UNIVERSIDADE DE SÃO PAULO

FACULDADE DE CIÊNCIAS FARMACÊUTICAS

Programa de Pós-graduação em Ciência dos Alimentos

Área de Bromatologia

Influence of pre- and post-harvest treatments on metabolite profiling of tomato *(Solanum lycopersicum)* during fruit ripening

(Versão Corrigida)

SILVIA LETICIA RIVERO MEZA

Tese para obtenção do Título de Doutor

Orientador: Prof. Dr. Eduardo Purgatto

São Paulo

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Silvia Leticia Rivero Meza

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I dedicated this work to Mita, Kike and Cuco (*in memorian*) for continually encouraging my dreams.

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RESUMO

MEZA, S. L. R. **Influência dos tratamentos pré- e pós-colheita no perfil de metabólitos do tomate (*Solanum lycopersicum*) durante o amadurecimento.** 2020. 167f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2020.

Durante o amadurecimento de frutos ocorrem mudanças sensoriais como na cor, textura e aroma assim como a produção de compostos benéficos para a saúde. Deste modo, alterações nos metabólitos primários e secundários foram avaliadas em frutos expostos aos tratamentos pré- e pós-colheita como estresse salino e tratamento hormonal, respectivamente, com o objetivo de melhorar a qualidade sensorial e nutricional do fruto. O tratamento salino foi aplicado em duas variedades tradicionais do Mediterrâneo denominadas 'Tomate Pimiento'(TP) e 'Muchamiel Aperado' (MA), usando como referência a cultivar comercial 'Moneymaker' (MM). Os tratamentos hormonais (etileno, metil jasmonato, 1-metilciclopropeno, e ambos 1-metilciclopropeno e metil jasmonato) foram aplicados em tomate Grape, usando os frutos não tratados como controle. Em relação ao tratamento pré-colheita, as plantas foram cultivadas sem sal (controle) e com moderado estresse salino (50 mM NaCl), o qual não afetou a produção das variedades. A variedade TP é de grande interesse devido seu elevado conteúdo de sólidos solúveis (SSC) no controle, o qual é ainda maior em sal, enquanto MA é muito atrativa devido seu elevado índice de produção Brix (SSC x produção), o que tem sido usada como medida geral da qualidade do fruto. A similaridade entre ambas variedades tradicionais foi encontrada no metabolismo primário, visto que aumentaram significativamente o conteúdo de sacarose com respeito a MM em fruto maduro de plantas cultivadas no controle e, especialmente, em condições de estresse salino. A diferença mais notável foi observada nos elevados níveis de aminoácidos totais em frutos TP, incluindo os três principais aminoácidos livres encontrados em tomate, GABA, glutamato e glutamina, que aumentaram mesmo sob salinidade. A respeito dos metabólitos secundários, a alteração mais interessante induzida pela salinidade foi o aumento em α-tocoferol encontrado nos frutos maduros de ambas variedades de tomate. Em relação ao tratamento pós-colheita, o etileno aumentou os níveis de frutose, sacarose e glicose, enquanto o metil jasmonato aumentou os ácidos orgânicos como ácidos cítrico e málico, e aminoácidos como ácido glutâmico, GABA, e fenilalanina no décimo dia após colheita (DAH); e ácidos graxos no 4 DAH. Ambos tratamentos induziram o acumulo de tocoferóis e fitosteróis no 10 DAH, e carotenoids (principalmente licopeno) no dia 10 e 21 DAH. Frutos tratados somente com 1-metilciclopropeno apresentaram redução significativa nos níveis dos metabolitos principalmente no dia 4 e 10 DAH. Frutos tratados com ambos1-metilciclopropeno e metil jasmonate exibiram menor impacto no atraso da produção de metabólitos do que frutos tratados somente com 1-metilciclopropeno. Além disso, esse tratamento demonstrou uma tendência em melhorar os níveis de açúcares (glicose, ácido glucárico e manose) no 10 DAH e ácidos orgânicos (ácidos propanoico e butanoico), carotenoides (licopeno, β-caroteno, luteína), tocoferóis (α-tocoferol, β-tocoferol e γ-tocoferol) e fitosteróis (β-sitosterol, stigmasterol and stigmastadienol) no 21 DAH. A maioria das alterações no perfil dos metabólitos está relacionada às características organolépticas e ao valor nutricional dos frutos, propondo que os tratamentos pré- e pós-colheita podem ser aplicados como ferramentas para melhorar os valores nutricionais e sensoriais do tomate.

Palavras-chave: *Solanum lycopersicum*, tratamentos pré- e pós-colheita, estresse salino, fitohormonios, amadurecimento, metabolitos primários e secundários, qualidade do fruto.

ABSTRACT

MEZA, S. L. R. **Influence of pre- and post-harvest treatments on metabolite profiling of tomato fruit *(Solanum lycopersicum)* during ripening**. 2020. 167f. Tese (Doutorado) – Faculdade de Ciências Farmacêuticas, Universidade de São Paulo, São Paulo, 2020.

During fruit ripening, sensory changes occur, such as in color, texture and aroma, as well as the production of beneficial compounds for health. Thus, changes in primary and secondary metabolites were analyzed in fruits, which were exposed to pre- and post-harvest treatments as well as salinity stress and hormonal treatment, respectively, aiming to improve the sensory and nutritional quality of the fruit. Salinity treatment was applied in two Mediterranean traditional tomato varieties named 'Tomate Pimiento' (TP) and 'Muchamiel Aperado' (MA), using as reference the commercial cultivar 'Moneymaker' (MM). Hormonal treatments (ethylene, methyl jasmonate, 1-methylcyclopropene, and both 1-methylcyclopropene and methyl jasmonate) were applied in Grape tomato fruits, using as control the no-treated fruits. Concerning pre-harvest treatment, plants were grown without salt (control) and with moderate salt stress (50 mM NaCl), which did not affect fruit yield in any variety. The variety TP is of great interest because of its high soluble solids content (SSC) in control, which is even higher in salt, while MA is very attractive because of its high Brix yield index (SSC x fruit yield), which has been used as overall fruit quality measure. Similitude between both traditional varieties were found for primary metabolism, as they significantly increased their sucrose contents with respect to MM in red ripe fruits from plants grown in control and, especially, salt stress conditions. The most remarkable difference was observed in the high constitutive levels of total amino acids in TP fruits, including the three major free amino acids found in tomato fruit, GABA, glutamate and glutamine, which even increased under salinity. On the subject of secondary metabolites, the most interesting change induced by salinity was the increase in α-tocopherol found in red ripe fruits of both tomato varieties. Regarding post-harvest treatment, ethylene increased fructose, sucrose and glucose, while methyl jasmonate increased organic acids such as citric and malic acids and amino acids such as glutamic acid, GABA and phenylalanine at 10 DAH; and fatty acids at 4 DAH. Both treatments induced the accumulation of tocopherols and phytosterols at 10 DAH, and carotenoids (mostly lycopene) at 10 and 21 DAH. Fruits treated with only 1-methylcyclopropene presented significant reduction in the levels of metabolites mainly at 4 and 10 DAH. Fruits treated with both 1-methylcyclopropene and methyl jasmonate exhibited a lower impact on delayed of metabolite production than treatment with 1-methylcyclopropene. In addition, it showed a tendency to improve the levels of sugars (glucose, glucaric acid and mannose) at 10 DAH and organic acids (propanoic and butanoic acids), carotenoids (lycopene, β-carotene, lutein), tocopherols (α-tocopherol, β-tocopherol, γ-tocopherol) and phytosterols (β-sitosterol, stigmasterol and stigmastadienol) at 21 DAH. Most of the changes in the metabolites profiling are related to the organoleptic and nutritional value of the fruits, proposing that the pre- and post-harvest treatments can be applied as tools to improve the nutritional and sensory value of tomato.

Keywords: *Solanum lycopersicum*, pre- and post-harvest treatments, salinity stress, phytohormones, fruit ripening, primary and secondary metabolites, fruit quality.

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1. INTRODUCTION

1.1 Fruit ripening and tomato

The developmental procedure of fruit requires a complex set up of interacting genes and signaling pathways. In fleshy fruits, it includes three separate stages, namely, fruit set, fruit development, and fruit ripening. Of these, fruit ripening has got the most attention from geneticists and breeders, once the important process triggers a whole set of biochemical pathways that makes the fruit attractive, desirable, and edible for consumers (Tripathi *et al*., 2016).

Tomato (*Solanum lycopersicum*) is one of the most important horticultural crops worldwide, being the second horticultural crop produced in the world after potato and the first one in yield terms (FAOSTAT, 2018). Tomato fruit has been used widely as a model system to understand the climacteric ripening (Zhang, Huber, & Rao, 2010). Climacteric fruits (tomato, apple, pear and melon) are characterized by a peak in respiration rate at the initiation of ripening associated with an autocatalytic biosynthesis of, and consequently an increase in, ethylene. Differently, non-climacteric fruits (grape, orange, and pineapple) are defined by the absence of ethylene-related respiratory peak (Figure 1) (Tripathi *et al*., 2016).

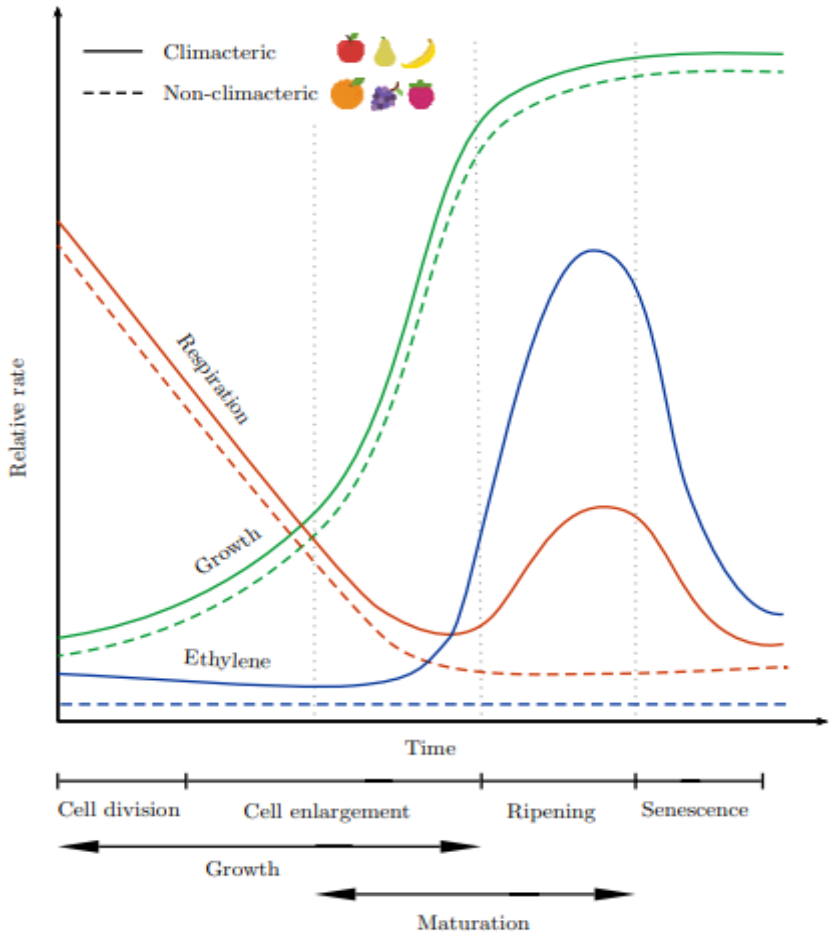

Figure 1. Relative rate of respiration, ethylene production, and growth in climacteric and non-climacteric fruits (Valente *et al*., 2019).

During fruit ripening several biological and biochemical alterations occur in consequence of changes in the metabolite profiling of tomato, such as in primary and secondary metabolism. These metabolic changes result in sugar accumulation, reduction in organic acids, production of volatiles responsible for flavor and aroma, development of characteristic color, texture change and fruit softening (Figure 2) (Bapat *et al*., 2010). All these alterations are responsible for the organoleptic quality attributes and make it desirable for consumption (Handa *et al*., 2012; Quinet *et al*., 2019). Organoleptic quality is a complex fruit quality characteristic including aspects of texture, taste and aroma. The flavor is cognized through a combination of odor, taste and sensation in the mouth, and

depends on the balance between sugars, organic acids, volatile compounds and free amino acids (Petró-Turza, 1986).

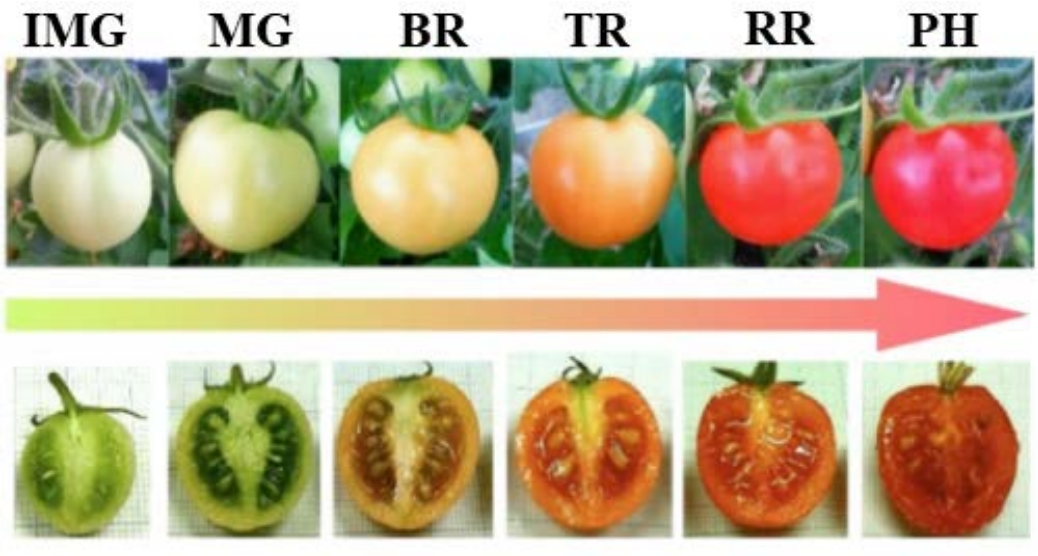

Figure 2. Tomato fruit ripening stages. Tomato fruits at the corresponding developmental stages: IMG, immature green; MG, mature green; BR, breaker; TR, turning; RR, red ripe; PH, postharvest (Adapted from Takizawa *et al*., 2014).

In addition, metabolite content changes in tomato fruit can also lead to alterations in the overall nutritional quality (Oms-Oliu *et al*., 2011). Several metabolites can be related to health benefits such as carotenoids (lycopene, β-carotene, lutein), tocopherols (α-, β- and γ-tocopherol) and phytosterols (β-sitosterol and stigmasterol). Carotenoids and tocopherols play an important role in human nutrition mainly due to antioxidant properties, and visual perception of ripe fruits, while phytosterols are associated in reducing LDL cholesterol and total cholesterol (Bramley, 2002; Almeida *et al.*, 2015; Moreau *et al*., 2018).

This complex process, which results in several changes in primary and secondary metabolism, is regulated by plant hormones such as ethylene, jasmonates, abscisic acid

and other phytohormones. These plant hormones control multiple processes as well as germination, organ senescence, stress responses and fruit ripening. In addition, ethylene plays an important role in climacteric fruits such as tomato fruit, including various metabolic changes during its ripening (Kumar *et al*., 2014; Prasanna *et al*., 2007).

