 1973.

TIWARI, Krishnaraj; PALIYATH, Gopinadhan. Microarray analysis of ripening-regulated gene expression and its modulation by 1-MCP and hexanal. **Plant Physiology and Biochemistry**, *[S. l.]*, v. 49, n. 3, p. 329–340, 2011. DOI: 10.1016/j.plaphy.2011.01.007.

VAN DE POEL, Bram et al. Targeted Systems Biology Profiling of Tomato Fruit Reveals Coordination of the Yang Cycle and a Distinct Regulation of Ethylene Biosynthesis during Postclimacteric Ripening . **Plant Physiology**, *[S. l.]*, v. 160, n. 3, p. 1498–1514, 2012. DOI: 10.1104/pp.112.206086.

WANG, Rufang; LAMMERS, Michiel; TIKUNOV, Yury; BOVY, Arnaud G.; ANGENENT, Gerco C.; DE MAAGD, Ruud A. The *rin, nor* and *Cnr* spontaneous mutations inhibit tomato fruit ripening in additive and epistatic manners. **Plant Science**, *[S. l.]*, v. 294, n. October 2019, p. 110436, 2020. DOI: 10.1016/j.plantsci.2020.110436. Disponível em: https://doi.org/10.1016/j.plantsci.2020.110436.

WANG, Xiaobei et al. PpIAA1 and PpERF4 form a positive feedback loop to regulate peach fruit ripening by integrating auxin and ethylene signals. **Plant Science**, *[S. l.]*, v. 313, p. 111084, 2021. DOI: 10.1016/j.plantsci.2021.111084.

WATKINS, Christopher B. Overview of 1-Methylcyclopropene Trials and Uses for Edible Horticultural Crops. **HortScience**, *[S. l.]*, v. 43, n. 1, p. 86–94, 2008. DOI: 10.21273/HORTSCI.43.1.86.

WELLBURN, Alan R. The Spectral Determination of Chlorophylls a and b, as well as Total Carotenoids, Using Various Solvents with Spectrophotometers of Different Resolution. **Journal of Plant Physiology**, *[S. l.]*, v. 144, n. 3, p. 307–313, 1994. DOI: 10.1016/S0176-1617(11)81192-2. Disponível em: http://dx.doi.org/10.1016/S0176-1617(11)81192-2.

YANG, R. F.; CHENG, T. S.; SHEWFELT, R. L. The Effect of High Temperature and Ethylene Treatment on the Ripening of Tomatoes. **Journal of Plant Physiology**, *[S. l.]*, v. 136, n. 3, p. 368–372, 1990. DOI: 10.1016/S0176-1617(11)80064-7.

YANG, Tianxia et al. Recoloring tomato fruit by CRISPR/Cas9-mediated multiplex gene editing. **Horticulture Research**, *[S. l.]*, v. 10, n. 1, 2023. DOI: 10.1093/HR/UHAC214. Disponível em: https://academic.oup.com/hr/article/10/1/uhac214/6705573. Acesso em: 21 abr. 2023.

YU, Jiaxuan; QIU, Kainan; SUN, Wenjing; YANG, Tuo; WU, Ting; SONG, Tingting; ZHANG, Jie; YAO, Yuncong; TIAN, Ji. A long noncoding RNA functions in high-light-induced anthocyanin accumulation in apple by activating ethylene synthesis. **Plant Physiology**, *[S. l.]*, v. 189, n. 1, p. 66–83, 2022. DOI: 10.1093/plphys/kiac049.

ZHANG, Zhijin; ZHANG, Haiwen; QUAN, Ruidan; WANG, Xue-Chen; HUANG, Rongfeng. Transcriptional Regulation of the Ethylene Response Factor LeERF2 in the Expression of Ethylene Biosynthesis Genes Controls Ethylene Production in Tomato and Tobacco . **Plant Physiology**, *[S. l.]*, v. 150, n. 1, p. 365–377, 2009. DOI: 10.1104/pp.109.135830.

ZHAO, Qiong; GUO, Hong-Wei. Paradigms and Paradox in the Ethylene Signaling Pathway and Interaction Network. **Molecular Plant**, *[S. l.]*, v. 4, n. 4, p. 626–634, 2011. DOI: 10.1093/mp/ssr042.

ZHOU, Xuesong; RAO, Sombir; WRIGHTSTONE, Emalee; SUN, Tianhu; LUI, Andy Cheuk Woon; WELSCH, Ralf; LI, Li. Phytoene Synthase: The Key Rate-Limiting Enzyme of Carotenoid Biosynthesis in Plants. **Frontiers in Plant Science**, *[S. l.]*, v. 13, 2022. DOI: 10.3389/fpls.2022.884720.

3. ALLELIC VARIATIONS IN CAROTENOGENESIS IMPACT THE ABSCISIC ACID AND ETHYLENE INTERPLAY DURING TOMATO POSTHARVEST RIPENING

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 phytoene 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.

References

ADASKAVEG, Jaclyn A.; SILVA, Christian J.; HUANG, Peng; BLANCO-ULATE, Barbara. Single and Double Mutations in Tomato Ripening Transcription Factors Have Distinct Effects on Fruit Development and Quality Traits. **Frontiers in Plant Science**, *[S. l.]*, v. 12, p. 672, 2021. DOI: 10.3389/FPLS.2021.647035/BIBTEX.

ALEXANDER, Lucille; GRIERSON, Donald. Ethylene biosynthesis and action in tomato: a model for climacteric fruit ripening. **Journal of Experimental Botany**, *[S. l.]*, v. 53, n. 377, p. 2039–2055,

2002. DOI: 10.1093/JXB/ERF072. Disponível em: https://academic.oup.com/jxb/article/53/377/2039/497226. Acesso em: 27 abr. 2022.

AVENDAÑO-VÁZQUEZ, Aida Odette et al. An Uncharacterized Apocarotenoid-Derived Signal Generated in ζ-Carotene Desaturase Mutants Regulates Leaf Development and the Expression of Chloroplast and Nuclear Genes in Arabidopsis. **The Plant Cell**, *[S. l.]*, v. 26, n. 6, p. 2524–2537, 2014. DOI: 10.1105/TPC.114.123349. Disponível em: https://academic.oup.com/plcell/article/26/6/2524/6098509. Acesso em: 16 abr. 2023.

BARICKMAN, T. Casey; KOPSELL, Dean A.; SAMS, Carl E. Abscisic acid increases carotenoid and chlorophyll concentrations in leaves and fruit of two tomato genotypes. **Journal of the American Society for Horticultural Science**, *[S. l.]*, v. 139, n. 3, p. 261–266, 2014. DOI: 10.21273/JASHS.139.3.261.

BARICKMAN, T. Casey; KOPSELL, Dean A.; SAMS, Carl E. Abscisic acid improves tomato fruit quality by increasing soluble sugar concentrations. **http://dx.doi.org/10.1080/01904167.2016.1231812**, *[S. l.]*, v. 40, n. 7, p. 964–973, 2017. DOI: 10.1080/01904167.2016.1231812. Disponível em: https://www.tandfonline.com/doi/abs/10.1080/01904167.2016.1231812. Acesso em: 19 abr. 2023.

BARTLEY, G. E.; SCOLNIK, P. A. cDNA cloning, expression during development, and genome mapping of PSY2, a second tomato gene encoding phytoene synthase. **The Journal of biological chemistry**, *[S. l.]*, v. 268, n. 34, p. 25718–21, 1993. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/8245008. Acesso em: 31 jul. 2019.