1.2 Primary metabolite profiling of tomato

1.2.1 Sugars

The soluble sugar content in tomato fruit can be measured by the soluble solid content (SSC) and shows a different behavior during post-harvest, which depends mainly on the species and storage conditions. The SSC value normally corresponds with variations in sugars present in ripe fruits such as glucose, fructose and sucrose (Pott *et al*., 2020).

Accumulation of sugars can be related to senescence processes. During ripening and senescence of the fruit, cross-talk between sugars and hormones involved in these processes as abscisic acid, ethylene and auxin, has been described by Jia *et al*. (2013) and Jia *et al*. (2016), and sucrose degradation during post-harvest storage can be critical for prompting senescence (Tang *et al*., 2016). Fruits contain not only the most abundant sugars (sucrose, glucose and fructose), but also minor sugars and alcohol derivatives such as myo-inositol, trehalose, sorbitol, galactinol and raffinose. These metabolites were found at lower levels; however they are important to fruit behavior during storage because they can alleviate the negative effects of the abiotic stresses underlying post-harvest conditions (Farcuh *et al*., 2018).

In addition, the accumulation of sugar during ripening can affect post-harvest water loss by interfering with cuticle growth. In spite of the complete molecular

mechanism has not been described, it was evidently recognized that sugar entry during fruit development affects the cell wall and cuticle structure, resulting in a drastic effect on tomato senescence (Vallarino *et al*., 2017). Moreover, the production of monosaccharides from cell wall disassembly can result in a process of tomato softening. Softening is a physiological process leading to decrease in firmness of ripe fruit. An excessive loss of firmness is result of overripening which prompt physical damage and pathogen attack, decreasing fruit quality (Wu *et al*., 2020).

Soluble sugars offer the primary carbon and energy source in reactive oxygen species metabolism, contributing to the generation of reducing power generation via the oxidative pentose phosphate pathway (Liu *et al*., 2013). Sugars imported by heterotrophic organs provide all of the carbon and energy for their growth (cell division and expansion), differentiation and maintenance. The excess of carbon is stored in soluble form as sugars in vacuoles or in polymeric forms including starch in plastids, or protein or oil in vesicles. To accomplish this, plants have evolved sophisticated mechanisms to sense, transport, metabolize and store sugars and sugar derivatives, and coordinate their availability with the many processes for consumption during plant development (Figure 3) (Patrick *et al*., 2013).

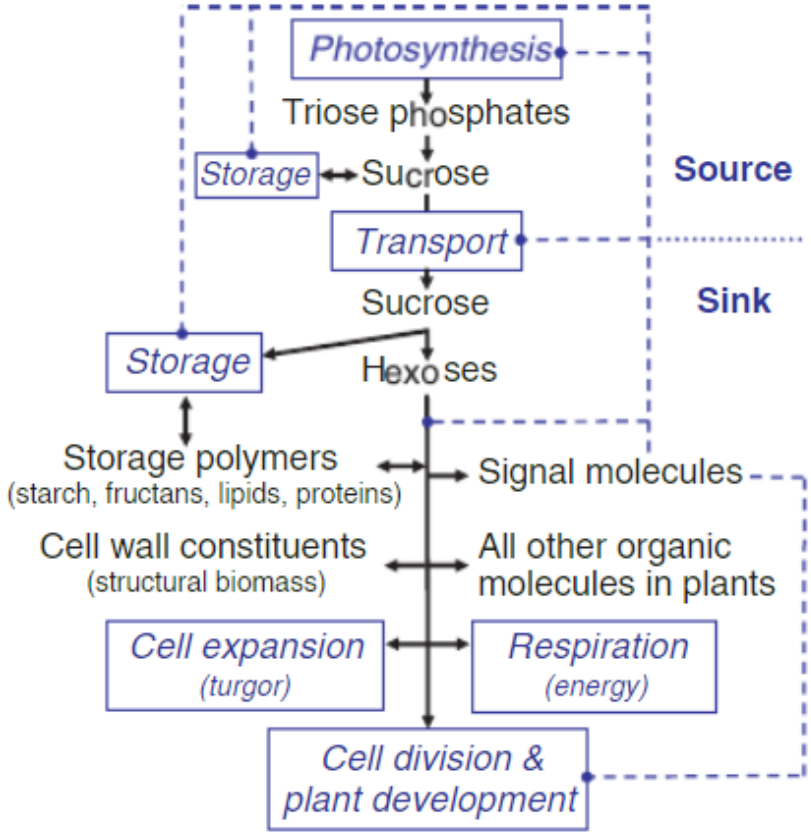

Figure 3. Metabolic process for sugars accumulation (Patrick *et al*., 2013)

1.2.2 Organic acids

Organic acids together with sugars are the main soluble components of ripe fruits. Several organic acids are related to fruit post-harvest metabolism. They present important role on taste, being related to sourness and contributing to the flavour. Sourness is generally attributed to proton release from acids, whereas their different anions each impart a distinct taste. Acidity is also one of the main ripening indices that determines the harvest date of fruits used either for direct consumption or industrial processing (Johanningsmeiner *et al*., 2005).

Many fruits accumulate organic acids in their flesh at certain stages of their development. The most abundant organic acids in fruits such as tomato are citric and malic acids. A characteristic feature of this accumulation in many fruits is that the

concentrations of these acids increase until the beginning of ripening and then decrease. In tomato, the levels of malic acid, among the most abundant organic acids can impact directly fruit shelf-life (Famiani *et al*., 2015).

In the flesh of several fruits, there is an increase in organic acid content during most of fruit development and ripening. Organic acids can be metabolized during ripening by the Krebs cycle (respiration), gluconeogenesis, fermentation to ethanol, amino acid synthesis/interconversion, and as a substrate for the synthesis of secondary metabolites such as pigments (Etienne *et al*., 2013; Famiani *et al*., 2015).

The Krebs cycle acids that accumulate within of fruits are synthesized within the flesh from sugars (Figure 4). Sugars can also derive from photosynthesis within the fruit, however, these sugars account for only a very small proportion of the sugars present in the fruit. The bulk of Krebs cycle acids present in fruits is synthesized from sugars. Products from the metabolism of sugars enter the glycolytic pathway and this converts them to phosphoenolpyruvate (PEP). For example, imported sucrose is often hydrolyzed to glucose and fructose by one of the invertases. These sugars are then phosphorylated by either glucokinase or fructokinase and enter glycolysis. In plants, the cytosolic enzyme phosphoenolpyruvate carboxylase (PEPC) is necessary for the synthesis of the Krebs cycle acids from sugars. It catalyzes the conversion of PEP to oxaloacetate. If the fruit is accumulating malate, oxaloacetate is transformed to malate by cytosolic malate dehydrogenase (NADMDH), and malate is transported across the tonoplast into the vacuole in which it is deposited. When citrate is accumulated one molecule of PEP is converted to oxaloacetate by PEPC. A second PEP is transformed to acetyl CoA by the sequential actions of pyruvate kinase and pyruvate dehydrogenase. Acetyl CoA and oxaloacetate are then combined by citrate synthase in the mitochondrion to give citrate. Citrate is transported across the tonoplast into the vacuole in which it is stored. The

amount of acids that are stored in the vacuole is the major determinant of the content of Krebs cycle acids in fruits. In the case of malate, it is thought that transport processes at the tonoplast are the key factor in defining the vacuolar content (Etienne *et al*., 2013).

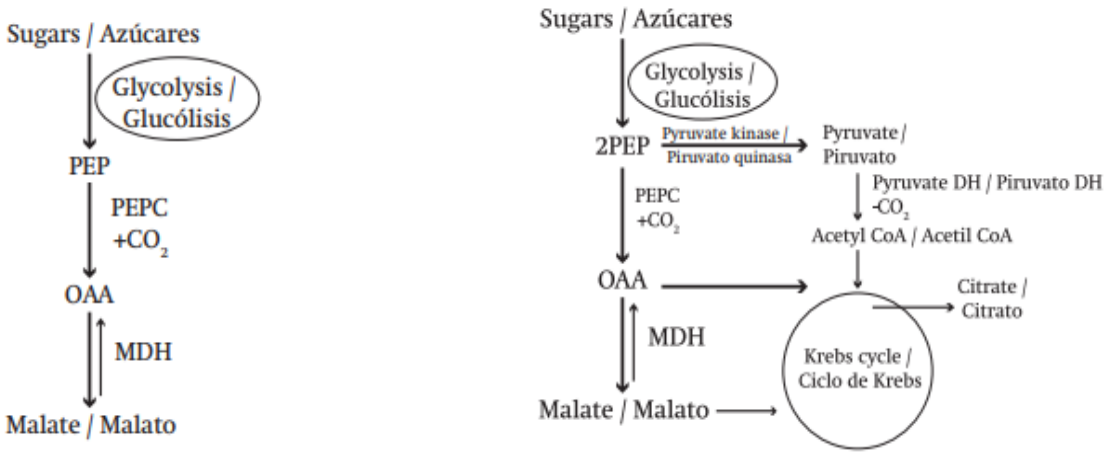

Figure 4. Simplified scheme showing malate and citrate synthesis. MDH, malate dehydrogenase; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PEPC, phosphoenolpyruvate carboxylase; Acetyl-CoA, acetyl-coenzyme A; DH, pyruvate dehydrogenase. (Famiani *et al*., 2015).

The catabolism of malate and citrate through respiration implies in the transformation of malate into pyruvate, by malic enzyme or through the combined action of malate dehydrogenase (MDH), phosphoenol-pyruvate carboxykinase pyruvate (PEPCK) and pyruvate kinase. Pyruvate is then metabolized by pyruvate dehydrogenase (DH) before entering the Krebs cycle (Figure 5). In addition, both malate and citrate can enter the Krebs cycle directly (Famiani *et al*., 2015).

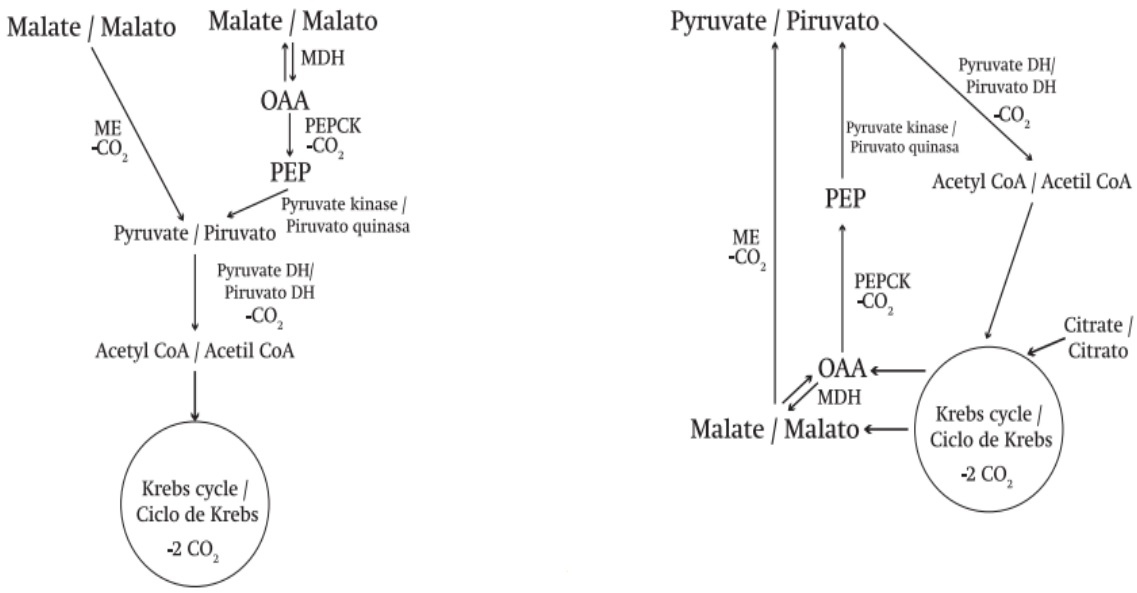

Figure 5. Simplified scheme showing malate and citrate catabolism by respiration. Acetyl-CoA, acetyl-coenzyme A; MDH, malate dehydrogenase; ME, malic enzyme; OAA, oxaloacetate; PEP, phosphoenolpyruvate; PEPCK, phosphoenol-pyruvate carboxykinase pyruvate; DH, pyruvate dehydrogenase (Famiani *et al*., 2015).

In addition, the organic acid content of the fruits can be affected by environmental factors and cultivation practices such as temperature, light intensity, cultivar, rootstock, mineral nutrition, and water availability. However, how these factors alter metabolism to bring about changes in organic acid content is in most cases uncertain (Etienne *et al*., 2013). The content of organic acids is also developmentally controlled and has been reported to increase during ripening. At all stages citric acid is the dominant organic acid but unripe green tomatoes may contain significant amounts of malic acid while its content in ripe fruits is fairly low. Similar to sugars, citric acid declines with progressing maturation after ripening while the content of malic acid remains relatively constant (Agius *et al*., 2018).

1.2.3 Amino acids

The level of amino acids is affected by post-harvest storage, since these metabolites are involved in several pathways induced during ripening. During senescence, amino acid catabolism can counteract the reduction in electron supply from the Krebs cycle (Pott *et al*., 2020). Free amino acids produced during tomato ripening play important roles both in the plant and in human health. Plant foods such as tomatoes contain high levels of free amino acids (Sorrequieta *et al*., 2009). Free amino acids represent a source of nitrogen and of nutritionally essential amino acids such as lysine, methionine, and threonine (Kumar *et al*., 2017).

Amino acids are important food compounds, contributing not only to the nutritive value of foods, but also providing several health benefits such as antimutagenicity as well as reduction in blood sugar and coronary heart diseases. Leucine, isoleucine and valine show to accelerate a recovery from muscle damages, soreness and fatigues after exercise Furthermore, they are involved in multiple functions as a source of energy and as precursors of proteins and other important molecules, contributing, at the same time, to the taste of foods (Odriozola-Serrano *et al*., 2013)

Not only sugars (glucose, fructose and sucrose) and organic acids (citric and malic acids) are responsible for the taste of tomato fruits, but also amino acids such as glutamate. Tomato contains higher content of glutamate procurer glutamic acid than other vegetables. Glutamte is recognized for umami taste and is rich in cheese, tomato and kelp (Zhang *et al*., 2015). Leucine, isoleucine and valine provide bitter taste. Glycine, alanine, and proline provide sweetness. L-serine gives mainly sweetness and minor umami taste, and D-serine an enantiomer of L-serine, gives sweet taste (Ito *et al*., 2017).

Glutamate is also a precursor of γ-aminobutyric acid (GABA) catalyzed by glutamate decarboxylase, which is the main pathway for GABA synthesis in plants (Liu

et al., 2020). There is a special interest in GABA identified in tomato as a health-related compound. Feeding spontaneously hypersensitive rats with a tomato cultivar with high GABA content reduced their systolic blood pressure (Yashimura *et al*., 2010). Multiple roles for GABA have been proposed for plants, including signaling, cell guidance, defense against insects, pH regulation, redox regulation, energy balance, stress responses, and in carbon and nitrogen metabolism. A role for GABA in mediating stress responses has been suggested because of its accumulation in plant tissues after exposure to a range of stresses including acidosis, mechanical damage, salinity, heat, cold and drought. GABA may play a major role in carbon and nitrogen metabolism and be an integral part of the Krebs cycle under both stress and non-stress conditions (Deewatthanawong *et al*., 2010).

Free amino acids have been recognized as aroma precursors and utilized for the synthesis of aroma components during fruit maturation (Ito *et al*., 2017). Aromatic amino acids in plants, phenylalanine, tyrosine and tryptophan, are vital components for protein synthesis (Figure 6). They also support a wide range of secondary metabolites such as pigments, alkaloids, hormones, and cell wall components, which are important for human nutrition as well as for plant development and growth (Filiz *et al*., 2019). Several of the most important tomato aroma volatiles, including 2-phenylacetaldehyde and 2-phenylethanol, are derived from phenylalanine (Tiemen *et al*., 2006).

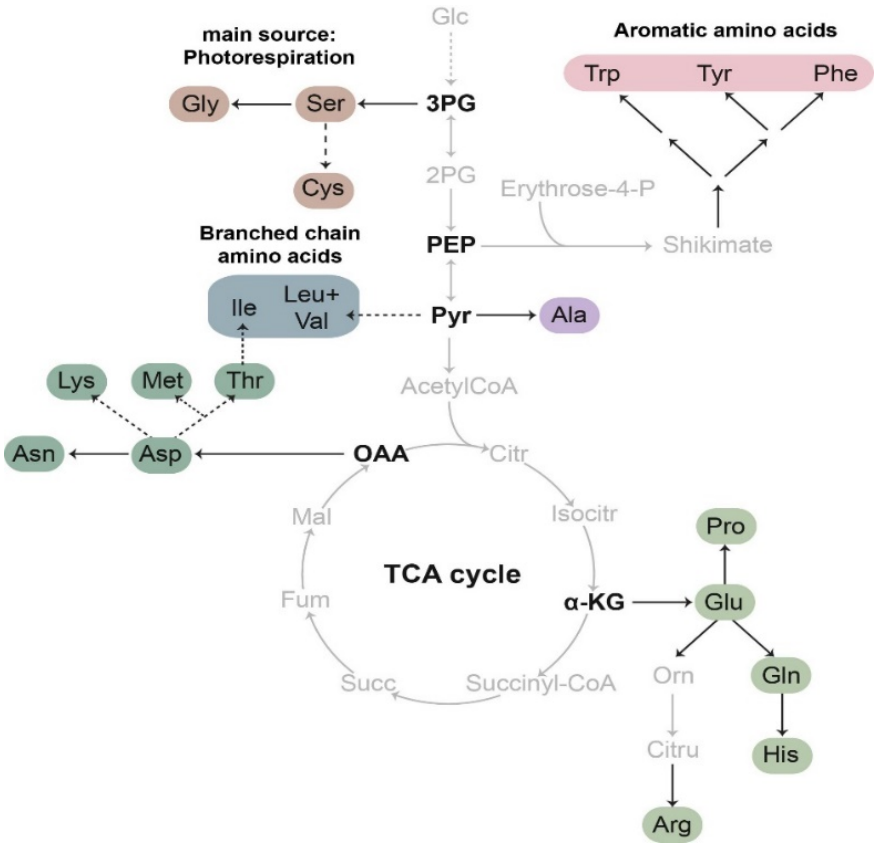

Figure 6. Biosynthesis of amino acids. 3PG, 3-phosphoglycerate, TCA, citric acid cycle; Gly, glycine; Ser, serine; Cys, cysteine; Leu, leucine; Val, valine; Thr, threonine; Met, methionine; Lys, lysine; Asn, asparagine; Asp, aspartic acid; Trp, tryptophan; Tyr, tyrosine; Phe, phenylalanine; Ala, alanine; Glc, glucose; PEP, phosphoenolpyruvate; Pyr, pyruvate; Pro, proline; Glu, glutamic acid; Gln, glutamine; Arg, arginine; His, histidine (Hildebrandt *et al*., 2015).

1.2.4 Fatty acids

Fatty acids are constituents of glycerol-containing lipids or glycerolipids, sphingolipids and extracellular lipids in plants. Lipid variety is attributed to the particular combination of polar head groups and hydrophobic fatty acid structures (Thelen & Ohlrogge, 2002). Although, more than 450 fatty acids are identified in plants, only five fatty acids occur in membrane lipids, including phospholipids and galactolipids and

triacylglycerols that include palmitic acid (16:0), stearic acid (18:0), oleic acid (18:1), linoleic acid (18:2), and α-linolenic acid (18:3) (Ohlrogge *et al*., 2018).

Fatty acids are known to have several functions. They act as structural constituents of membranes, serving as an energy storage and form of carbon, and acting in signal transduction networks. In addition, fatty acids is recognized for their inference in the evolutionary achievement of terrestrial habitats by plants as mediators of interactions with arbuscular mycorrhizal fungi and as constituents of the plant cuticle, which protects plants from water loss. All these functions in plant lipids is due to the extreme structural and compositional diversity of fatty acids in plant lipids (Cahoonm & Li-Beisson, 2020).

In plants, the reactions of fatty acid synthesis are located in plastids, which are plant-specific organelles bound by an envelope double membrane. Priming and elongation of nascent acyl chains requires acetyl- and malonyl-CoA, respectively, as direct precursors (Figure 7). The fatty acid synthase machinery is similar to prokaryotes in that the enzymatic components are separable polypeptides rather than large multifunctional polypeptides as found in animals and fungi. For the synthesis of fatty acids, the first desaturation step for fatty acids is catalyzed by a plastidial stearoyl-acyl carrier protein desaturase. Termination of plastidial fatty acid chain elongation is catalyzed by acyl-ACP thioesterases, which hydrolyze acyl chains from plastidial stearoyl-acyl carrier protein. After termination, free fatty acids are activated to CoA esters, exported from the plastid, and assembled into glycerolipids at the endoplasmic reticulum. In addition, further modifications (desaturation, hydroxylation, elongation, etc.) occur in the endoplasmic reticulum (Thelen & Ohlrogge, 2002).

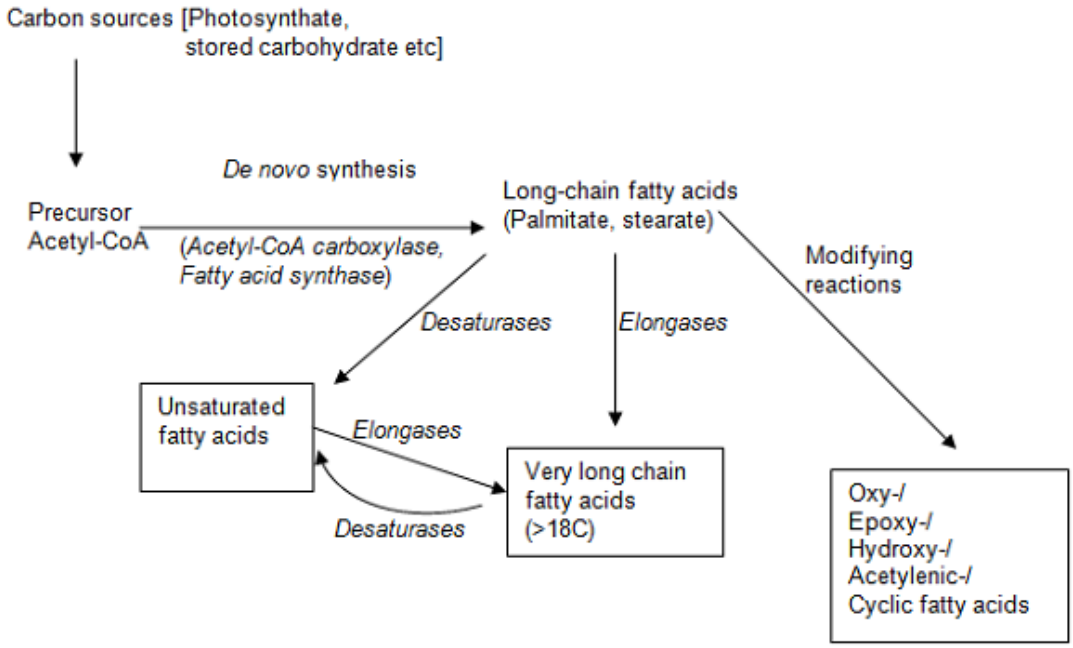

Figure 7. Biosynthesis of fatty acids (Thelen & Ohlrogge, 2002).

In addition, fatty acid-derived volatiles, responsible for aldehyde, alcohol and ester accumulation, the last being the predominant class of aromatic compounds in fruits of several species. Some fatty acids are precursor of flavor compounds in tomato such cis-3-hexenol, 1-hexanol, hexanal, cis-3-hexenal, and 2-nonena (Wang *et al*., 2001). In tomato, C6 volatiles are synthesized from the polyunsaturated fatty acids linoleic acid (C18:2) and linolenic acid (C18:3) through the successive action of the enzymes lipoxygenase (LOX), hydroperoxide lyase (HPL) and alcohol dehydrogenase (ADH). Fatty acid desaturase 7 (FAD7) is an omega-3 FAD that desaturates linoleic acid (C18:2) to generate linolenic acid (C18:3) (Li *et al*., 2018) (Figure 8).

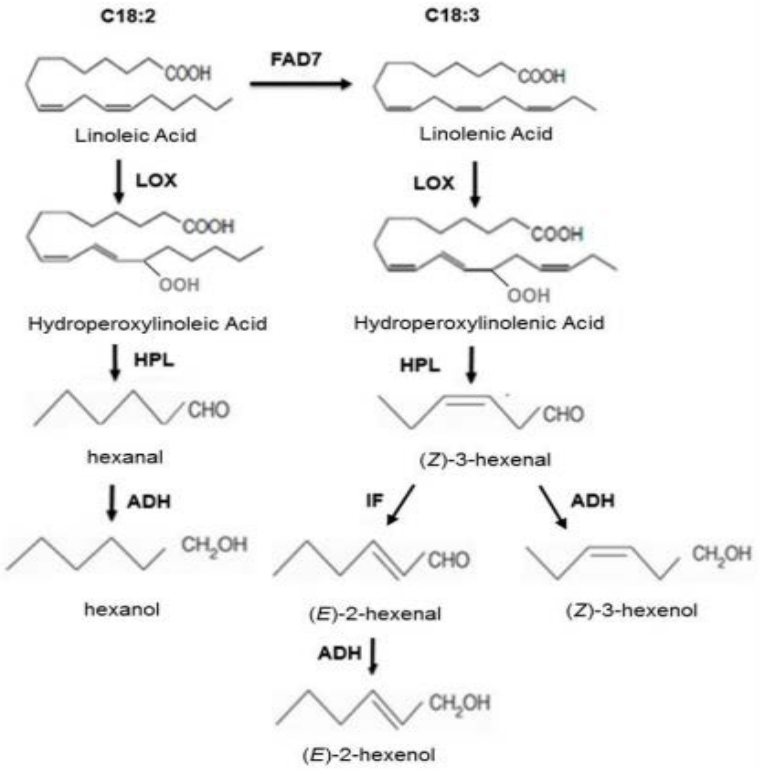

Figure 8: Synthesis of C6 volatiles from polyunsaturated fatty acids in tomato. LOX, lipoxygenase; HPL, hydroperoxide lyase; ADH, alcohol dehydrogenase; FAD7, fatty acid desaturase 7 (Li *et al*., 2018).

1.3 Secondary metabolite profiling of tomato

1.3.1 Carotenoids

Tomatoes provide a variety of nutrients such as antioxidants that contribute to the prevention of chronic diseases. Tomato contains higher amounts of carotenoids such as lycopene, β-carotene and lutein. Lycopene is a type of metabolite with antioxidant properties, which is beneficial in reducing the incidence of some chronic diseases like cancer and many other cardiovascular disorders. β-Carotene is an effective dietary precursor of vitamin A, its deficiency can lead to xerophthalmia, blindness and premature

death (Mayne, 1996). Furthermore, they are essential components of some pigment-protein complexes and are precursors of abscisic acid (Parry *et al*., 1990).

Other carotenoids are associated to alleviate age-related diseases when consumed in adequate amounts in the diet, possibly due to their potent properties as antioxidants (Mordi, 1993). For instance, zeaxanthin and lutein are related to protection against macular degeneration, while lycopene is associated to reduce incidence of prostate cancer when high ingestion of tomato fruits is included in the diet (Giovannucci, 1999; Seddon *et al*., 1994). Tomato sauce decreases the amount of DNA damage in white blood cells and prostate tissues of people with prostate cancer (Chen *et al*., 2001). As the tomato fruit is a source of lycopene, its accumulation in tomatoes has received attention, with the aim of raising its levels through genetic manipulation or plant breeding.

In addition, carotenoids are also important to the color of tomato fruit and is responsible for acting as attractants to pollinators and for seed dispersal. Visible changes in tomato involves degreening and accumulation of carotenoids. This phenomenon is associated with a chloroplast to chromoplast transition (Egea *et al*., 2011) (Figure 9).

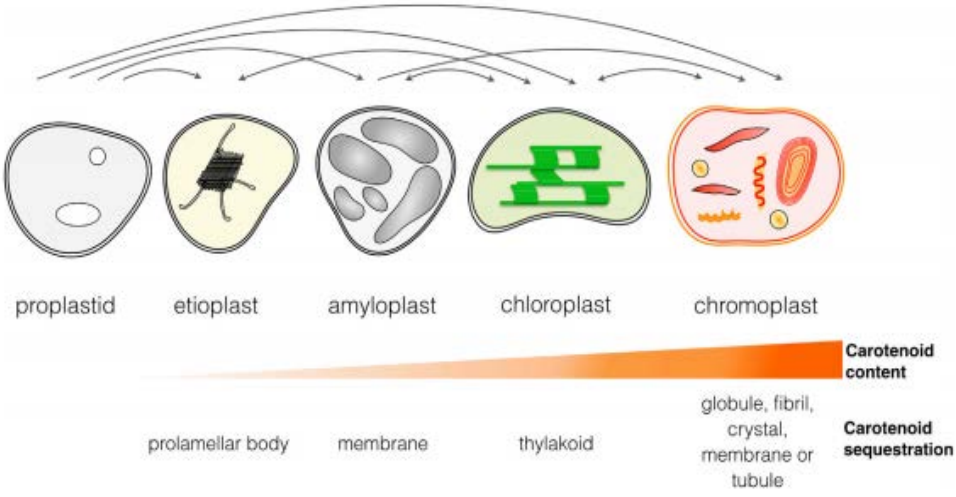

Figure 9. Type of plastid and its relationship to carotenoid accumulation (Sun *et al*., 2018).

Carotenoids are metabolites that are common to photosynthetic tissues such as lycopene, β-carotene and xanthophylls. Carotenoids participate in light harvesting in photosynthetic membranes and protect the photosynthetic apparatus from excessive light energy by quenching triplet chlorophylls, superoxide anion radicals and singlet oxygen in the chloroplast (Niyogi, 1999).

Carotenoid biosynthesis depends on the supply of building blocks, isopentenyl diphosphate (IPP), and its isomer dimethylallyl diphosphate (DMAPP), in higher plants. Plants have two distinct routes for IPP and DMAPP biosynthesis: the mevalonic acid (MVA) pathway in the cytosol and the methylerythritol 4-phosphate (MEP) pathway in plastids. The reaction chains of carotenoid biosynthesis occur in plastids, and carotenogenesis is dependent on precursors produced via MEP pathway. Furthermore, the MEP pathway is also associated with the synthesis of isoprene, diterpenes, the side chains of the chlorophylls, other key photosynthesis-related compounds (plastoquinones, phylloquinones, and tocopherols), hormones (gibberellins, ABA, and strigolactones), and monoterpenes (Figure 10).

The biosynthesis of linear C40 lycopene from geranylgeranyl diphosphate (GGPP), carotenoid precursor, is formed by the condensation of three isopentenyl diphosphate and one dimethylallyl diphosphate molecules. At the beginning of carotenoid biosynthesis is the condensation of two GGPP to produce phytoene that is catalyzed by phytoene synthase. Subsequently, lycopene is formed from phytoene by a series of desaturation and isomerization reactions catalyzed by phytoene desaturase, ζ-carotene desaturase, ζ-carotene isomerase and carotenoid isomerase. Lycopene ε-cyclase and lycopene β-cyclase are involved in cyclization of lycopene which gives rise to α-carotene and β-carotene identified as orange pigments (Pott *et al*., 2019; Sun *et al*., 2018).