BASTÍAS, Adriana; LÓPEZ-CLIMENT, María; VALCÁRCEL, Mercedes; ROSELLO, Salvador; GÓMEZ-CADENAS, Aurelio; CASARETTO, José A. Modulation of organic acids and sugar content in tomato fruits by an abscisic acid-regulated transcription factor. **Physiologia Plantarum**, *[S. l.]*, v. 141, n. 3, p. 215–226, 2011. DOI: 10.1111/J.1399-3054.2010.01435.X. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1399-3054.2010.01435.x. Acesso em: 19 abr. 2023.

BENSCHOP, Joris J.; MILLENAAR, Frank F.; SMEETS, Maaike E.; VAN ZANTEN, Martijn; VOESENEK, Laurentius A. C. J.; PEETERS, Anton J. M. Abscisic Acid Antagonizes Ethylene-Induced Hyponastic Growth in Arabidopsis. **Plant Physiology**, *[S. l.]*, v. 143, n. 2, p. 1013–1023, 2007. DOI: 10.1104/PP.106.092700. Disponível em: https://academic.oup.com/plphys/article/143/2/1013/6106634. Acesso em: 19 abr. 2023.

CASALS, Joan; RIVERA, Ana; SABATÉ, Josep; DEL CASTILLO, Roser Romero; SIMÓ, Joan. Cherry and Fresh Market Tomatoes: Differences in Chemical, Morphological, and Sensory Traits and Their Implications for Consumer Acceptance. **Agronomy 2019, Vol. 9, Page 9**, *[S. l.]*, v. 9, n. 1, p. 9, 2018. DOI: 10.3390/AGRONOMY9010009. Disponível em: https://www.mdpi.com/2073-4395/9/1/9/htm. Acesso em: 19 abr. 2023.

CHEN, Chong; ZHANG, Meng; ZHANG, Mingyue; YANG, Minmin; DAI, Shanshan; MENG, Qingwei; LV, Wei; ZHUANG, Kunyang. ETHYLENE-INSENSITIVE 3-LIKE 2 regulates β-carotene and ascorbic acid accumulation in tomatoes during ripening. **Plant Physiology**, *[S. l.]*, 2023. DOI: 10.1093/plphys/kiad151.

CHENG, Wan-Hsing; CHIANG, Ming-Hau; HWANG, San-Gwang; LIN, Pei-Chi. Antagonism between abscisic acid and ethylene in Arabidopsis acts in parallel with the reciprocal regulation of their metabolism and signaling pathways. **Plant Molecular Biology**, *[S. l.]*, v. 71, n. 1–2, p. 61–80, 2009. DOI: 10.1007/s11103-009-9509-7.

CHRISTMANN, Alexander; HOFFMANN, Thomas; TEPLOVA, Irina; GRILL, Erwin; MÜLLER, Axel. Generation of active pools of abscisic acid revealed by in vivo imaging of water-stressed Arabidopsis. **Plant physiology**, *[S. l.]*, v. 137, n. 1, p. 209–219, 2005. DOI: 10.1104/PP.104.053082. Disponível em: https://pubmed.ncbi.nlm.nih.gov/15618419/. Acesso em: 17 abr. 2023.

CONTI, Alessio; PANCALDI, Simonetta; FAMBRINI, Marco; MICHELOTTI, Vania; BONORA, Angelo; SALVINI, Mariangela; PUGLIESI, Claudio. A Deficiency at the Gene Coding for ζ-Carotene Desaturase Characterizes the Sunflower non dormant-1 Mutant. **Plant and Cell Physiology**, *[S. l.]*, v. 45, n. 4, p. 445–455, 2004. DOI: 10.1093/PCP/PCH052. Disponível em: https://academic.oup.com/pcp/article/45/4/445/1922027. Acesso em: 16 abr. 2023.

CORONA, Vittorio; ARACRI, Benedetto; KOSTURKOVA, Georgina; BARTLEY, Glenn E.; PITTO, Letizia; GIORGETTI, Lucia; SCOLNIK, Pablo A.; GIULIANO, Giovanni. Regulation of a carotenoid biosynthesis gene promoter during plant development. **The Plant Journal**, *[S. l.]*, v. 9, n. 4, p. 505–512, 1996. DOI: 10.1046/J.1365-313X.1996.09040505.X. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-313X.1996.09040505.x. Acesso em: 22 abr. 2023.

DIRETTO, Gianfranco et al. Manipulation of β-carotene levels in tomato fruits results in increased ABA content and extended shelf life. **Plant Biotechnology Journal**, *[S. l.]*, v. 18, n. 5, p. 1185–1199, 2020. DOI: 10.1111/pbi.13283. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.13283. Acesso em: 3 jun. 2021.

EGEA, I.; BARSAN, C.; BIAN, W.; PURGATTO, E.; LATCHE, A.; CHERVIN, C.; BOUZAYEN, M.; PECH, J. C. Chromoplast Differentiation: Current Status and Perspectives. **Plant and Cell Physiology**, *[S. l.]*, v. 51, n. 10, p. 1601–1611, 2010. DOI: 10.1093/pcp/pcq136. Disponível em: https://academic.oup.com/pcp/article-lookup/doi/10.1093/pcp/pcq136. Acesso em: 3 set. 2019.

EGEA, Isabel; BIAN, Wanping; BARSAN, Cristina; JAUNEAU, Alain; PECH, Jean Claude; LATCHÉ, Alain; LI, Zhengguo; CHERVIN, Christian. Chloroplast to chromoplast transition in tomato fruit: Spectral confocal microscopy analyses of carotenoids and chlorophylls in isolated plastids and time-lapse recording on intact live tissue. **Annals of Botany**, *[S. l.]*, v. 108, n. 2, p. 291–297, 2011. DOI: 10.1093/aob/mcr140. Disponível em: /pmc/articles/PMC3143050/?report=abstract. Acesso em: 19 jan. 2021.

ERNESTO BIANCHETTI, Ricardo; SILVESTRE LIRA, Bruno; SANTOS MONTEIRO, Scarlet; DEMARCO, Diego; PURGATTO, Eduardo; ROTHAN, Christophe; ROSSI, Magdalena; FRESCHI, Luciano. Fruit-localized phytochromes regulate plastid biogenesis, starch synthesis, and carotenoid metabolism in tomato. **Journal of Experimental Botany**, *[S. l.]*, v. 69, n. 15, p. 3573–3586, 2018. DOI: 10.1093/JXB/ERY145. Disponível em: https://academic.oup.com/jxb/article/69/15/3573/4975406. Acesso em: 23 abr. 2023.

FRASER, Paul D.; KIANO, Joy W.; TRUESDALE, Mark R.; SCHUCH, Wolfgang; BRAMLEY, Peter M. Phytoene synthase-2 enzyme activity in tomato does not contribute to carotenoid synthesis in ripening fruit. **Plant Molecular Biology**, *[S. l.]*, v. 40, n. 4, p. 687–698, 1999. DOI: 10.1023/A:1006256302570.

FRAY, Rupert G.; GRIERSON, Donald. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. **Plant Molecular Biology**, *[S. l.]*, v. 22, n. 4, p. 589–602, 1993. DOI: 10.1007/BF00047400.

FRAY, Rupert G.; WALLACE, Andrew; FRASER, Paul D.; VALERO, Daniel; HEDDEN, Peter; BRAMLEY, Peter M.; GRIERSON, Donald. Constitutive expression of a fruit phytoene synthase gene in transgenic tomatoes causes dwarfism by redirecting metabolites from the gibberellin pathway. **The Plant Journal**, *[S. l.]*, v. 8, n. 5, p. 693–701, 1995. DOI: 10.1046/J.1365-313X.1995.08050693.X. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1046/j.1365-313X.1995.08050693.x. Acesso em: 16 abr. 2023.