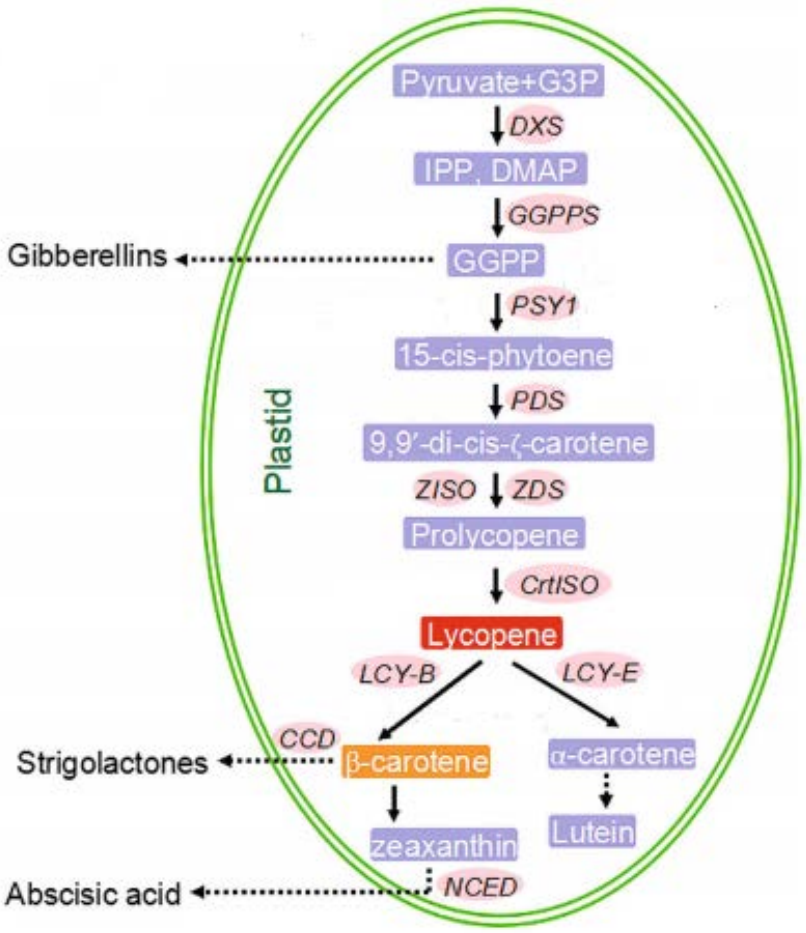

Figure 10. Biosynthesis of carotenoids from MEP pathway. CCD, carotenoid cleavage dioxygenases; CrtISO, carotene isomerase; DMAPP, dimethylallyl diphosphate; DXS, 1-deoxy-d-xylulose 5-phosphate synthase; G3P, glyceraldehyde 3- phosphate; GGPP, geranylgeranyl diphosphate; GGPPS, geranylgeranyl pyrophosphate synthase; IPP, isopentenyl diphosphate; LCY-B, lycopene b-cyclase; LCY-E, lycopene ε-cyclase; NCED, 9-cis-expoxycarotenoid dioxygenases; PDS, phytoene desaturase; PSY1, phytoene synthase; ZDS, z-carotene desaturase; ZISO, z-carotene isomerase (Adapted from Liu *et al*., 2015).

In addition, apocarotenoids are metabolites formed by oxidative cleavage of carotenoid cleavage dioxygenases and non-enzymatic cleavage of carotenoid molecules between the C9 and C10 position. These metabolites include phytohormones and volatile

compounds such as α- and β-ionone, 6-methyl-5-hepten-2-one, and geranylacetone. The volatiles compounds from carotenoid pathway play an important role in the aroma not only in tomato but also in melon and apricot (Figure 11) (Pott *et al*., 2019; Wang *et al*., 2019; Tieman *et al*., 2017).

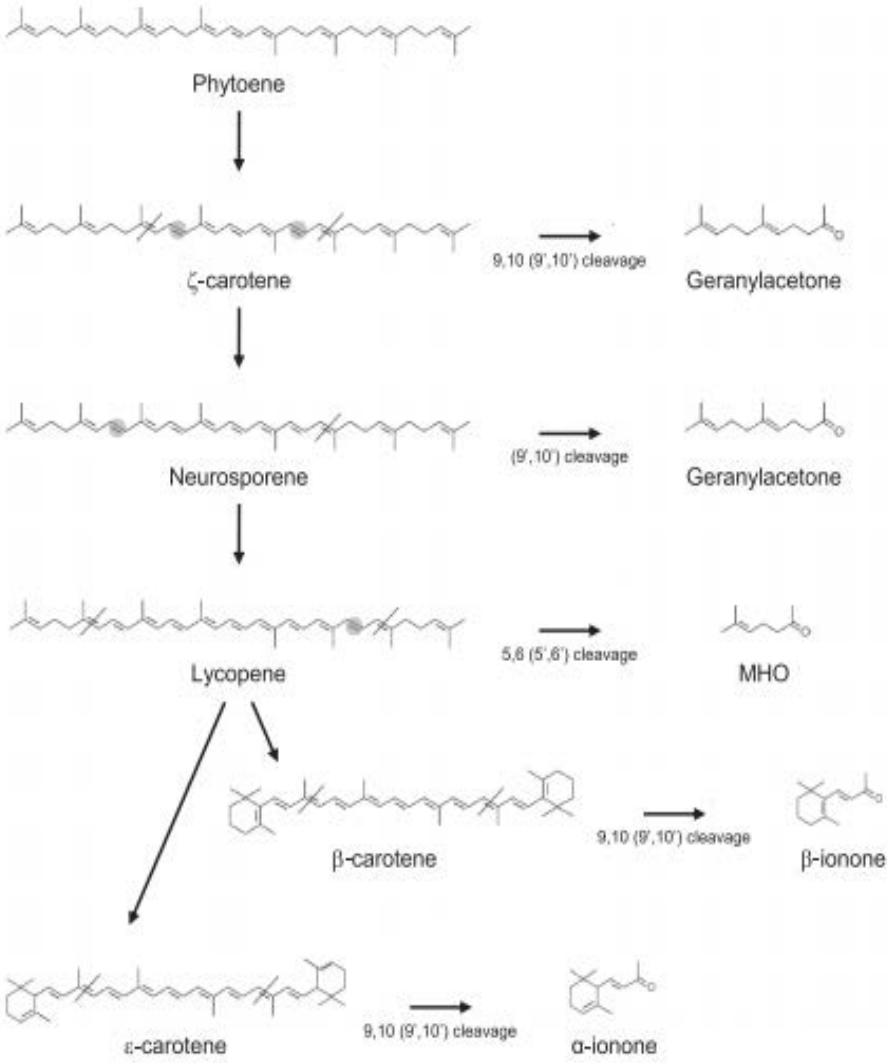

Figure 11. Biosynthesis of volatiles compounds from carotenoid pathway. MHO, 6-methyl-5-hepten-2-one. (Adapted from Lashbrooke *et al*., 2013).

1.3.2 Chlorophylls

Chlorophyll is one of the most abundant biological molecules on earth and plays an essential role in global carbon cycling through plant solar energy capture and photosynthetic carbon fixation (Kim *et al*., 2013). Green bacteria presents more than 100 structures of chlorophyll and some of them are structurally characterized species such as chlorophyll *a*, *b*, *c*, *d*, and *f*. Chlorophyll *a* and *b* are predominate in photosynthetic organisms, and chlorophyll *c*, *d*, and *f* in some microalgae, algae and several classes of photosynthetic bacteria, which differ by only one functional side group attached to the tetrapyrrole ring. Phytol is a diterpene alcohol with a long hydrophobic hydrocarbon tail for anchoring chlorophyll to pigment-binding proteins (Lin & Charng, 2021). The absorption properties of chlorophyll depend on the number of conjugated double bounds and the use of different substituents on the pyrrole rings which is responsible for the change of colors and bioactive potential (Zepka *et al*., 2019).

The chlorophylls are used commercially as a food additive, the monitoring of agricultural production and ocean primary productivity. It is well known that the natural color of these molecules are the main economic attribute. However, chlorophylls have drawn attention not only due to their chromatic properties, but also for their potential health benefits. The characteristics of these bioactive compounds are associated to their chemical structure. For instance, the presence of the phytyl chain rises the molecule hydrophobicity and its aggregative properties. In addition, other rearrangements in different chlorophylls can modify the absorption and bioactive properties (Pérez-Gálvez *et al*., 2017). The biological activities of chlorophylls are related to cancer prevention, antioxidant, antimutagenic, antimicrobial and anti-inflammation activities, mutagen trapping, modulation of xenobiotic metabolism and induction of apoptosis (Zepka *et al*., 2019).

Two distinct biochemical pathways generate chlorophyll (Figure 12). The tetrapyrrole biosynthetic pathway produces chlorophyllide, a chlorin moiety synthesized from protoporphyrin after Mg^{2+} incorporation. The MEP metabolic pathway is responsible for the isoprenoid phytol tail of chlorophyll, derived from geranylgeranyl diphosphate (GGPP). Chlorophyll synthase esterifies the hydrophobic hydrocarbon chain of phytyl diphosphate to chlorophyllide. A second, nonplastidic, isoprenoid synthesis pathway, the mevalonate pathway (MEV), also exists in plants. Although pathway intermediates may be exchanged between the MEP and MEV pathways, the majority of isoprenoids are thought to be derived primarily from one pathway or the other (Kim *et al*., 2013).

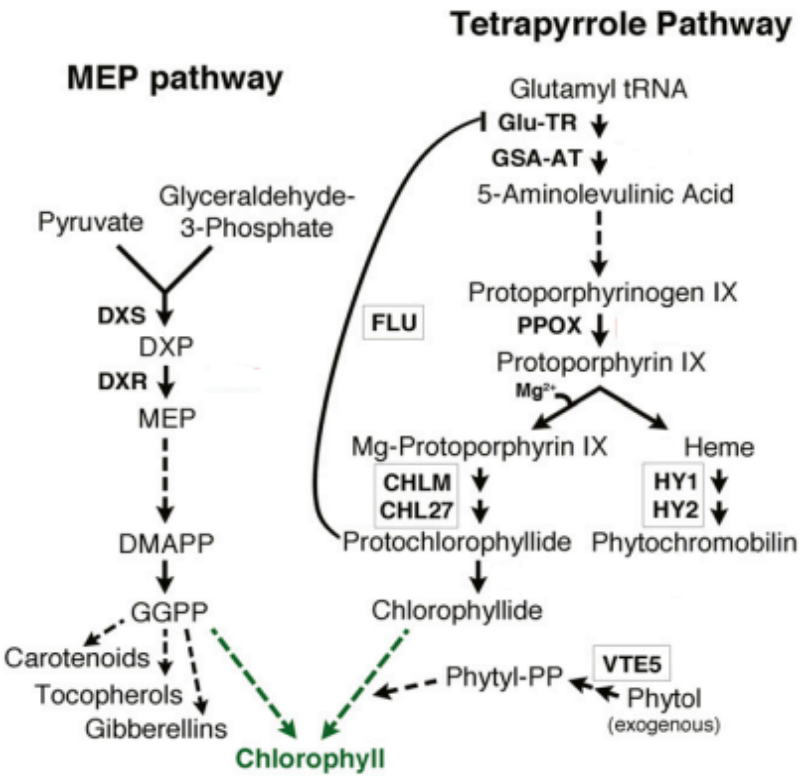

Figure 12. Biosynthesis of chlorophyll from MEP and Tetrapyrrole pathways. DXS, 1-deoxy-d-xylulose 5-phosphate synthase; DXP, deoxyxylulose 5-P synthase; DXR, deoxyxylulose-5-P reductoisomerase; DMAPP, dimethylallyl pyrophosphate;

GGPP, geranylgeranyl pyrophosphate; Glu-TR, glutamyl-tRNA reductase; GSA-AT, glutamate semialdehyde aminotransferase; PPOX, protoporphyrinogen oxidase; CHLM, magnesium-protoporphyrin O-methyltransferase; VTE5, enzyme phosphatidate cytidylyltransferase (Adapted from Kim *et al*., 2013).

1.3.3 Tocopherols

Tocopherols (Vitamin E) are potent lipid soluble antioxidant molecules, synthesized by photosynthetic organisms that reduce free-radical damage to membrane lipids by scavenging peroxyl radicals. Tocopherols can also act as scavengers of singlet oxygen and are responsible for the protection of the photosynthetic apparatus from oxygen toxicity (Almeida *et al*., 2015). Higher doses of vitamin E can be associated to cancer reduction, immune response and cardiovascular benefits. Tocopherols consist of four isoforms, α-, β-, γ-, and δ-tocopherol, differentiated by the number and position of methyl groups on the chromanol ring. Among the tocopherols family, α-tocopherol presents the highest vitamin E activity that are important to human health, which is retained and distributed throughout the human body (Li *et al*., 2010).

For the reason that tocopherols and tocotrienols are amphipathic molecules consisting of a polar chromanol ring and a lipophilic isoprenyl tail, these molecules are considered to be important antioxidants that scavenge reactive oxygen species and lipid peroxyl radicals in lipophilic environments (Seo *et al*., 2011). Several lines of evidence indicate that tocopherols have an essential function in protecting polyunsaturated fatty acids in membrane lipids and oils against various oxidative stresses, such as seed storage and low temperature (Maeda *et al*., 2008).

Tocopherols are isoprenoid-derived compounds that are synthesized from the condensation of a chromanol ring and a prenyl side-chain from the shikimate and MEP

pathways, respectively (Figure 13). MEP pathway also provides precursors for other plastid isoprenoids, such as chlorophylls and carotenoids. The biosynthesis of tocopherol occur in plastids of the higher plants. Homogentisic acid, derived from shikimate pathway, and phytyldiphosphate (PDP), from the MEP pathway, are responsible for the biosynthesis of tocopherols. Homogentisic acid is formed from the tyrosine aromatic amino acid catabolite phydroxyphenylpyruvate (HPP) by the cytosolic enzyme 4-hydroxyphenylpyruvate dioxygenase (HPPD). Condensation of homogentisic acid and phytyldiphosphate is catalyzed by homogentisate phytyltransferases (VTE2). The product of this reaction, 2-methyl-6-phytylbenzoquinol (MPBQ), is the first phytylquinol intermediate in the pathway and can be methylated to 2,3-dimethyl-6-phytyl-1, 4-benzoquinol (DMPBQ) by MPBQ methyltransferase (VTE3). Both MPBQ and DMPBQ are substrates for tocopherol cyclase (VTE1) that result in δ- and γ-tocopherol, respectively. Both δ- and γ-tocopherol can be methylated by γ-tocopherol methyltransferase (VTE4) to form β- and α-tocopherol, respectively (Li *et al*., 2010).

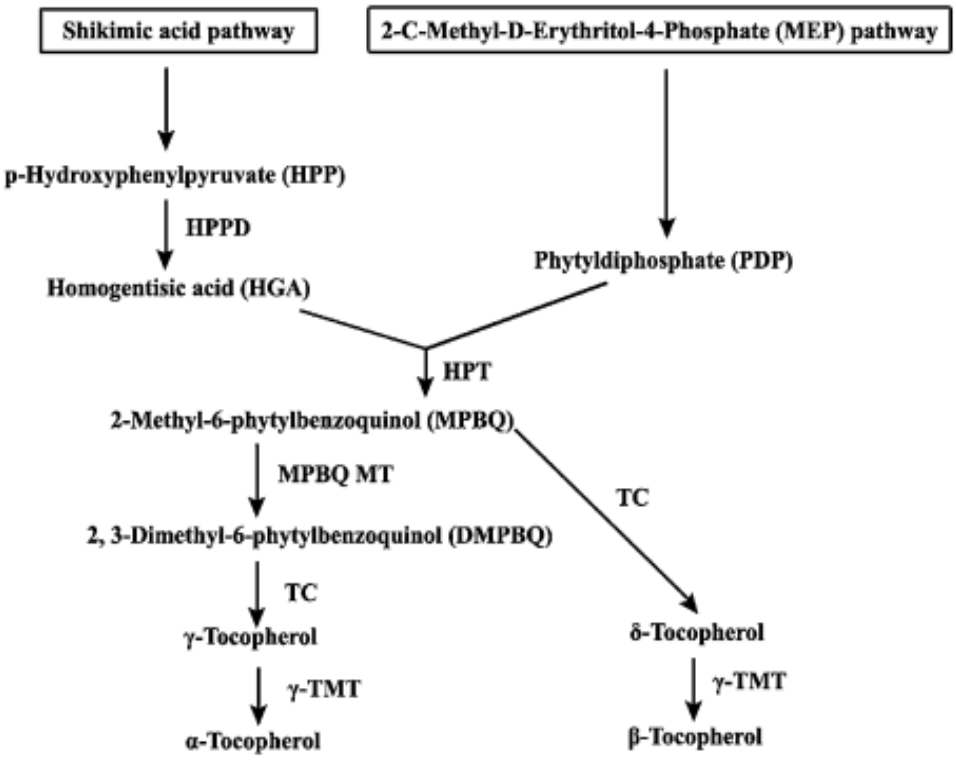

Figure 13. Biosynthesis of tocopherols from shikimic acid and MEP pathways. HPPD, 4-hydroxyphenylpyruvate dioxygenase; HPT, homogentisate phytyltransferase; MPBQ MT, 2-methyl-6-phytyl-1,4-benzoquinone methyltransferase; TC, tocopherol cyclase; γ-TMT, γ-tocopherol methyltransferase (Li *et al*., 2010).

1.3.4 Phytosterols

Phytosterols are characteristic metabolites present in the cell walls of the plants. Phytosterols are divided into 24-methylsterols and 24-ethylsterols in accordance to different alkyl groups at the chiral center C-24. Around 250 types of phytosterols have been found in plants; however, β-sitosterol (29 carbons), stigmasterol (28 carbons) and campesterol (28 carbons) are the most abundant in most plants. Campesterol and β-sitosterol present a methyl and ethyl group at C-24, respectively and stigmasterol an additional double bond at C-22. Besides their major importance as membrane components, phytosterols serve as precursors for brassinosteroids, signalling molecules

involved in growth and development. Tomatoes present around 5 mg/100 g of total phytosterols, which include β-sitosterol, campesterol, stigmasterol, sitostanol and campestanol (Valitova *et al*., 2016).

Phytosterols are members of the triterpene family: they are composed of a tetracyclic structure and a side chain in position C-17. The structure of phytosterols is highly similar to the structure of cholesterol, which is by far the most abundant sterol found in animal cells. Plant cells also contain cholesterol, though its levels generally remain under 1% of total sterols. Unlike animal cells, several hundreds of different sterols have been identified in plant cells. These differ primarily in the substitutions in C-4 as well as C-24 on the side chain, unsaturation of the side chain and rings, as well as conjugation of the C-3 alcoholic hydroxyl group with not only fatty acids but also phenolic acids and carbohydrates (Moreau *et al*., 2018).

In addition to these three most common plant sterols more than 250 different plant sterols have been reported. Sterols can occur in the “free” unbound OH sterol lipid class. Sterols can also occur as conjugates when the OH group is covalently bound via an ester bond or via a glycosidic linkage to glucose and occasionally other sugars. The four common types of sterol conjugate sterol lipid classes are sterol esters (esterified to fatty acids), hydroxycinnamate sterol esters (mainly esterified to ferulic or p-coumaric acid), steryl glucosides and acylated sterol glucosides (Piironen *et al*., 2000).

Phytosterols in plants are produced by a bifurcated sterol biosynthetic pathway involving a common precursor, mainly via cycloartenol pathway (Figure 14). The first step is the mevalonate (MVA) pathway shared in plants, fungi and animals, which converts acetyl-CoA into squalene with mevalonate as an intermediate to generate isoprenoids. Even though MEP pathway has also been found in higher plants to produce

isoprenoids, studies have confirmed sterols are generated via MVA pathway (Vranová *et al*., 2013).

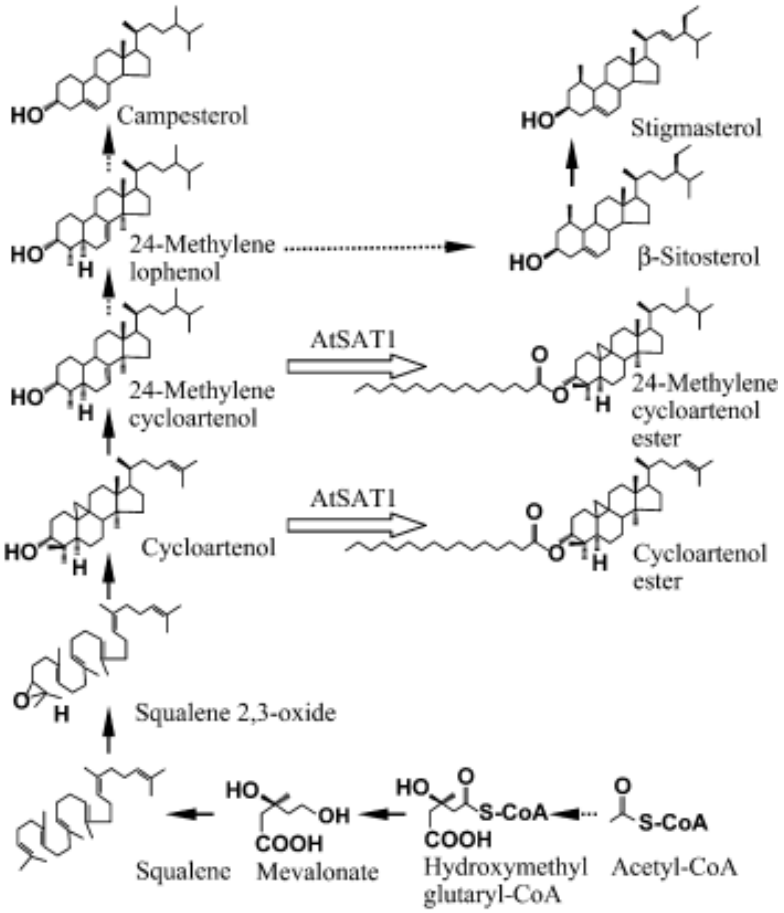

Figure 14. Biosynthesis of phytosterols from mevalonate (MVA) pathway (Chen *et al*., 2007).

Cycloartenol is the first tetracyclic sterol precursors in plant sterol biosynthesis pathway and is considered an intermediate in the phytosterol biosynthesis pathway equivalent to lanosterol in yeast and animal systems. After cycloartenol, several major biochemical steps including methyltransferase, demethylation, and desaturation reactions need to take place before the final products of β-sitosterol and campesterol can be formed. The elevated expression of AtSAT1 could enhance the esterification of cycloartenol, thereby removing cycloartenol from the main stream of sterol biosynthetic pathway into

the formation of esters. For one thing, campesterol, a 24-methylsterol generated from 24-methylene cholesterol is the precursor of brassinosteroid. For another, β-sitosterol, a 24-ethylsterol, is a bifunctional enzyme, catalyzing both Δ24 (28) isomerization and Δ24 (25) reduction in brassinosteroid biosynthesis pathway. Then, β-sitosterol transforms in stigmasterol via CYP710A (Chen *et al*., 2007).

The consumption of phytosterols can decrease intestinal cholesterol absorption, prompting a reduction in plasma total and LDL cholesterol levels. Plants stanols have been showed to be more effective in reducing cholesterol absorption than sterols. The hypocholesterolemic effects of phytosterols have been known since about 1950, when a fall of about 27% was observed in the plasma cholesterol levels of 26 healthy subjects supplemented with 5–10 g/day of phytosterols, for two weeks. Other studies revealed that doses over 10 g/day of phytosterols taken for 3–5 weeks can reduce blood cholesterol concentrations around to 20% (Thompson & Grundy, 2005). However, Marangoni & Poli (2010) demonstrated that the consumption of 1.6–2 g/day incorporated in foods were enough to decrease cholesterol absorption from the gut by 30%, and plasma LDL cholesterol concentration by 8–10%, being an effective cholesterol-lowering agents. These metabolites are able to decrease cholesterol absorption from the gut due to their structural similarity with cholesterol. In the last years, phytosterols were purified and added in several foods, aiming to achieve functional properties with hypocholesterolemic activity (Marangoni & Poli, 2010).

1.4 Pre-harvest treatment of tomatoes

1.4.1 Salinity stress

Salinity has been reported to affect fruit quality by inducing metabolic changes in response to the stressful condition (Fanciullino *et al*., 2014; Rouphael *et al*., 2018). Plants have developed several strategies to counteract the rise in salt concentration, including the accumulation of osmotically active metabolites and specific secondary metabolites, but the effect of salinity can vary depending on the species or varieties studied as well as on the salt concentration (Albaladejo *et al*., 2017).

Recently, Quinet *et al*. (2019) summarized some recent studies regarding the impact of abiotic stress on primary and secondary metabolism of tomato fruits and concluded that understanding tomato responses to salt stress and how it behaves regarding accumulation of metabolites is crucial to maximize productivity and fruit quality. One of the problems limiting progress in developing tomatoes containing high levels of health-promoting compounds is genetic erosion, which reflects the loss of variation in crops due to the modernization of agriculture (D'Esposito *et al*., 2017).

Recently, Zhu *et al*. (2018) illustrated how breeding changed the tomato fruit metabolome. Plant breeding was long ago carried out by farmers who selected for specific adaptation traits leading to the generation of traditional varieties, but later on they were replaced by a small number of cultivars in modern plant breeding, being the plant genetic sources for food more genetically vulnerable than ever before (Dwivedi *et al*., 2017). Because of their nearer genetic proximity to modern cultivars than their wild relatives, landraces or traditional varieties, originated by adaptive responses to local habitats, are considered a valuable resource for many traits of agronomic interest as well as traits of fruit nutritional quality (Gascuel *et al*., 2017; Moles *et al*., 2019).

From a physiological point of view, plants have a limited supply of essential resources, which they must split among different competing physiological functions. This multiple use of limited resources creates resources allocation trade-offs (Munns *et al*.,

2020). When the level of salt stress induces reductions in plant growth and fruit yield, such resources could be invested in a greater degree for metabolites synthesis. Therefore, changes in the metabolome might be different depending on the stress levels. Massaretto *et al*., (2018) investigated the changes in the metabolome induced by high salt stress level (100 mM NaCl), where fruit yield was significantly reduced but fruit quality was improved. From an agronomic point of view, the improvement of fruit quality in response to salt stress should be achieved without affecting fruit yield by using moderate salt stress levels. Currently, salt stress can be applied in a controlled manner in greenhouses, which may represent an innovative strategy for enhancement of the crop product quality (Toscano *et al*., 2019).

1.5 Post-harvest treatment of tomatoes

1.5.1 Ethylene

Many of the physiological changes described in fruit ripening section are under the severe control of ethylene hormone, which is autocatalytically, produced during climacteric ripening. Ethylene is a gaseous plant hormone that plays an important role in various physiological processes during plant growth, including a considerable metabolic changes during the ripening of climacteric fruits (Lin *et al*., 2009; Bapat *et al*., 2010). In a commercial point of view, the control of fruit ripening is frequently reached through early harvest, by controlling the post-harvest storage atmosphere and by genetic selection for slow or late ripening varieties (Matas *et al*., 2009).

Climacteric and non-climacteric fruits demonstrate similar modifications in several metabolic processes, which yield in color alterations, sugar accumulation, texture change, fruit softening and volatiles compounds production. However, the distinction of

climacteric and non-climacteric fruits is not determined by phylogeny (Giovannoni, 2004). The climacteric fruits as tomato show a rise in the rate of respiration at the beginning of ripening concomitant with the increased biosynthesis of ethylene (Paul *et al*., 2012).

Even though ethylene is important for the development of ripening attributes in climacteric fruit, it is not obligatory for the metabolic changes in non-climacteric fruits such as grape, citrus, and strawberry (Oeller *et al*., 1991; Giovannoni, 2004; Bapat *et al*., 2010). These considerations have raised a remarkable discussion on ethylene-dependent and -independent processes during fruit ripening. Substantial information has begun to emerge on ethylene regulation of the ripening process in climacteric fruits (Pnueli *et al*., 1994; Cara and Giovannoni, 2008; Bapat *et al*., 2010).

The biochemical pathway of ethylene synthesis in plants starts from methionine, which is then S-adenosylated to form S-adenosylmethionine by S-adenosylmethionine synthetase (Figure 15). S-adenosylmethionine is subsequently metabolized to 1-aminocyclopropane-1-carboxylic acid (ACC) and 5-methylthioadenosine (MTA) by ACC synthase (ACS). ACC, the first committed intermediate in the ethylene biosynthesis pathway, is transformed to ethylene by ACC oxidase (ACO). MTA, the other product of ACS reaction, enters the methionine cycle. Genes encoding ACS and ACO were first identified from zucchini and tomato fruit, respectively. Even though numerous genes involved in methionine metabolism show altered expression during plant development and in response to the environment, typically activities of ACS and in some cases ACO are generally considered the rate-limiting steps in ethylene production in fruit. ACS and ACO belong to a family of genes that have been characterized in tomato, melon, apple, banana, pear, kiwifruit, peach, and persimmon (Cara and Giovannoni, 2008; Lin *et al*., 2009; Bapat *et al*., 2010),

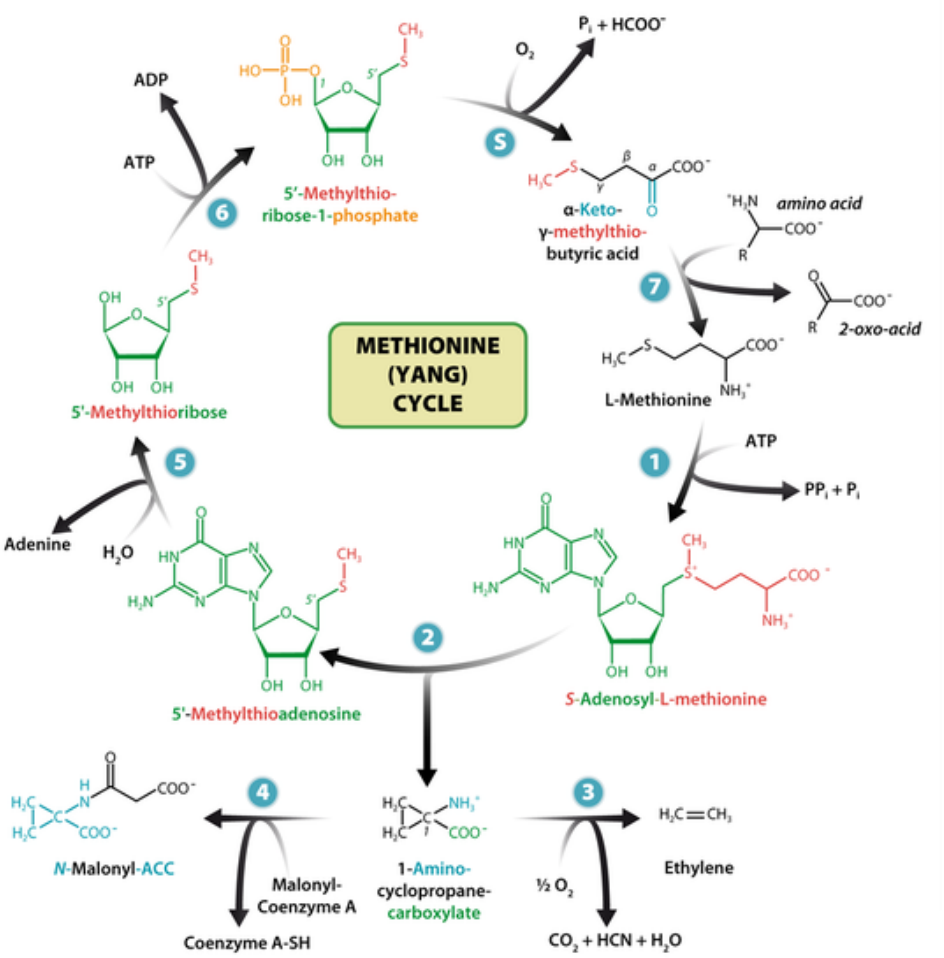

Figure 15. Biosynthesis of ethylene from methionine (Yang e Hoffman, 1984; Karlova *et al.*, 2014).