FREITAS-ASTUA, Juliana; ASTUA-MONGE, Gustavo; POLSTON, Jane Elisabeth; HIEBERT, Ernest. A simple and reliable method for the screening of transgenic tobacco plants. **Pesquisa Agropecuária Brasileira**, *[S. l.]*, v. 38, n. 7, p. 893–896, 2003. DOI: 10.1590/S0100-204X2003000700015. Disponível em: http://www.scielo.br/j/pab/a/gXFWCWJT7R7CytfnymVJJnm/?lang=en. Acesso em: 18 abr. 2023.

GALPAZ, Navot; WANG, Qiang; MENDA, Naama; ZAMIR, Dani; HIRSCHBERG, Joseph. Abscisic acid deficiency in the tomato mutant high-pigment 3 leading to increased plastid number and higher fruit lycopene content. **Plant Journal**, *[S. l.]*, v. 53, n. 5, p. 717–730, 2008. DOI: 10.1111/j.1365-313X.2007.03362.x. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1365-313X.2007.03362.x. Acesso em: 27 maio. 2021.

GARG, Naveen; CHEEMA, Devinder Singh. Assessment of fruit quality attributes of tomato hybrids involving ripening mutants under high temperature conditions. **Scientia Horticulturae**, *[S. l.]*, v. 131, n. 1, p. 29–38, 2011. DOI: 10.1016/J.SCIENTA.2011.09.024.

GIULIANO, Giovanni; BARTLEY, Glenn E.; SCOLNIK, Pablo A. Regulation of Carotenoid Biosynthesis during Tomato Development. **The Plant Cell**, *[S. l.]*, v. 5, p. 379–387, 1993. Disponível em: https://academic.oup.com/plcell/article/5/4/379/5984490. Acesso em: 28 abr. 2022.

GUPTA, A.; UPADHYAY, Rakesh K.; PRABHAKAR, Rakhi; TIWARI, Neerja; GARG, Rashmi; SANE, Vidhu A.; SANE, Aniruddha P. SlDREB3, a negative regulator of ABA responses, controls seed germination, fruit size and the onset of ripening in tomato. **Plant Science**, *[S. l.]*, v. 319, p. 111249, 2022. a. DOI: 10.1016/J.PLANTSCI.2022.111249.

GUPTA, Prateek; RODRIGUEZ-FRANCO, Marta; BODANAPU, Reddaiah; SREELAKSHMI, Yellamaraju; SHARMA, Rameshwar. Phytoene synthase 2 in tomato fruits remains functional and contributes to abscisic acid formation. **Plant Science**, *[S. l.]*, v. 316, p. 111177, 2022. b. DOI: 10.1016/J.PLANTSCI.2022.111177.

HAUSER, Felix; WAADT, Rainer; SCHROEDER, Julian I. Evolution of Abscisic Acid Synthesis and Signaling Mechanisms. **Current Biology**, *[S. l.]*, v. 21, n. 9, p. R346–R355, 2011. DOI: 10.1016/J.CUB.2011.03.015.

JI, Kai et al. SlNCED1 and SlCYP707A2: key genes involved in ABA metabolism during tomato fruit ripening. **Journal of Experimental Botany**, *[S. l.]*, v. 65, n. 18, p. 5243–5255, 2014. DOI: 10.1093/JXB/ERU288. Disponível em: https://academic.oup.com/jxb/article/65/18/5243/2884963. Acesso em: 17 abr. 2023.

JIA, Kun Peng; BAZ, Lina; AL-BABILI, Salim. From carotenoids to strigolactones. **Journal of Experimental Botany**, *[S. l.]*, v. 69, n. 9, p. 2189–2204, 2018. DOI: 10.1093/JXB/ERX476. Disponível em: https://academic.oup.com/jxb/article/69/9/2189/4743512. Acesso em: 16 abr. 2023.

JIANG, Fangling et al. Disassembly of the fruit cell wall by the ripening-associated polygalacturonase and expansin influences tomato cracking. **Horticulture Research 2019 6:1**, *[S. l.]*, v. 6, n. 1, p. 1–15, 2019. DOI: 10.1038/s41438-018-0105-3. Disponível em: https://www.nature.com/articles/s41438-018-0105-3. Acesso em: 21 abr. 2023.

KAI, Wenbin; WANG, Juan; LIANG, Bin; FU, Ying; ZHENG, Yu; ZHANG, Wenbo; LI, Qian; LENG, Ping. PYL9 is involved in the regulation of ABA signaling during tomato fruit ripening. **Journal of Experimental Botany**, *[S. l.]*, v. 70, n. 21, p. 6305, 2019. DOI: 10.1093/JXB/ERZ396. Disponível em: /pmc/articles/PMC6859720/. Acesso em: 22 abr. 2023.

KLEE, Harry J.; GIOVANNONI, James J. Genetics and Control of Tomato Fruit Ripening and Quality Attributes. **Annual Review of Genetics**, *[S. l.]*, v. 45, n. 1, p. 41–59, 2011. DOI: 10.1146/annurev-genet-110410-132507.

KOU, Xiao Hong; ZHOU, Jia Qian; WU, Cai E.; YANG, Sen; LIU, Ye Fang; CHAI, Li Ping; XUE, Zhao Hui. The interplay between ABA/ethylene and NAC TFs in tomato fruit ripening: a review. **Plant Molecular Biology 2021 106:3**, *[S. l.]*, v. 106, n. 3, p. 223–238, 2021. DOI: 10.1007/S11103-021-01128-W. Disponível em: https://link.springer.com/article/10.1007/s11103-021-01128-w. Acesso em: 22 abr. 2023.

KOVÁCS, Katalin; FRAY, Rupert G.; TIKUNOV, Yury; GRAHAM, Neil; BRADLEY, Glyn; SEYMOUR, Graham B.; BOVY, Arnaud G.; GRIERSON, Donald. Effect of tomato pleiotropic ripening mutations on flavour volatile biosynthesis. **Phytochemistry**, *[S. l.]*, v. 70, n. 8, p. 1003–1008, 2009. DOI: 10.1016/J.PHYTOCHEM.2009.05.014.

LIU, Lihong; SHAO, Zhiyong; ZHANG, Min; WANG, Qiaomei. Regulation of Carotenoid Metabolism in Tomato. **Molecular Plant**, *[S. l.]*, v. 8, n. 1, p. 28–39, 2015. DOI: 10.1016/J.MOLP.2014.11.006. Disponível em: https://www.sciencedirect.com/science/article/pii/S1674205214000070. Acesso em: 31 jul. 2019.

LÓPEZ-RÁEZ, Juan Antonio et al. Tomato strigolactones are derived from carotenoids and their biosynthesis is promoted by phosphate starvation. **New Phytologist**, *[S. l.]*, v. 178, n. 4, p. 863–874, 2008. DOI: 10.1111/J.1469-8137.2008.02406.X. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1111/j.1469-8137.2008.02406.x. Acesso em: 16 abr. 2023.

MARTÍN RODRIGUEZ, José Ángel; LEÓN MORCILLO, Rafael; VIERHEILIG, Horst; ANTONIO OCAMPO, Juan; LUDWIG-MÜLLER, Jutta; GARCÍA GARRIDO, José Manuel. Mycorrhization of the *notabilis* and *sitiens* tomato mutants in relation to abscisic acid and ethylene contents. **Journal of Plant Physiology**, *[S. l.]*, v. 167, n. 8, p. 606–613, 2010. DOI: 10.1016/J.JPLPH.2009.11.014.