The members in each gene family are differentially expressed during development and in response to environmental cues (Cara and Giovannoni, 2008; Lin *et al.*, 2009). Nine ACS and five ACO genes were identified in tomato such as ACS (SlACS1A, SlACS2, SlACS4, and SlACS6) and ACO (SlACO1, SlACO3, and SlACO4) members are differentially expressed during tomato fruit development. SlACS6 is principally expressed in the green fruit and is related with the production of ethylene during the early stages of fruit development. SlACS1A is similarly expressed in green fruit but at lower concentrations than SlACS6. SlACS1A and SlACS4 are induced along

side ripening transition and proposed to be responsible for the induction of SlACS2, a gene implicated in the autocatalytic production of ethylene during the ripening process. The autocatalytic ethylene production may exert negative feedback inhibition on early ethylene production by reducing the expression of SlACS1A and SlACS6 (Handa *et al*., 2012).

Ethylene signaling involves a unique pathway that consists of the following main steps: (i) ethylene is perceived by an ethylene receptor complex at the endoplasmic reticulum (ER) membrane; (ii) ethylene detection triggers cleavage of a key protein in the complex, ETHYLENE-INSENSITIVE2 (EIN2); (iii) the cleaved soluble portion of EIN2 is involved in repressing the translation of two regulatory F-box proteins, which would otherwise target two master transcription factors for degradation by the 26S proteasome; and (iv) rapid stabilization of the two transcription factors results in the regulation of gene expression (Chang, 2016). The pathway relies heavily on negative regulation and post-translational controls. For example, as explained below, the ethylene receptors repress responses when no ethylene is detected (as opposed to activating responses when ethylene is detected), and the repression of ethylene responses involves protein phosphorylation and protein turnover (Figure 16).

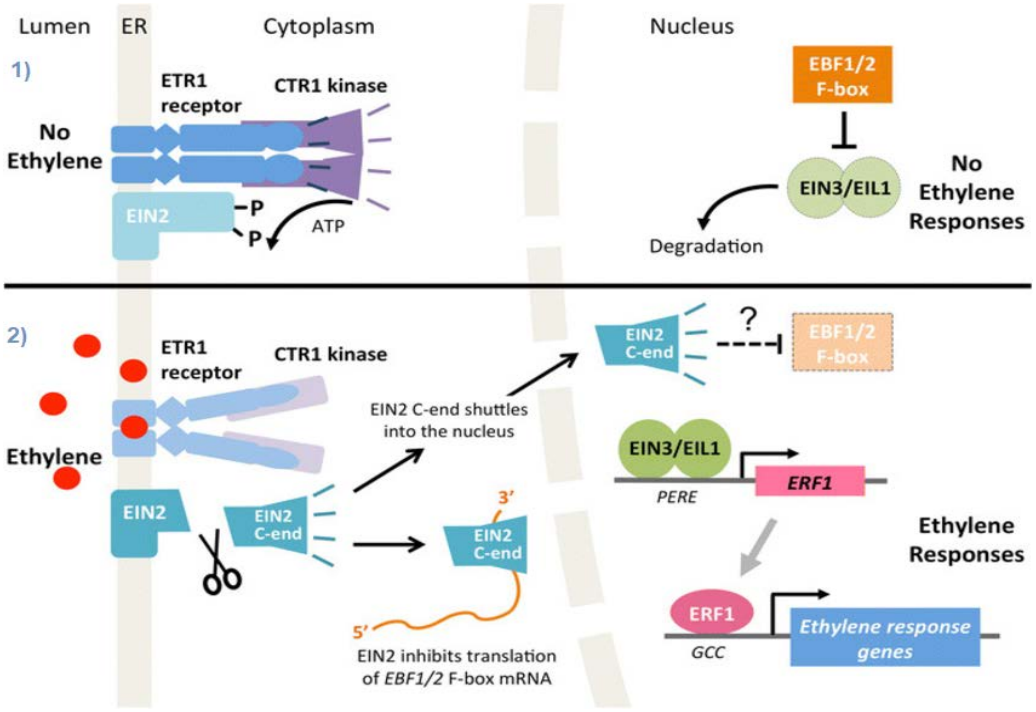

Figure 16. Pathway of ethylene-signaling in the absence of ethylene signal (1) and when ethylene is detected (2). ETR1, receptors of ehtylene; CTR1, protein kinase; EIN2, ethylene insensitive; F-box (EBF1/2), protein; EIL, EIN3-like protein; ERF1, ethylene response factor (Chang, 2016).

1.5.2 1-Methylcyclopropene

The 1-methylcyclopropene (C4H6) is an important inhibitor of ethylene action and is successfully employed in postharvest ripening control of climacteric fruits. The high responsiveness of climacteric fruits to 1-methylcyclopropene is due to the ethylene perception requirement for the initiation and progression of fruit ripening (Zhang *et al.*, 2009), and it has been applied to prolong the shel-life of tomato (Guillén *et al.*, 2007).

The use of 1-methylcyclopropene, an ethylene antagonist, can retain firmness, delay lycopene production and consequently color development of tomato fruits. However, the main effect of tomato exposure to 1-methylcyclopropene is the delay in respiration and ethylene production rates during ripening, consequently delaying ripening

and ripening related processes, such as softening, color change, starch breakdown (Li *et al*., 2016).

The 1-methylcyclopropene is one of the most frequently employed cyclopropenes for vegetable preservation, since it is stable at room temperature and active at low concentrations, without being toxic. This antagonist of ethylene has been an alternative growth regulator in the conservation of plant products, but its effect is dependent on the genotype, application interval, concentration and maturity stage at time of application (Blankenship & Dole, 2003).

This simple organic compound probably acts as a competitor of ethylene, blocking its access to the ethylene-binding receptor . The 1-methylcyclopropene not only blocks the response of basal level of ethylene, but also the auto-induced production of ethylene (Figure 17). Auto-inductive ethylene production, a response of ethylene itself, means triggered production of ethylene in presence of ethylene itself due to its promotory effect on two most important ethylene biosynthesizing enzymes such as ACC-synthase (ACS) and ACC oxidase (ACO). In this way, 1-methylcyclopropene treatment can be considered as one of the most efficient ways in managing various ethylene-mediated responses in fruits, vegetables, and flowers (Watkins, 2006; Guillen *et al*., 2006; Paul & Pandey, 2013).

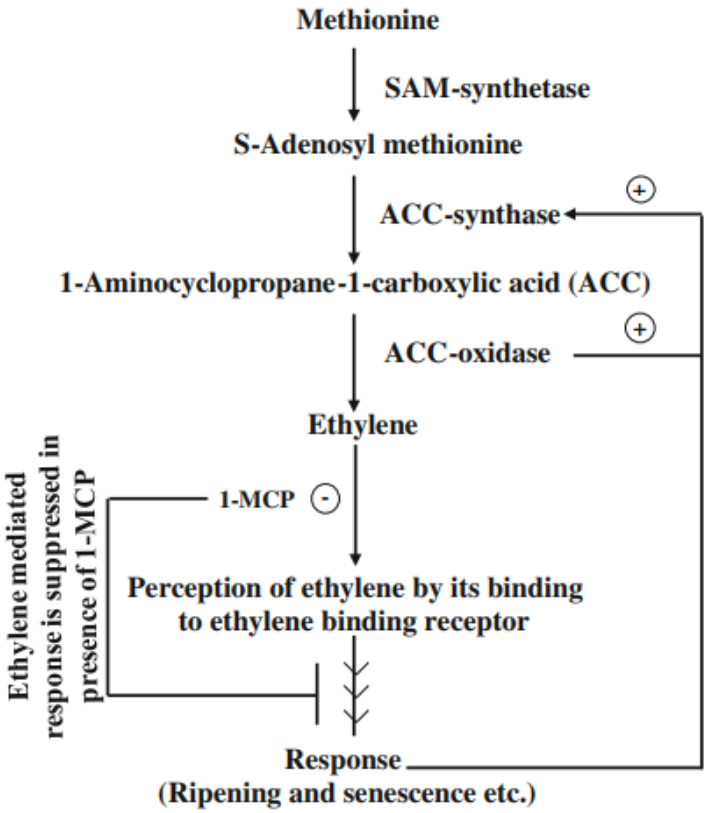

Figure 17. Action of 1-methylciclopropene on the ethylene biosynthesis. 1-methylciclopropene blocks the receptor of the ethylene and thereby its action. (-) means inhibition or suppression, (↓) means blockage of action pathway in presence of 1-methylciclopropene and (+) means induction in presence of ethylene and in absence of 1-methylciclopropene (Paul & Pandey, 2013).

1.5.3 Methyl jasmonate

Jasmonates such as jasmonic acid and its methyl ester, methyl jasmonate, have been suggested as plant growth regulators and have been demonstrated to elicit defense responses from the plant as well as antibacterial and antifungal activities. Moreover, jasmonates can promote biological activity through its interaction with other phytohormones. Application of exogenous jasmonate stimulate climacteric tomato fruits ripening by increasing ethylene and respiration rates, and changing metabolites profile

remarkable by chlorophyll degradation, β-carotene synthesis and aroma compounds production. The changes directly affect fruit color, texture and flavor (Fan, Mattheis, & Fellman, 1998).

Jasmonates play an important role in signaling plant defenses and interact with other phytohormones in prompting biological activity. Although exogenous application of jasmonate affects ethylene production and other ripening-related processes, a role for endogenous jasmonates in regulating climacteric fruit ripening has not been established. Moreover, little is known about the impacts of jasmonates on the biosynthesis of ehtylene enzymes, specifically, 1-aminocyclopropane-1- carboxylic acid (ACC) oxidase and ACC synthase (Zhai *et al*., 2017).

The study the role of jasmonates in fruits is extremely important due to their considerable levels found in fruits, and their involvement in the regulation of ripening-related processes, as well as the relationship of jasmonates and ethylene in regulating a number of plant physiological processes. Exogenous jasmonate hormone modulate the biosynthesis of ethylene in a developmental- and concentration dependent technique and increase the endogenous jasmonate levels during the initiation of climacteric ripening, indicating a role for jasmonates during the onset of climacteric fruit ripening (Fan, Mattheis, & Fellman, 1998).

The biosynthesis of jasmonates happens through octadecanoid pathway, in which translocation of lipid intermediates takes place from chloroplast membranes into cytoplasm and before into peroxisomes (Figure 18). The jasmonate biosynthesis starts from the oxidation of α-linolenic acid (C18:3) released from chloroplast membranes by the action of phospholipases (PLD). In chloroplast membrane, linolenic acid is transformed into 12-oxophytodienoic acid (OPDA) in three steps. In the subsequent dioxygenation steps, a chloroplastic 13-lipoxygenases (LOXs; 13-LOX and 9-LOX)

oxidize α-linolenic acid (α-LA) and generate the 13-hydroperoxy derivative of LA hydroperoxy octadecadienoic acids. Subsequently, 13-hydroperoxy is dehydrated with the support of enzyme allene oxide synthase (AOS; EC 4.2.1.92) into the 12-oxo phytodienoic acid (12-OPDA), also identified as allene oxide. In the peroxisome, oxidation of exported OPDA to 3-oxo-2-2′(Z)-pentenyl)-cyclopentane-1-octanoic acid (OPC:8) is executed by the enzyme OPDA reductase (OPR; EC 1.3.1.4). Finally, the carbon side chain from the precursor molecule is shortened through three cycles of β-oxidation in the peroxisome. Three of LOXs specifically, LOX2, LOX3 and LOX4 have been reported in *Arabidopsis thaliana* genome. Both AOS and AOC are chloroplastic enzymes. However, OPDA is exported from chloroplast to persoxisome and potentially involves the carrier COMATOSE1/PEROXIMAL 1/PEROXISOME ABC TRANSPORTER (ABC CTS1/PXA1/ PED3) (Per *et al*.,2018).

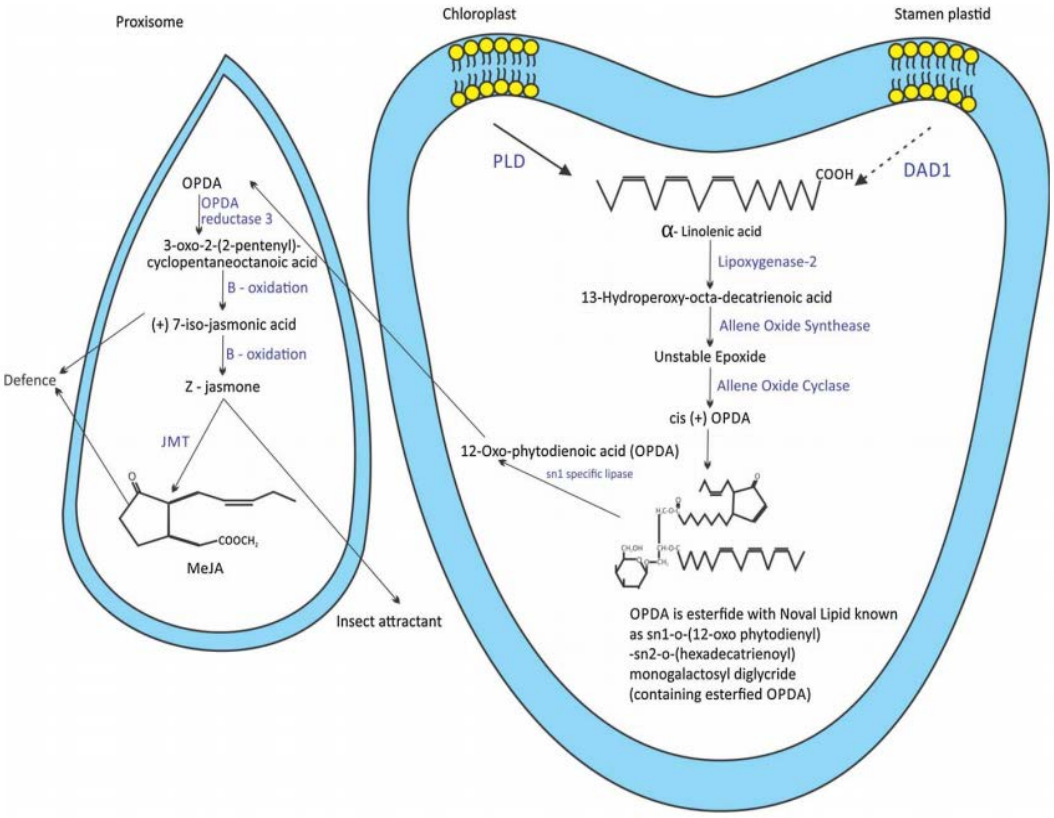

Figure 18. Biosynthesis of methyl jasmonate from α-linolenic acid. PLD, phospholipase; OPDA, 12-oxophytodienoic acid; JMT, jasmonic acid carboxyl methyltransferase; MeJA, methyl jasmonate (Per *et al*., 2018).