MCQUINN, Ryan P.; GAPPER, Nigel E.; GRAY, Amanda G.; ZHONG, Silin; TOHGE, Takayuki; FEI, Zhangjun; FERNIE, Alisdair R.; GIOVANNONI, James J. Manipulation of ZDS in tomato exposes carotenoid- and ABA-specific effects on fruit development and ripening. **Plant Biotechnology Journal**, *[S. l.]*, v. 18, n. 11, p. 2210–2224, 2020. DOI: 10.1111/pbi.13377. Disponível em: www.arabidopsis.org; Acesso em: 28 maio. 2021.

MENG, Chen; YANG, Dongyue; MA, Xiaocui; ZHAO, Weiyang; LIANG, Xiaoqing; MA, Nana; MENG, Qingwei. Suppression of tomato SlNAC1 transcription factor delays fruit ripening. **Journal of Plant Physiology**, *[S. l.]*, v. 193, p. 88–96, 2016. DOI: 10.1016/J.JPLPH.2016.01.014.

MI, Jianing et al. A manipulation of carotenoid metabolism influence biomass partitioning and fitness in tomato. **Metabolic Engineering**, *[S. l.]*, v. 70, p. 166–180, 2022. DOI: 10.1016/J.YMBEN.2022.01.004.

MIZRAHI, Yosef; DOSTAL, Herbert C.; MCGLASSON, William B.; CHERRY, Joe H. Effects of Abscisic Acid and Benzyladenine on Fruits of Normal and rin Mutant Tomatoes. **Plant Physiology**, *[S. l.]*, v. 56, n. 4, p. 544–546, 1975. DOI: 10.1104/PP.56.4.544. Disponível em: https://academic.oup.com/plphys/article/56/4/544/6074382. Acesso em: 18 abr. 2023.

MOU, Wangshu; LI, Dongdong; BU, Jianwen; JIANG, Yuanyuan; KHAN, Zia Ullah; LUO, Zisheng; MAO, Linchun; YING, Tiejin. Comprehensive Analysis of ABA Effects on Ethylene Biosynthesis and Signaling during Tomato Fruit Ripening. **PLOS ONE**, *[S. l.]*, v. 11, n. 4, p. e0154072, 2016. DOI: 10.1371/journal.pone.0154072. Disponível em: https://dx.plos.org/10.1371/journal.pone.0154072. Acesso em: 28 maio. 2020.

NITSCH, L. et al. ABA-deficiency results in reduced plant and fruit size in tomato. **Journal of Plant Physiology**, *[S. l.]*, v. 169, n. 9, p. 878–883, 2012. DOI: 10.1016/j.jplph.2012.02.004. Disponível em: https://pubmed.ncbi.nlm.nih.gov/22424572/. Acesso em: 28 maio. 2021.

ORSI, Bruna. **Variações alélicas que afetam a carotenogênese em tomateiro alteram o amadurecimento e a suscetibilidade dos frutos ao fungo Botrytis cinerea**. 2018. Biblioteca Digital de Teses e Dissertações da Universidade de São Paulo, Piracicaba, 2018. DOI: 10.11606/D.11.2018.TDE-08102018-171720. Disponível em: http://www.teses.usp.br/teses/disponiveis/11/11144/tde-08102018-171720/. Acesso em: 16 abr. 2023.

ORSI, Bruna; SESTARI, Ivan; PRECZENHAK, Ana Paula; TESSMER, Magda Andréia; DA SILVA SOUZA, Mayara Adja; HASSIMOTTO, Neuza Mariko Aymoto; KLUGE, Ricardo Alfredo. Allelic variations in the tomato carotenoid pathway lead to pleiotropic effects on fruit ripening and nutritional quality. **Postharvest Biology and Technology**, *[S. l.]*, v. 181, p. 111632, 2021. DOI: 10.1016/J.POSTHARVBIO.2021.111632.

PECKER, I.; CHAMOVITZ, D.; LINDEN, H.; SANDMANN, G.; HIRSCHBERG, J. A single polypeptide catalyzing the conversion of phytoene to zeta-carotene is transcriptionally regulated during tomato fruit ripening. **Proceedings of the National Academy of Sciences of the United States of America**, *[S. l.]*, v. 89, n. 11, p. 4962–6, 1992. DOI: 10.1073/pnas.89.11.4962. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/1594600. Acesso em: 31 jul. 2019.

PECKER, Iris; GABBAY, Rachel; CUNNINGHAM, Francis X.; HIRSCHBERG, Joseph. Cloning and characterization of the cDNA for lycopene fl-cyclase from tomato reveals decrease in its expression during fruit ripening. **Plant Molecular Biology**, *[S. l.]*, v. 30, p. 807–819, 1996.

PINO, Lilian E.; LOMBARDI-CRESTANA, Simone; AZEVEDO, Mariana S.; SCOTTON, Danielle C.; BORGO, Lucélia; QUECINI, Vera; FIGUEIRA, Antonio; PERES, Lázaro E. P. The Rg1 allele as a valuable tool for genetic transformation of the tomato "Micro-Tom" model system. **Plant Methods**, *[S. l.]*, v. 6, n. 1, p. 1–11, 2010. DOI: 10.1186/1746-4811-6-23/TABLES/1. Disponível em: https://plantmethods.biomedcentral.com/articles/10.1186/1746-4811-6-23. Acesso em: 18 abr. 2023.

POMA, Betsabé Antezana; MALUF, Wilson Roberto; GOUVEIA, Beatriz Tome; DE OLIVEIRA, Alisson Marcel Souza; FERREIRA, Rodolfo de Paula Duarte; CARVALHO, Regis de Castro. Fruit color and post-harvest shelf life in tomato affected by the og, nor, and rin allleles. **Pesquisa Agropecuária Brasileira**, *[S. l.]*, v. 52, n. 9, p. 743–750, 2017. DOI: 10.1590/S0100-204X2017000900006. Disponível em: http://www.scielo.br/j/pab/a/wjfWHHgpSdMJRGdMHpDYP8F/abstract/?lang=en. Acesso em: 22 abr. 2023.

ROCK, C. D.; ZEEVAART, J. A. D. The aba mutant of Arabidopsis thaliana is impaired in epoxy-carotenoid biosynthesis. **Proceedings of the National Academy of Sciences**, *[S. l.]*, v. 88, n. 17, p. 7496–7499, 1991. DOI: 10.1073/PNAS.88.17.7496. Disponível em: https://www.pnas.org/doi/abs/10.1073/pnas.88.17.7496. Acesso em: 16 abr. 2023.

RONEN, G.; CARMEL-GOREN, L.; ZAMIR, D.; HIRSCHBERG, J. An alternative pathway to beta -carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. **Proceedings of the National Academy of Sciences of the United States of America**, *[S. l.]*, v. 97, n. 20, p. 11102–7, 2000. DOI: 10.1073/pnas.190177497. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10995464. Acesso em: 31 jul. 2019.

RONEN, G.; COHEN, M.; ZAMIR, D.; HIRSCHBERG, J. Regulation of carotenoid biosynthesis during tomato fruit development: expression of the gene for lycopene epsilon-cyclase is down-regulated during ripening and is elevated in the mutant Delta. **The Plant journal : for cell and molecular biology**, *[S. l.]*, v. 17, n. 4, p. 341–51, 1999. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10205893. Acesso em: 31 jul. 2019.

SAIKA, Hiroaki et al. Ethylene Promotes Submergence-Induced Expression of OsABA8ox1 , a Gene that Encodes ABA 8'-Hydroxylase in Rice. **Plant and Cell Physiology**, *[S. l.]*, v. 48, n. 2, p. 287–298, 2007. DOI: 10.1093/PCP/PCM003. Disponível em: https://academic.oup.com/pcp/article/48/2/287/2329757. Acesso em: 19 abr. 2023.

SÉRINO, Sylvie; GOMEZ, Laurent; COSTAGLIOLA, Guy; GAUTIER, Hélène. HPLC Assay of Tomato Carotenoids: Validation of a Rapid Microextraction Technique. **Journal of Agricultural and Food Chemistry**, *[S. l.]*, v. 57, n. 19, p. 8753–8760, 2009. DOI: 10.1021/jf902113n. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/19769393. Acesso em: 31 jul. 2019.

SOTO, Alvaro; RUIZ, Karina B.; RAVAGLIA, Daniela; COSTA, Guglielmo; TORRIGIANI, Patrizia. ABA may promote or delay peach fruit ripening through modulation of ripening- and hormone-related gene expression depending on the developmental stage. **Plant Physiology and Biochemistry**, *[S. l.]*, v. 64, p. 11–24, 2013. DOI: 10.1016/J.PLAPHY.2012.12.011.

STEPANOVA, Anna N.; YUN, Jeonga; LIKHACHEVA, Alla V.; ALONSO, Jose M. Multilevel Interactions between Ethylene and Auxin in Arabidopsis Roots. **The Plant Cell**, *[S. l.]*, v. 19, n. 7, p. 2169–2185, 2007. DOI: 10.1105/TPC.107.052068. Disponível em: https://academic.oup.com/plcell/article/19/7/2169/6092110. Acesso em: 17 abr. 2023.

SU, Liyan et al. Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance. **BMC Plant Biology**, *[S. l.]*, v. 15, n. 1, 2015. DOI: 10.1186/S12870-015-0495-4.

SUN, Liang et al. Suppression of 9-cis-Epoxycarotenoid Dioxygenase, Which Encodes a Key Enzyme in Abscisic Acid Biosynthesis, Alters Fruit Texture in Transgenic Tomato. **Plant Physiology**, *[S. l.]*, v. 158, n. 1, p. 283–298, 2012. a. DOI: 10.1104/PP.111.186866. Disponível em: https://academic.oup.com/plphys/article/158/1/283/6109076. Acesso em: 17 abr. 2023.

SUN, Liang; YUAN, Bing; WANG, Ling; CUI, Mengmeng; WANG, Qi; LENG, Ping. Fruit-specific RNAi-mediated Suppression of SlNCED1 Increases Both Lycopene and β-Carotene Contents in Tomato Fruit. **Journal of Experimental Botany**, *[S. l.]*, v. 63, n. 8, p. 3097–3108, 2012. b. DOI: 10.1093/jxb.

SUN, Zairen; GANTT, Elisabeth; CUNNINGHAM, Francis X. Cloning and functional analysis of the beta-carotene hydroxylase of *Arabidopsis thaliana*. **The Journal of biological chemistry**, *[S. l.]*, v.

271, n. 40, p. 24349–24352, 1996. DOI: 10.1074/JBC.271.40.24349. Disponível em: https://pubmed.ncbi.nlm.nih.gov/8798688/. Acesso em: 22 abr. 2023.

TAO, Xiaoya; WU, Qiong; AALIM, Halah; LI, Li; MAO, Linchun; LUO, Zisheng; YING, Tiejin. Effects of Exogenous Abscisic Acid on Bioactive Components and Antioxidant Capacity of Postharvest Tomato during Ripening. **Molecules**, *[S. l.]*, v. 25, n. 6, 2020. DOI: 10.3390/MOLECULES25061346. Disponível em: /pmc/articles/PMC7144105/. Acesso em: 16 abr. 2023.

TAYLOR, I. B.; LINFORTH, R. S. T.; AL NAIEB, R. J.; BOWMAN, W. R.; MARPLES, B. A. The wilty tomato mutants flacca and sitiens are impaired in the oxidation of ABA-aldehyde to ABA. **Plant, Cell and Environment**, *[S. l.]*, 1988. DOI: 10.3/JQUERY-UI.JS. Disponível em: https://agris.fao.org/agris-search/search.do?recordID=GB19900031258. Acesso em: 17 abr. 2023.

TAYLOR, Ian B.; SONNEVELD, Tineke; BUGG, Timothy D. H.; THOMPSON, Andrew J. Regulation and Manipulation of the Biosynthesis of Abscisic Acid, Including the Supply of Xanthophyll Precursors. **Journal of Plant Growth Regulation 2005 24:4**, *[S. l.]*, v. 24, n. 4, p. 253–273, 2005. DOI: 10.1007/S00344-005-0070-6. Disponível em: https://link.springer.com/article/10.1007/s00344-005-0070-6. Acesso em: 16 abr. 2023.

TIWARI, Krishnaraj; PALIYATH, Gopinadhan. Microarray analysis of ripening-regulated gene expression and its modulation by 1-MCP and hexanal. **Plant Physiology and Biochemistry**, *[S. l.]*, v. 49, n. 3, p. 329–340, 2011. DOI: 10.1016/j.plaphy.2011.01.007.

WENG, Lin; ZHAO, Fangfang; LI, Rong; XU, Changjie; CHEN, Kunsong; XIAO, Han. The Zinc Finger Transcription Factor SlZFP2 Negatively Regulates Abscisic Acid Biosynthesis and Fruit Ripening in Tomato. **Plant Physiology**, *[S. l.]*, v. 167, n. 3, p. 931–949, 2015. DOI: 10.1104/PP.114.255174. Disponível em: https://academic.oup.com/plphys/article/167/3/931/6113768. Acesso em: 21 abr. 2023.

WU, Qiong; BAI, Jiawei; TAO, Xiaoya; MOU, Wangshu; LUO, Zisheng; MAO, Linchun; BAN, Zhaojun; YING, Tiejin; LI, Li. Synergistic effect of abscisic acid and ethylene on color development in tomato (Solanum lycopersicum L.) fruit. **Scientia Horticulturae**, *[S. l.]*, v. 235, p. 169–180, 2018. DOI: 10.1016/j.scienta.2018.02.078.

YANG, Sen; ZHOU, Jiaqian; WATKINS, Christopher B.; WU, Caie; FENG, Yanchun; ZHAO, Xiaoyang; XUE, Zhaohui; KOU, Xiaohong. NAC transcription factors SNAC4 and SNAC9 synergistically regulate tomato fruit ripening by affecting expression of genes involved in ethylene and abscisic acid metabolism and signal transduction. **Postharvest Biology and Technology**, *[S. l.]*, v. 178, p. 111555, 2021. DOI: 10.1016/j.postharvbio.2021.111555. Disponível em: https://doi.org/10.1016/j.postharvbio.2021.111555. Acesso em: 23 abr. 2023.

YIN, Cui Cui et al. Ethylene Responses in Rice Roots and Coleoptiles Are Differentially Regulated by a Carotenoid Isomerase-Mediated Abscisic Acid Pathway. **The Plant Cell**, *[S. l.]*, v. 27, n. 4, p. 1061–1081, 2015. DOI: 10.1105/TPC.15.00080. Disponível em: https://academic.oup.com/plcell/article/27/4/1061/6101473. Acesso em: 16 abr. 2023.