Jasmonate can be metabolized into a diversity of products that include the methyl ester (MeJA), amide conjugates with amino acids, conjugate with sugars and hydroxylated and didehydro derivatives. Several plant species have been used to reproduce the proteins and enzymes required for jasmonate biosynthesis and perception and some of them have also been crystallized such as 13-AOS, AOC, ACYL-CoA-OXIDASE1, JAR1, 13-LOX, OPR3 and the SCFCOI1–JAZ-coreceptor complex (Kombrink, 2012).

2. JUSTIFICATIVE AND STRUCTURE OF THE THESIS

This topic has as objective to explain the structure of this work. The results of the thesis has been divided into three sections, which described the pre-harvest (**Section 1**) and post-harvest treatments (**Section 2 and 3**) applied in tomato fruit.

Tomato is one of the most important horticultural crops worldwide, being the second horticultural crop produced in the world after potato and the first one in yield terms (FAOSTAT, 2018). Tomato contains metabolites that play important role in human health as well as lycopene, a type of carotenoid with antioxidant properties, which is beneficial in reducing the incidence of some chronic diseases like cancer and many other cardiovascular disorders. Due to the importance of tomato fruit for human health given its so high consumption per capita, the identification of tomato varieties able to exhibit higher accumulation of primary and secondary metabolites in their fruits is currently a priority objective (Hou *et al*., 2020; Rowland *et al*., 2020).

In this context, pre- and post-harvests treatments can be applied with the objective to improve fruit quality and nutritional value of tomato fruit by increasing primary and secondary metabolites. However, its known that post-harvest qualities of tomatoes are dependent not only on post-harvest handling and treatment methods but also on many preharvest factors such as genetic and environmental conditions. Many post-harvest quality losses are result of many preharvest factors such as salinity stress (Massaretto *et al*., 2018). However, the use of moderate salt concentration in irrigation can reduce the losses in fruit yield and moisture content of fruit, for example. It is therefore important to know the pre-harvest factors that can produce superior qualities in fruits during harvest whilst using appropriate postharvest handling and treatment methods to maintain the quality after harvest. Therefore, on **Section 1** we demonstrated that the use of moderate salt concentration (50 mM NaCl) can improve fruit quality and nutritional

value by increasing in amino acid and α-tocopherol contents while fruit yield was maintained.

Recently, Quinet *et al*. (2019) summarized some recent studies regarding the impact of abiotic stress on primary and secondary metabolism of tomato fruits, and concluded that understanding tomato responses to salt stress and how it behaves regarding accumulation of metabolites is crucial to maximize productivity and fruit quality. The global consequences of the rapidly changing climate make it clear that the environment is gradually becoming unsuitable for crop growth and development. Soil salinity is among the characteristics being altered by climate change, accounting for remarkable global reductions in crop growth and productivity (Shrivastava and Kumar, 2015). Therefore, improving plant tolerance to salinity stress is one of the current major objectives in achieving sustainable agriculture. This requires an understanding of the inherent mechanisms in plants that enable continued survival and growth when subjected to changes in soil salinity.

In the following sections, we emphasis the knowledge about post-harvest treatment of tomato as well as the exogenous use of phytohormones. In the **Section 2**, it was verified that exogenous application of ethylene and methyl jasmonate in Grape tomato can stimulate primary and secondary metabolites responsible for fruit quality and nutritional value. Treatment with ethylene showed increased mainly fructose, sucrose and glucose during ripening; while methyl jasmonate improved several metabolites such as citric and malic acids, and some amino acids responsible for taste and aroma, and led to accumulation of lycopene.

However, by physiological point of view, it is interesting to know if the action of exogenous methyl jasmonate in accumulate metabolites during ripening was or not dependent of endogenous ethylene. Thus, in the **Section 3**, we described the changes in

the metabolite profiling in tomato with ethylene receptor blocked by 1-methylcyclopropene. Another treatment with both 1-methylcyclopropene and methyl jasmonate was conducted to observe if an exogenous methyl jasmonate can improve the levels of metabolites in fruits with ethylene receptors blocked. Thus, fruits treated with both 1-methylcyclopropene and methyl jasmonate exhibited a lower impact in the production of metabolites than fruit treated only with 1-methylcyclopropene, mainly at 4 and 10 day after harvest. However, this treatment showed a tendency to improve the levels of carotenoids, tocopherols and phytosterols at 21 day after harvest.

3. OBJECTIVES

3.1 General objectives

Evaluating the changes of metabolite profiling of tomato *(Solanum lycopersicum)* exposed to post-harvest and pre-harvest treatments during ripening.

3.2 Specific objectives

- Analyzing the changes in primary and secondary metabolism of traditional tomato landraces growing under salinity stress;

- Analyzing the effect of exogenous ethylene and methyl jasmonate on primary and secondary metabolite profile of grape tomato fruit during ripening;

- Analyzing the effect of exogenous methyl jasmonate on primary and secondary metabolite profile of grape tomato fruit with ethylene inhibited by 1-methylciclopropene during ripening

4. MATERIAL AND METHODS

4.1 Plant Material and treatments

4.1.1 Tomatoes and pre-harvest treatment

The tomato traditional varieties or landraces ‘Tomate Pimiento’ ('TP') and ‘Muchamiel Aperado’ ('MA') were selected due to their peculiar fruit morphology. The seeds of 'TP' and 'MA' were supplied by the Agroecology Network of the Region of Murcia (RAERM). The commercial cultivar ‘Moneymaker’ (‘MM’) was used as reference of the assays.

Seeds were germinated in seedbeds in darkness, in a 2:1 (v/v) mixture of peat:perlite, at 28ºC temperature and 90% relative humidity (RH), placed in a controlled conditions growth chamber. After emergence, seedlings were grown in the same growth chamber with environmental conditions of 18-25ºC temperature, 50-70% RH, with a photoperiod of 16 h light/8 h darkness. A photosynthetic photon flux (400-700 nm) of 345 µmol m^{-2} s^{-1} at the plant level was provided by fluorescent tubes (Luminux daily light 58W and Fluora 58W, Osram, Madrid, Spain). During this period plants were irrigated daily with half-strength Hoagland solution (Hoagland and Arnon, 1950).

A spring-summer culture was carried out in a greenhouse located at the campus of the University of Murcia (Espinardo, Region of Murcia, Spain), which offered controlled culture conditions. At the 4^{th}-leaf developmental stage (30 days after sowing) plants were transplanted to the greenhouse; fourteen plants per variety were grown in plastic pots containing 18 L of a peat/perlite mixture in a 2:1 volume ratio. The fertigation solution (Hoagland solution) was prepared in 2000 L tanks with local irrigation water (EC=0.9 dSm^{-1}), and pH and EC was regularly monitored. Fertigation of plants was

achieved through a drip irrigation system, with 3 L h^{-1} drippers. At the 6^{th}-leaf stage fertigation solution supplemented with 50 mM NaCl was applied to seven plants of each tomato variety for 80 days, while the other seven plants per variety were irrigated without salt (control condition). Temperature and RH were daily registered and they daily oscillated between 30ºC-15ºC and 40%-60% (day/night), respectively. A complete randomized design was used with seven plants per variety for each treatment (0 and 50 mM NaCl).

Just at the beginning of salt treatment plant height, number of leaves and chlorophylls content and fluorescence were determined to check homogeneity of plants. The last two physiological parameters were determined as described in García-Abellán *et al*. (2015).

To determine fruit yield and its two components, fruit number and fruit weight, ripe fruits from 1^{st} to 3^{rd} truss of each plant were collected, weighed and counted. Harvest index was also estimated by weighting shoot weight of plants at the end of the campaign.

For analysis of fruit quality standard parameters (soluble solids content and titratable acidity), red ripe (RR) fruits at commercial stage were harvested. Together with these fruits, mature-green (MG) fruits that have reached their final size were also harvested for mineral and metabolites analyses. Material from MG and RR fruits were cut up, frozen and homogenized in liquid N_2 and stored at -80ºC until analyses were done. One part of frozen material was lyophilized for analysis and water content. For each variety, fruit stage and treatment three biological replicates of 10 fruits each were analysed.

4.1.2 Tomatoes and post-harvest treatment

Mature green tomato (*Solanum lycopersicum* cv. Grape) fruits (N = 1200) with uniform size and without injury were collected from a commercial standard greenhouse in Ibiúna (23°39'21" S; 47°13'22" W), São Paulo, Brazil, and transported on ice to the postharvsest facilities. Fruits were washed by tap water and drained at room temperature after sterilization with 0.1 % sodium hypochlorite aqueous solution during 15 minutes. Four biological replicates were applied in the experiment and each of them were composed by 100 fruits. Grape tomato fruits were randomly separated into five groups (N = 400 by group): 1) control group (CTRL), without any treatment; 2) treated ethylene group (ETHY); 3) treated methyl jasmonate group (MeJA); 4) treated 1-methylciclopropene group (MCP); and 5) treated 1-methylciclopropene and methyl jasmonate group (MCP+MeJA). Ethylene treatments were performed using a 100 ppm ethylene gaseous hormone for 24 hours. Methyl jasmonate solution (100 ppm) was applied in a filter paper left on the wall of the chamber for evaporation. For the MCP treatment, it was followed the instructions of the manufacturer for “manual addition”: 2.45 g of 1-methylcyclopropene (powder, 3.3% w/w active ingredient, Smart-Fresh, AgroFresh Inc., Philadelphia, USA) was weighted and transferred to a 500 mL Erlenmeyer flask, capped with a rubber stopper. Using a syringe, 75 mL of deionized water was added to the flask, dissolving the powder and releasing the 1-methylcyclopropene gas. The flask was placed in the chamber, the stopper was removed and, immediately, the chamber was closed. A small fan was placed in the chamber, directed to the flask to aid the dispersion of the gas. For the MCP+MeJA group, methyl jasmonate (Sigma-Aldrich, Saint Louis, USA) was applied to a filter paper left on the chamber wall for evaporation (100 ppm, final concentration in gas phase), and 1-methylcyclopropene treatment was made as described above. Treatments were conducted for the second time 12 h after the first exposure to the hormone, totaling 24 h of treatment. During the experiments, fruits

were left to ripen spontaneously in a 323 L chamber at constant temperature (20 ± 2 ºC) and humidity (80 ± 5% RH) in a 16-hour-day/8-hour-night cycle.Samples of 10 fruits from each replicate were randomly taken at 4 (breaker), 10 (red) and 21 (postharvest) days after harvest (DAH), considering the control group as reference. Samples were frozen in liquid nitrogen and stored at -80 °C for subsequent analyses.

4.2 Soluble Solids Content and Titratable Acidity Determinations

For soluble solids content (SSC) analysis, an aliquot of the frozen tomato fruit samples was thawed and filtered through a nylon membrane filter. The supernatant was collected and used to measure SSC using a refractometer with automatic temperature compensation (ATAGO PR-101 digital, Tokyo, Japan) and the result was expressed as °Brix at 20°C. Titratable acidity (TA) was determined from the juice obtained, in triplicate, by taking 5 g of the thawed fruit mesocarp homogenate and adding 45 mL of distilled water, followed by pH titration with 0.1 M NaOH up to pH 8.1 and the result was expressed as grams of citric acid per 100 g of fresh weight (%). Fruit quality indexes such as Maturation Index (SSC/TA ratio) and Brix Yield index (SSC x fruit yield) were calculated.

4.3 Ethylene emission

Ethylene emission was performed by placed five Grape tomato fruits in airtight glass containers of 600 mL at 25 °C for 1h. Then, five samples of 1 mL of gas produced in the headspace were collected with gastight syringes through a rubber septum. A gas chromatography with a flame-ionization detector (GC-FID) (Agilent Technologies, HP-6890) and HP-Plot Q column (30 m x 0.53 mm x 40 µm) were used to evaluate ethylene emission. Temperatures of injector and detector were equally established at 250 °C, and

the oven at 30 °C. The helium gas flow was set at 1 $mL.min^{-1}$ and the injections were performed in pulsed splitless mode.

4.4 Fruit surface color

Fruit surface color measure was conducted as described by Fabi *et al.* (2007). The colorimeter HunterLab ColorQuest XE instrument (Hunter Associates Laboratories) measure in terms of *L**, *a** and *b** space. The experimental data were treated to obtain values of °hue angle. Three measurements were made at equator of six Grape tomato fruits.

4.5 Analysis of Cations by ICP-OES

The fruit samples were dried lyophilized, milled to powder and digested during 24 h in a concentrated HNO_3: $HClO_4$ (2:1 v/v) solution. Na^+, K^+, Ca^{2+} and Mg^{2+} contents were determined by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) in an ICAP 6500 DUO/IRIS Intrepid II XLD equipment (Thermo Scientific, Waltham, MA, USA). The ICP-OES analysis was carried out at the Ionomics Platform of CEBAS-CSIC (Murcia, Spain).

4.6 Analysis of metabolite profiling

4.6.1 Primary metabolites by ^{1}H-nuclear magnetic resonance (NMR) spectroscopy analysis

The components of primary metabolism were analyzed as described by Choi *et al*. (2004, 2006) with slight modifications. One mL H_2O:MeOH 1:1 (v/v) was added to 50 mg of lyophilized fruit sample and vortexed for 1 min, sonicated for 1 min and

subsequently centrifuged (11,000 x *g* at 4°C for 20 min). The supernatant was collected in a 2 mL microtube and dried with a rotary vacuum evaporator. The dried extract was reconstituted in 800 µL of a D_2O phosphate buffer (100 mM KH_2PO_4, pH 6.0) containing 0.01% of TSP (0.58 mM trimethyl silyl propionic acid sodium salt) as internal standard and vortexed for 1 min. The mixture was centrifuged (16,100 x *g* at 4°C for 5 min) and 600 µL of the supernatant was transferred to an NMR tube for further analysis. All ^1H NMR spectra were recorded at 298 K on a Bruker AVIII HD 500 NMR spectrometer (500.13 MHz for ^1H) equipped with a 5 mm CPP BBO cryogenic probe (Bruker Biospin, Germany). ^1H spectra were referenced to TSP signal (δ = 0.00 ppm), whereas ^{13}C spectra were referenced to CH-1 resonance of α-D-glucose (δ = 93.10 ppm). For each sample, 32 scans were recorded with the following parameters: 0.126 Hz/point, pulse width = 4.0 µs (30°) and relaxation delay =1.0 s. FIDs were Fourier transformed with LB=0.5 HZ, GB=0, and PC=1.0 and peak integral was used for quantitative analysis. The whole peak intensities in every 0.02 ppm in ^1H NMR spectra in the range of δ 0.30–12.0 were used as variables. ^1H NMR spectra were manually corrected for phase and baseline distortions using TOPSPIN (v3.2, Bruker Biospin). Peak-fitting on the resulting spectra was performed using a computer algorithm associated with Chenomx NMR Suite 8.1 software to generate concentrations of primary metabolites detected in plant material (Chenomx, Edmonton, AB, Canada). The region δ = 4.67-5.15 was discarded to eliminate the effects of imperfect water presaturation. The spectral areas of all buckets were normalized to the weight of extracts employed for measurements. The intensities of the selected ^1H resonances due to hydro-alcoholic metabolites were measured with respect to the intensity of TSP signal used as internal standard with a concentration of 0.58 mM. The metabolites analysis was performed at the Metabolomics Platform of CEBAS-CSIC (Murcia, Spain).