YU, Yanwen; WANG, Juan; LI, Shenghui; KAKAN, Xiamusiya; ZHOU, Yun; MIAO, Yuchen; WANG, Fangfang; QIN, Hua; HUANG, Rongfeng. Ascorbic Acid Integrates the Antagonistic Modulation of Ethylene and Abscisic Acid in the Accumulation of Reactive Oxygen Species. **Plant Physiology**, *[S. l.]*, v. 179, n. 4, p. 1861–1875, 2019. DOI: 10.1104/PP.18.01250. Disponível em: https://academic.oup.com/plphys/article/179/4/1861/6116812. Acesso em: 19 abr. 2023.

ZHANG, Mei; YUAN, Bing; LENG, Ping. The role of ABA in triggering ethylene biosynthesis and ripening of tomato fruit. **Journal of Experimental Botany**, *[S. l.]*, v. 60, n. 6, p. 1579–1588, 2009. DOI: 10.1093/JXB/ERP026. Disponível em: https://academic.oup.com/jxb/article/60/6/1579/512178. Acesso em: 28 abr. 2022.

ZOU, Jian et al. Co-silencing of ABA receptors (SlRCAR) reveals interactions between ABA and ethylene signaling during tomato fruit ripening. **Horticulture Research**, *[S. l.]*, v. 9, 2022. DOI: 10.1093/HR/UHAC057. Disponível em: https://academic.oup.com/hr/article/doi/10.1093/hr/uhac057/6603453. Acesso em: 17 abr. 2023.

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 disasembly, 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.

References

ADIE, Bruce; CHICO, José Manuel; RUBIO-SOMOZA, Ignacio; SOLANO, Roberto. Modulation of Plant Defenses by Ethylene. **Journal of Plant Growth Regulation**, *[S. l.]*, v. 26, n. 2, p. 160–177, 2007. DOI: 10.1007/s00344-007-0012-6.

ARIAS, R.; LEE, T. C.; SPECCA, D.; JANES, H. Quality comparison of hydroponic tomatoes (*Lycopersicon esculentum*) ripened on and off vine. **Journal of Food Science**, *[S. l.]*, v. 65, n. 3, p. 545–548, 2000. DOI: 10.1111/J.1365-2621.2000.TB16045.X.

BAPAT, Vishwas A.; TRIVEDI, Prabodh K.; GHOSH, Antara; SANE, Vidhu A.; GANAPATHI, Thumballi R.; NATH, Pravendra. Ripening of fleshy fruit: Molecular insight and the role of ethylene. **Biotechnology Advances**, *[S. l.]*, v. 28, n. 1, p. 94–107, 2010. DOI: 10.1016/j.biotechadv.2009.10.002.

BARICKMAN, T. Casey; KOPSELL, Dean A.; SAMS, Carl E. Abscisic acid improves tomato fruit quality by increasing soluble sugar concentrations.

http://dx.doi.org/10.1080/01904167.2016.1231812, *[S. l.]*, v. 40, n. 7, p. 964–973, 2017. DOI: 10.1080/01904167.2016.1231812. Disponível em: https://www.tandfonline.com/doi/abs/10.1080/01904167.2016.1231812. Acesso em: 19 abr. 2023.

BARRY, Cornelius S.; LLOP-TOUS, M. Immaculada; GRIERSON, Donald. The Regulation of 1-Aminocyclopropane-1-Carboxylic Acid Synthase Gene Expression during the Transition from System-1 to System-2 Ethylene Synthesis in Tomato. **Plant Physiology**, *[S. l.]*, v. 123, n. 3, p. 979–986, 2000. DOI: 10.1104/pp.123.3.979.

BECKLES, Diane M. Factors affecting the postharvest soluble solids and sugar content of tomato (*Solanum lycopersicum* L.) fruit. **Postharvest Biology and Technology**, *[S. l.]*, v. 63, n. 1, p. 129–140, 2012. DOI: 10.1016/J.POSTHARVBIO.2011.05.016.

BRECHT, Jeffrey K. Locular Gel Formation in Developing Tomato Fruit and the Initiation of Ethylene Production. **HortScience**, *[S. l.]*, v. 22, n. 3, p. 476–479, 1987. DOI: 10.21273/HORTSCI.22.3.476. Disponível em: https://journals.ashs.org/hortsci/view/journals/hortsci/22/3/article-p476.xml. Acesso em: 4 abr. 2023.

CHEN, Chong; ZHANG, Meng; ZHANG, Mingyue; YANG, Minmin; DAI, Shanshan; MENG, Qingwei; LV, Wei; ZHUANG, Kunyang. ETHYLENE-INSENSITIVE 3-LIKE 2 regulates β-carotene and ascorbic acid accumulation in tomatoes during ripening. **Plant Physiology**, *[S. l.]*, 2023. DOI: 10.1093/plphys/kiad151.

CHERVIN, Christian. Should Starch Metabolism Be a Key Point of the Climacteric vs. Non-climacteric Fruit Definition? **Frontiers in Plant Science**, *[S. l.]*, v. 11, 2020. DOI: 10.3389/fpls.2020.609189.

COLÉOU, Thierry; POUPON, Manuel; AZBEL, Kostia. Unsupervised seismic facies classification: A review and comparison of techniques and implementation. **The Leading Edge**, *[S. l.]*, v. 22, n. 10, p. 942–953, 2003. DOI: 10.1190/1.1623635.

CRUZ, Aline Bertinatto; BIANCHETTI, Ricardo Ernesto; ALVES, Frederico Rocha Rodrigues; PURGATTO, Eduardo; PERES, Lazaro Eustaquio Pereira; ROSSI, Magdalena; FRESCHI, Luciano. Light, Ethylene and Auxin Signaling Interaction Regulates Carotenoid Biosynthesis During Tomato Fruit Ripening. **Frontiers in Plant Science**, *[S. l.]*, v. 9, 2018. DOI: 10.3389/fpls.2018.01370.

DIRETTO, Gianfranco et al. Manipulation of β-carotene levels in tomato fruits results in increased ABA content and extended shelf life. **Plant Biotechnology Journal**, *[S. l.]*, v. 18, n. 5, p. 1185–1199, 2020. DOI: 10.1111/pbi.13283. Disponível em: https://onlinelibrary.wiley.com/doi/full/10.1111/pbi.13283. Acesso em: 3 jun. 2021.

FRAY, Rupert G.; GRIERSON, Donald. Identification and genetic analysis of normal and mutant phytoene synthase genes of tomato by sequencing, complementation and co-suppression. **Plant Molecular Biology**, *[S. l.]*, v. 22, n. 4, p. 589–602, 1993. DOI: 10.1007/BF00047400.

GAUTIER, Hélène; DIAKOU-VERDIN, Vicky; BÉNARD, Camille; REICH, Maryse; BURET, Michel; BOURGAUD, Frédéric; POËSSEL, Jean Luc; CARIS-VEYRAT, Catherine; GÉNARD, Michel. How Does Tomato Quality (Sugar, Acid, and Nutritional Quality) Vary with Ripening

Stage, Temperature, and Irradiance? **Journal of Agricultural and Food Chemistry**, *[S. l.]*, v. 56, n. 4, p. 1241–1250, 2008. DOI: 10.1021/jf072196t.

GIOVANELLI, G.; LAVELLI, V.; PERI, C.; NOBILI, S. THE ANTIOXIDANT ACTIVITY OF TOMATO. II. EFFECTS OF VINE AND POST-HARVEST RIPENING. **Acta Horticulturae**, *[S. l.]*, n. 542, p. 211–216, 2001. DOI: 10.17660/ActaHortic.2001.542.26.

GIULIANO, Giovanni; BARTLEY, Glenn E.; SCOLNIK, Pablo A. Regulation of Carotenoid Biosynthesis during Tomato Development. **The Plant Cell**, *[S. l.]*, v. 5, p. 379–387, 1993. Disponível em: https://academic.oup.com/plcell/article/5/4/379/5984490. Acesso em: 28 abr. 2022.

GÓMEZ, Perla; ÁNGELES FERRER, M.; PABLO FERNÁNDEZ-TRUJILLO, J.; CALDERÓN, Antonio; ARTÉS, Francisco; EGEA-CORTINES, Marcos; WEISS, Julia. Structural changes, chemical composition and antioxidant activity of cherry tomato fruits (cv. Micro-Tom) stored under optimal and chilling conditions. **Journal of the Science of Food and Agriculture**, *[S. l.]*, v. 89, n. 9, p. 1543–1551, 2009. DOI: 10.1002/JSFA.3622.

GONZALEZ, Carla; RÉ, Martín D.; SOSSI, María L.; VALLE, Estela M.; BOGGIO, Silvana B. Tomato cv. 'Micro-Tom' as a model system to study postharvest chilling tolerance. **Scientia Horticulturae**, *[S. l.]*, v. 184, p. 63–69, 2015. DOI: 10.1016/j.scienta.2014.12.020.

GUPTA, A.; UPADHYAY, Rakesh K.; PRABHAKAR, Rakhi; TIWARI, Neerja; GARG, Rashmi; SANE, Vidhu A.; SANE, Aniruddha P. SlDREB3, a negative regulator of ABA responses, controls seed germination, fruit size and the onset of ripening in tomato. **Plant Science**, *[S. l.]*, v. 319, p. 111249, 2022. DOI: 10.1016/J.PLANTSCI.2022.111249.

KAI, Wenbin; WANG, Juan; LIANG, Bin; FU, Ying; ZHENG, Yu; ZHANG, Wenbo; LI, Qian; LENG, Ping. PYL9 is involved in the regulation of ABA signaling during tomato fruit ripening. **Journal of Experimental Botany**, *[S. l.]*, v. 70, n. 21, p. 6305, 2019. DOI: 10.1093/JXB/ERZ396. Disponível em: /pmc/articles/PMC6859720/. Acesso em: 22 abr. 2023.

KLEE, Harry J.; GIOVANNONI, James J. Genetics and Control of Tomato Fruit Ripening and Quality Attributes. **Annual Review of Genetics**, *[S. l.]*, v. 45, n. 1, p. 41–59, 2011. DOI: 10.1146/annurev-genet-110410-132507.

KOTÍKOVÁ, Zora; LACHMAN, Jaromír; HEJTMÁNKOVÁ, Alena; HEJTMÁNKOVÁ, Kateřina. Determination of antioxidant activity and antioxidant content in tomato varieties and evaluation of mutual interactions between antioxidants. **LWT - Food Science and Technology**, *[S. l.]*, v. 44, n. 8, p. 1703–1710, 2011. DOI: 10.1016/J.LWT.2011.03.015. Disponível em: https://www.sciencedirect.com/science/article/pii/S0023643811001022. Acesso em: 3 set. 2019.

KURINA, A. B.; SOLOVIEVA, A. E.; KHRAPALOVA, I. A.; ARTEMYEVA, A. M. Biochemical composition of tomato fruits of various colors. **Vavilov Journal of Genetics and Breeding**, *[S. l.]*, v. 25, n. 5, p. 514, 2021. DOI: 10.18699/VJ21.058. Disponível em: /pmc/articles/PMC8453365/. Acesso em: 28 abr. 2022.

LENUCCI, Marcello S.; CADINU, Daniela; TAURINO, Marco; PIRO, Gabriella; DALESSANDRO, Giuseppe. Antioxidant Composition in Cherry and High-Pigment Tomato Cultivars.

Journal of Agricultural and Food Chemistry, *[S. l.]*, v. 54, n. 7, p. 2606–2613, 2006. DOI: 10.1021/JF052920C. Disponível em: https://pubs.acs.org/doi/full/10.1021/jf052920c. Acesso em: 6 abr. 2023.

LI, Tao; HEUVELINK, Ep; MARCELIS, Leo F. M. Quantifying the source/sink balance and carbohydrate content in three tomato cultivars. **Frontiers in Plant Science**, *[S. l.]*, v. 6, 2015. DOI: 10.3389/fpls.2015.00416.

LIU, Lihong; SHAO, Zhiyong; ZHANG, Min; WANG, Qiaomei. Regulation of Carotenoid Metabolism in Tomato. **Molecular Plant**, *[S. l.]*, v. 8, n. 1, p. 28–39, 2015. DOI: 10.1016/J.MOLP.2014.11.006. Disponível em: https://www.sciencedirect.com/science/article/pii/S1674205214000070. Acesso em: 31 jul. 2019.

MCMURCHIE, E. J.; MCGLASSON, W. B.; EAKS, I. L. Treatment of Fruit with Propylene gives Information about the Biogenesis of Ethylene. **Nature**, *[S. l.]*, v. 237, n. 5352, p. 235–236, 1972. DOI: 10.1038/237235a0.

MCQUINN, Ryan P.; GAPPER, Nigel E.; GRAY, Amanda G.; ZHONG, Silin; TOHGE, Takayuki; FEI, Zhangjun; FERNIE, Alisdair R.; GIOVANNONI, James J. Manipulation of ZDS in tomato exposes carotenoid- and ABA-specific effects on fruit development and ripening. **Plant Biotechnology Journal**, *[S. l.]*, v. 18, n. 11, p. 2210–2224, 2020. DOI: 10.1111/pbi.13377. Disponível em: www.arabidopsis.org; Acesso em: 28 maio. 2021.

MIN, Dedong; LI, Zilong; FU, Xiaodong; WANG, Jihan; LI, Fujun; LI, Xiaoan; ZHANG, Xinhua. Integration of transcriptomic and metabonomic reveals molecular differences of sweetness and aroma between postharvest and vine ripened tomato fruit. **Food Control**, *[S. l.]*, v. 139, p. 109102, 2022. DOI: 10.1016/j.foodcont.2022.109102.

ORSI, Bruna. **Variações alélicas que afetam a carotenogênese em tomateiro alteram o amadurecimento e a suscetibilidade dos frutos ao fungo Botrytis cinerea**. 2018. Biblioteca Digital de Teses e Dissertações da Universidade de São Paulo, Piracicaba, 2018. DOI: 10.11606/D.11.2018.TDE-08102018-171720. Disponível em: http://www.teses.usp.br/teses/disponiveis/11/11144/tde-08102018-171720/. Acesso em: 16 abr. 2023.

ORSI, Bruna; SESTARI, Ivan; PRECZENHAK, Ana Paula; TESSMER, Magda Andréia; DA SILVA SOUZA, Mayara Adja; HASSIMOTTO, Neuza Mariko Aymoto; KLUGE, Ricardo Alfredo. Allelic variations in the tomato carotenoid pathway lead to pleiotropic effects on fruit ripening and nutritional quality. **Postharvest Biology and Technology**, *[S. l.]*, v. 181, p. 111632, 2021. DOI: 10.1016/J.POSTHARVBIO.2021.111632.

OSORIO, Sonia; CARNEIRO, Raphael T.; LYTOVCHENKO, Anna; MCQUINN, Ryan; SØRENSEN, Iben; VALLARINO, José G.; GIOVANNONI, James J.; FERNIE, Alisdair R.; ROSE, Jocelyn K. C. Genetic and metabolic effects of ripening mutations and vine detachment on tomato fruit quality. **Plant Biotechnology Journal**, *[S. l.]*, v. 18, n. 1, p. 106–118, 2020. DOI: 10.1111/pbi.13176.

OZGEN, Senay; SEKERCI, Saziye; KORKUT, Recep; KARABIYIK, Tugba. The tomato debate: Postharvest-ripened or vine ripe has more antioxidant? **Horticulture, Environment, and Biotechnology**, *[S. l.]*, v. 53, n. 4, p. 271–276, 2012. DOI: 10.1007/s13580-012-0001-y.

RE, R.; PELLEGRINI, N.; PROTEGGENTE, A.; PANNALA, A.; YANG, M.; RICE-EVANS, C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. **Free radical biology & medicine**, *[S. l.]*, v. 26, n. 9–10, p. 1231–7, 1999.

RONEN, G.; CARMEL-GOREN, L.; ZAMIR, D.; HIRSCHBERG, J. An alternative pathway to beta -carotene formation in plant chromoplasts discovered by map-based cloning of beta and old-gold color mutations in tomato. **Proceedings of the National Academy of Sciences of the United States of America**, *[S. l.]*, v. 97, n. 20, p. 11102–7, 2000. DOI: 10.1073/pnas.190177497. Disponível em: http://www.ncbi.nlm.nih.gov/pubmed/10995464. Acesso em: 31 jul. 2019.

SEYMOUR, Graham B.; ØSTERGAARD, Lars; CHAPMAN, Natalie H.; KNAPP, Sandra; MARTIN, Cathie. Fruit Development and Ripening. **Annual Review of Plant Biology**, *[S. l.]*, v. 64, n. 1, p. 219–241, 2013. DOI: 10.1146/annurev-arplant-050312-120057.

SU, Liyan et al. Carotenoid accumulation during tomato fruit ripening is modulated by the auxin-ethylene balance. **BMC Plant Biology**, *[S. l.]*, v. 15, n. 1, 2015. DOI: 10.1186/S12870-015-0495-4.

SUN, Liang et al. Suppression of 9-cis-Epoxycarotenoid Dioxygenase, Which Encodes a Key Enzyme in Abscisic Acid Biosynthesis, Alters Fruit Texture in Transgenic Tomato. **Plant Physiology**, *[S. l.]*, v. 158, n. 1, p. 283–298, 2012. DOI: 10.1104/PP.111.186866. Disponível em: https://academic.oup.com/plphys/article/158/1/283/6109076. Acesso em: 17 abr. 2023.

TADESSE, T.; SEYOUM WORKNEH, T.; WOLDETSADIK, K. Effect of varieties on changes in sugar content and marketability of tomato stored under ambient conditions. **African Journal of Agricultural Research**, *[S. l.]*, v. 7, n. 14, p. 2124–2130, 2012. DOI: 10.5897/AJAR11.1216. Disponível em: http://www.academicjournals.org/AJAR. Acesso em: 3 abr. 2023.

TAO, Xiaoya; WU, Qiong; AALIM, Halah; LI, Li; MAO, Linchun; LUO, Zisheng; YING, Tiejin. Effects of Exogenous Abscisic Acid on Bioactive Components and Antioxidant Capacity of Postharvest Tomato during Ripening. **Molecules**, *[S. l.]*, v. 25, n. 6, 2020. DOI: 10.3390/MOLECULES25061346. Disponível em: /pmc/articles/PMC7144105/. Acesso em: 16 abr. 2023.

VOGEL, J. T.; TIEMAN, D. M.; SIMS, C. A.; ODABASI, A. ..; CLARK, D. G.; KLEE, H. J. Carotenoid content impacts flavor acceptability in tomato (*Solanum lycopersicum*). **Journal of the science of food and agriculture**, *[S. l.]*, v. 90, n. 13, p. 2233–2240, 2010. DOI: 10.1002/JSFA.4076. Disponível em: https://pubmed.ncbi.nlm.nih.gov/20661902/. Acesso em: 30 ago. 2021.

WENG, Lin; ZHAO, Fangfang; LI, Rong; XU, Changjie; CHEN, Kunsong; XIAO, Han. The Zinc Finger Transcription Factor SlZFP2 Negatively Regulates Abscisic Acid Biosynthesis and Fruit Ripening in Tomato. **Plant Physiology**, *[S. l.]*, v. 167, n. 3, p. 931–949, 2015. DOI: 10.1104/PP.114.255174. Disponível em: https://academic.oup.com/plphys/article/167/3/931/6113768. Acesso em: 21 abr. 2023.

WU, Qiong; BAI, Jiawei; TAO, Xiaoya; MOU, Wangshu; LUO, Zisheng; MAO, Linchun; BAN, Zhaojun; YING, Tiejin; LI, Li. Synergistic effect of abscisic acid and ethylene on color development in tomato (*Solanum lycopersicum* L.) fruit. **Scientia Horticulturae**, *[S. l.]*, v. 235, p. 169–180, 2018. DOI: 10.1016/j.scienta.2018.02.078.

YANG, Tianxia et al. Recoloring tomato fruit by CRISPR/Cas9-mediated multiplex gene editing. **Horticulture Research**, *[S. l.]*, v. 10, n. 1, 2023. DOI: 10.1093/HR/UHAC214. Disponível em: https://academic.oup.com/hr/article/10/1/uhac214/6705573. Acesso em: 21 abr. 2023.

YOO, Hee et al. Inferring the Genetic Determinants of Fruit Colors in Tomato by Carotenoid Profiling. **Molecules**, *[S. l.]*, v. 22, n. 5, p. 764, 2017. DOI: 10.3390/molecules22050764. Disponível em: http://www.mdpi.com/1420-3049/22/5/764. Acesso em: 31 jul. 2019.

YOO, Hee Ju; KIM, Jin-Hyun; PARK, Kyoung-Sub; SON, Jung Eek; LEE, Je Min. Light-Controlled Fruit Pigmentation and Flavor Volatiles in Tomato and Bell Pepper. **Antioxidants**, *[S. l.]*, v. 9, n. 1, p. 14, 2019. DOI: 10.3390/antiox9010014.

ZHANG, Chi; DUAN, Wenyi; CHEN, Kunsong; ZHANG, Bo. Transcriptome and methylome analysis reveals effects of ripening on and off the vine on flavor quality of tomato fruit. **Postharvest Biology and Technology**, *[S. l.]*, v. 162, p. 111096, 2020. DOI: 10.1016/j.postharvbio.2019.111096.

ZHOU, Jiaqi; CHEN, Bixuan; ALBORNOZ, Karin; BECKLES, Diane M. Postharvest handling induces changes in fruit DNA methylation status and is associated with alterations in fruit quality in tomato (*Solanum lycopersicum* L.). **Scientia Horticulturae**, *[S. l.]*, v. 283, p. 110090, 2021. DOI: 10.1016/j.scienta.2021.110090.

ZHOU, JiaQian; ZHAO, XiaoYang; YANG, Sen; WU, Cai E.; XUE, ZhaoHui; KOU, XiaoHong. Differential roles of SNAC4 and SNAC9 in ABA-mediated softening during tomato fruit ripening. **Scientia Horticulturae**, *[S. l.]*, v. 310, p. 111685, 2023. DOI: 10.1016/j.scienta.2022.111685.

ZIELIŃSKI, H.; KOZŁOWSKA, H. Antioxidant activity and total phenolics in selected cereal grains and their different morphological fractions. **Journal of Agricultural and Food Chemistry**, *[S. l.]*, v. 48, n. 6, p. 2008–2016, 2000. DOI: 10.1021/jf990619o. Disponível em: https://pubmed.ncbi.nlm.nih.gov/10888490/. Acesso em: 5 jan. 2021.