4.6.2 Primary and secondary metabolites by GC-MS analysis

4.6.2.1 Extraction and derivatization of polar metabolites

The extraction and drivatization of polar metabolites were conducted as described by Lisec *et al.* (2006). For the extraction process 100 mg of frozen pericarp powder was mixed with an 100% distilled methanol at –20 °C (1400 μL) and ribitol (200 $\mu g.mL^{-1}$, internal standard) (60 μL). The mixture was vortexed, incubated in a thermomixer at 950 rpm for 10 min at 70 °C, centrifugated at 11000 g for 10 min, and the supernatant collected. In the upper phase was added chloroform at –20 °C (750 μL) and Milli-Q water (1500 μL), following of mixture and centrifiguation at 2200 g for 15 min. The upper hydrophilic phase (150 μL) were collected and dried under nitrogen gas The derivatization of samples consisted in the addition of 20 $mg.mL^{-1}$ metoxyamine hydrochloride (Sigma–Aldrich Chemical Co., St. Louis, MO, USA) (40 μL) and pyridine with subsequent incubation in an orbital shaker at 1000 rpm and 37 °C for 2 h. Consecutive, N-methyl-N-(trimethylsilyl) tri-fluoroacetamide (MSTFA) (70 μL) was added to the sample and incubated in an orbital shaker at 1000 g and 37 °C for 30 min. Finally, the derivatized samples were moved into glass vials and run on the GC-MS. A pool of polar metabolite external standards (1 $mg.mL^{-1}$, Sigma–Aldrich) was applied in order to certify the identified metabolites by mass spectra comparison: D-glucose; D-fructose; maltose; sucrose; D-galactose; myo-inositol; citric acid; L-alanine; L-serine; L-proline; L-aspartate; L-glutamate (Kind *et al.*, 2009).

4.6.2.2 Extraction and derivatization of non-polar metabolites

Non-polar metabolite extraction and derivatization was performed based on the methods previously described by Bligh & Dyer (1959), Ichihara *et al.* (2010) and Fiehn *et al.* (2000). For the extraction process 1000 mg of frozen pericarp powder was mixed with chloroform (1250 μL), methanol (2500 μL), n-tridecane (800 $\mu g.mL^{-1}$, internal standard)(20 μL), following of vortex for 10 s and incubation on ice for 30 min. Then 1.5% sodium sulfate (1250 μL) and chloroform (1250 μL) were added to the mixture, incubated on ice for 5 min and centrifugated at 4 °C for 1000 g and 15 min. The upper polar phase was collected and dried under nitrogen gas. The sample was redissolved in hexane (1000 μL), toluene (200 μL), methanol (1500 μL) and 8% cloridric acid (300 μL), mixed for 10 s and incubated for 1.5h at 100 °C. After that, hexane (1000 μL) and Milli-Q water was added to the sample and mixed. The hexane phase was separated and dried under nitrogen gas. The sample was redissolved in hexane (80 μL) and pyridine (20 μL), and derivateized with MSTFA (40 μL). Finally, the derivatized samples were moved into glass vials and run on the GC-MS. A pool of fatty acid methyl esters (FAME) external standards (Sigma–Aldrich) was applied in order to certify the identified metabolites by mass spectra comparison: methyl laurate (C12:0, 0.8 $mg.mL^{-1}$); methyl tetradecanoate (C14:0, 0.8 $mg.mL^{-1}$); methyl palmitate (C16:0, 0.8 $mg.mL^{-1}$); methyl octadecanoate (C18:0, 0.4 $mg.mL^{-1}$); methyl arachidate (C20:0, 0.4 $mg.mL^{-1}$); methyl docosanoate (C22:0, 0.4 $mg.mL^{-1}$); methyl lignocerate (C24:0, 0.4 $mg.mL^{-1}$); methyl linoleate (C 18:2, 0.4 $mg.mL^{-1}$); (Z) -9-oleyl methyl ester (C 18:1, 0.4 $mg.mL^{-1}$); methyl linolenate (C 18:3, 0.4 $mg.mL^{-1}$) and methyl palmitoleate (C 16:1, 0.8 $mg.mL^{-1}$) (Kind *et al.*, 2009).

4.6.2.3 Determination of polar and non-polar metabolites

Derivatized samples were evaluated on a gas chromatography–mass spectrometry (Agilent GC-MS 5977, Agilent Technologies, CA, USA) as described by

Kind *et al.* (2009). Trimethylsilyl (TMS) derivatives analyses on GC-MS followed the protocol described by Kind *et al.* (2009). TMS derivatives (1 μL) was injected into an injector at 230 °C and split-less mode. The oven temperature ramp applied was 80 °C (initial temperature), held for 2 min, heating at 15 °C.min^{-1} to 330 °C and held for 6 min. The electron impact ionization mass spectrometer was setted to: 70 eV of ionization voltage; 250 °C of ion source temperature; 250 °C of injection port temperature; 70–600 m/z at 20 scans.s^{-1} of mass scan range. The column used was a HP5ms column (30 m x 0.25 m x 0.25 μm). The flow rate of helium gas was 2 mL.min^{-1}. Acquisition, deconvolution, and analyses of experimental data were processed by Mass Hunter software (Agilent, CA, EUA). For retention index (RI) comparsion and data validation was used the NIST mass spectral library (NIST 2011, Gaithersburg, MD, USA). Some of the identified metabolites were also confirmed by mass spectral comparison with the authentic external standards previously described.

4.6.3 Secondary metabolites by UHPLC and HPLC analysis

4.6.3.1 Extraction of carotenoids

Extraction of carotenoids was conducted by the method described by Sérino *et al.* (2009). Frozen pericarp powder sample (200 mg) was mixed with 100 μL of 30% NaCl (w:v) solution and 200 μL of dichlorometane, and then vortexed for 1 min. Successively, in the mixture was added 500 μL of hexane:ether (1:1), stirred for 1 min and centrifuged (13000 g at 4 °C for 5 min). The supernatant was collected in a 2 mL microtube. This protocol was repeated three times and the organic phases were pooled together. The remaining hexane phase was evaporated under N_2 atmosphere. The dried carotenoid extract was reconstituted in ethyl acetate for liquid chromatographic analysis.

All sample solutions were filtered through Millex 0.2 μm nylon membrane syringe filters prior to their introduction into the UHPLC or HPLC equipment.

4.6.3.2 Extraction of α-tocopherol

The analysis of α-tocopherol was also carried out by UHPLC. The extraction procedure was performed as described by Almeida *et al*. (2011). First, 250 mg of freshly frozen fruit material was extracted with 750 μL of methanol. The resulting mixture was vortexed for 1 min and then 500 μL of chloroform was added, stirred for another min and incubated on ice for 10 min in darkness. 500 μL of saline Tris buffer (50 mM Tris pH 7.5/1 M NaCl) was added. After the solution was vortexed for 1 min this was centrifuged 3,000 x *g* at 4°C for 5 min. The chloroform phase was recovered and the methanol phase (remaining pellet) was re-extracted with 1 mL of chloroform repeating the above mentioned steps. Chloroform phases were pooled and adjusted to a final volume of 2 mL. The pooled chloroform phase was evaporated under N_2 atmosphere. The dried tocopherol extract was reconstituted in 0.2 ml of injection solvent CH_2Cl_2/MeOH (2:1, v/v) for liquid chromatographic analysis. All sample solutions were filtered through Millex 0.2 μm nylon membrane syringe filters prior to injection into the UHPLC equipment (Millipore, Bedford, MA, USA).

4.6.3.3 UHPLC determination

The dried carotenoid extract was reconstituted in 300 μL with the injection solvent [acetonitrile (ACN)/MeOH (7:3, v/v)]/acetone (6.7:3.3 v/v), for liquid chromatographic analysis. All sample solutions were filtered through Millex 0.2 μm nylon membrane syringe filters prior to injection into the UHPLC equipment (Millipore,

Bedford, MA, USA). UHPLC analysis was carried out using an Acquity I Class Ultra Performance LC system connected to a TUV detector measuring absorbance at 286 and 450 nm (Waters, Milford, MA, USA). UHPLC separations were performed on a reversed-phase column Acquity UPLC C18 BEH 130 Å, 1.7 μm, 2.1 × 100 mm (Waters), using the method described by Rivera *et al*. (2013). Identification was carried out by comparison of retention time values and spectral properties of samples with those from authentic standards, purchased from Sigma Chemicals Co. (St. Louis, MO, USA) and CaroteNature (Lupsingen, Switzerland), and reference spectra. Standard stock solutions of major carotenoids present in fruits of tomato plants were prepared using HPLC-grade ethanol (neoxanthin, violaxanthin and lutein) or hexane (phytoene, β-carotene, lycopene). Before use aliquots of each stock solution were diluted in their respective HPLC-grade solvent and each concentration was determined by UV-VIS absorption at their maximum absorbance wavelengths using the extinction coefficients (ε) described by Rivera *et al*. (2012). Calibration was fulfilled by dose-response curves constructed from the standard solutions.

The tocopherol content was determined using a Waters I-Class HPLC system coupled with a TUV detector. Separation was carried out on a normal-phase column BEH C18 (50 mm, 2.1 mm and 1.7 μm mesh) using an isocratic solvent system (mobile phase) consisting of 60:40 ACN/MeOH (v/v). The column temperature was set at 30°C, the flow rate was 0.5 mL min^{-1} and the total runtime was 5.0 min including column equilibration. Eluting compounds were detected by UV detector at 292 nm wavelength. Identification and quantification of α-tocopherol was achieved by comparison with retention time and peak area of authentic standard purchased from Sigma-Aldrich (St. Louis, MO, USA). Standard stock solution of α-tocopherol was prepared by dissolving 2.54 mg in 1.0 mL

EtOH HPLC-degree. Before use aliquots were diluted in ACN/MeOH (60/40, v/v) and calibration was fulfilled by dose-response curves constructed from the standard solutions.

4.6.3.4 HPLC determination

The HPLC (Infinity 1260 HPLC, Agilent Technologies, USA) was coupled to a diode array detector (DAD) equipped with YMC Carotenoid HPLC C30 (5 µm x 250 mm x 4.6 mm) column under optimized conditions described by Souza *et al.* (2019). Identification was carried out by comparison of retention time values and spectral properties of samples with those from authentic standards, purchased from Sigma–Aldrich: Lycopene, β-carotene and lutein. Calibration was fulfilled by dose-response curves constructed from the standard solutions. Each concentration was determined by calculating the peak area and comparing it to the corresponding calibration curve.

4.6.3.5 Analysis of Chlorophylls

The contents of chlorophylls a and b in fruits were determined by the method of Nagata and Yamashita (1992) using frozen samples. One g of thawed sample was homogenized with 20 mL of acetone:hexane (2:3 v/v) and centrifuged at 3,000 x *g* for 10 min at 4°C. Absorbance (A) at 663 and 645 nm wavelengths was measured using a spectrophotometer. Chlorophyll a and b were calculated according to the equations:

$$Chorophyll\ a\ =\ 0.999\ A663 - 0.0989\ A645$$

$$Chorophyll\ b\ =\ 1.77\ A645 - 0.328\ A663$$

4.7 Statistical Analysis

Experimental data were expressed as mean ± standard deviation (SD) of four biological replicates per day and treatment. Statistical analysis was performed by one-way analysis of variance (ANOVA) and Tukey´s test was applied to establish significant differences among mean values at $P < 0.05$, using the Minitab 19.0 software package. For multivariate analysis, PCA-biplot and heatmap were performed on data matrixes and used to ascertain the overall variability among: cultivars and treatments (for pre-harvest treatment) and days and treatments (for post-harvest treatment). Multivariate analysis was produced using the Metaboanalyst 4.0 server (Chong *et al*., 2018). The raw data obtained by ^{1}H-RMN analysis were normalized by median, processed using generalized log transformation (log 2) and then mean-centered and divided by the square root of deviation of each variable (Pareto scaling). The raw data obtained by GC-MS analysis were normalized by ribitol or n-tridecane (internal polar and non-polar standards, respectively) area, processed using generalized log transformation (log 2) and then mean-centered and divided by the square root of deviation of each variable (Pareto scaling). The univariate analysis of fold change was also performed using the Metaboanalyst 4.0 server to evaluate significant differences among accumulated metabolites in fruits.

5. SECTION 1: EFFECT OF SALINITY STRESS ON METABOLITE PROFILING OF TOMATO

Traditional tomato varieties improve fruit quality without affecting fruit yield under moderate salt stress

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5.1 Objective

Understanding the mechanisms adopted by plants to counteract salt stress, involving both primary and secondary metabolisms, is the key step in order to make use of salt stress as a tool to improve the sensorial and nutritional properties of crops, however its generally is accompanied by productivity losses. Thus, it is very interesting to implement strategies aiming at enhancing fruit quality of tomato by means of growing plants in moderate salt stress that allows for a sustainable fruit yield. Here, agronomic responses and fruit quality traits, including primary and secondary metabolites, were analyzed in fruits of two Mediterranean traditional tomato varieties named “Tomate Pimiento” (“TP”) and “Muchamiel Aperado” (“MA”), with very different fruit shape and size between them, and using as reference the commercial cultivar “Moneymaker” (“MM”). Plants were grown without salt (control) and with moderate salt stress (50 mM NaCl).

5.2 Results

5.2.1 Agronomic response and fruit sensory attributes of traditional vs commercial tomato varieties

The traditional tomato varieties ‘Tomate Pimiento’ ('TP') and ‘Muchamiel Aperado’ ('MA') were selected for the distinct morphology of their fruits; 'TP' fruits are mid-sized and elongated, and similar in shape to bell peppers, whereas 'MA' ones have large ribbed fruits that are pear-shaped with numerous locules. In contrast, the commercial cultivar ‘Moneymaker’ (‘MM’) used as reference has round-shape fruits with two locules (Figure 1A). Vegetative development was similar in 'TP', 'MA', and 'MM' before the application of salt stress (Supplementary Figure S1A); the only difference was

the greater plant height of 'MA' owing to a larger internode distance, although leaf number was similar for the three genotypes (Supplementary Figure S1B). Moreover, leaf chlorophyll content and chlorophyll fluorescence, two important physiologic traits related to salinity tolerance, were similar for 'TP', 'MA', and 'MM' (Supplementary Figure S1B). Given these characteristics, both landraces represent excellent materials to study the response of salt stress at the reproductive level.

Given that salt stress can variably affect the fruit yield of each truss and because the duration of exposure to salt treatment is longer for the upper trusses, fruit yield and its components (fruit number and fruit weight) were estimated for the first three trusses (Figure 1B). Although there was a clear trend of decreasing fruit yield with exposure to moderate salinity (50 mM NaCl), especially in 'MM' and 'MA', there were no significant differences in yield between the control and salt conditions in any genotype. 'MA' had the highest fruit yield because it had the highest fruit weight, which was only significantly affected by salinity at the 3rd truss. Regarding fruit number, neither landrace was affected by salinity at any truss, and in 'MM' it was only significantly reduced at the 3rd truss. These results show that the moderate salt stress applied in our study had a slight effect on the reproductive development of tomato plants without significantly altering fruit yield and harvest index (Figures 1B and 2B). However, harvest index varied across genotypes: 'TP' had the lowest value, which was attributable to its highest vegetative biomass (Figure 2A), whereas the high harvest index of 'MA' was mainly due to its highest fruit yield (Figure 1B).

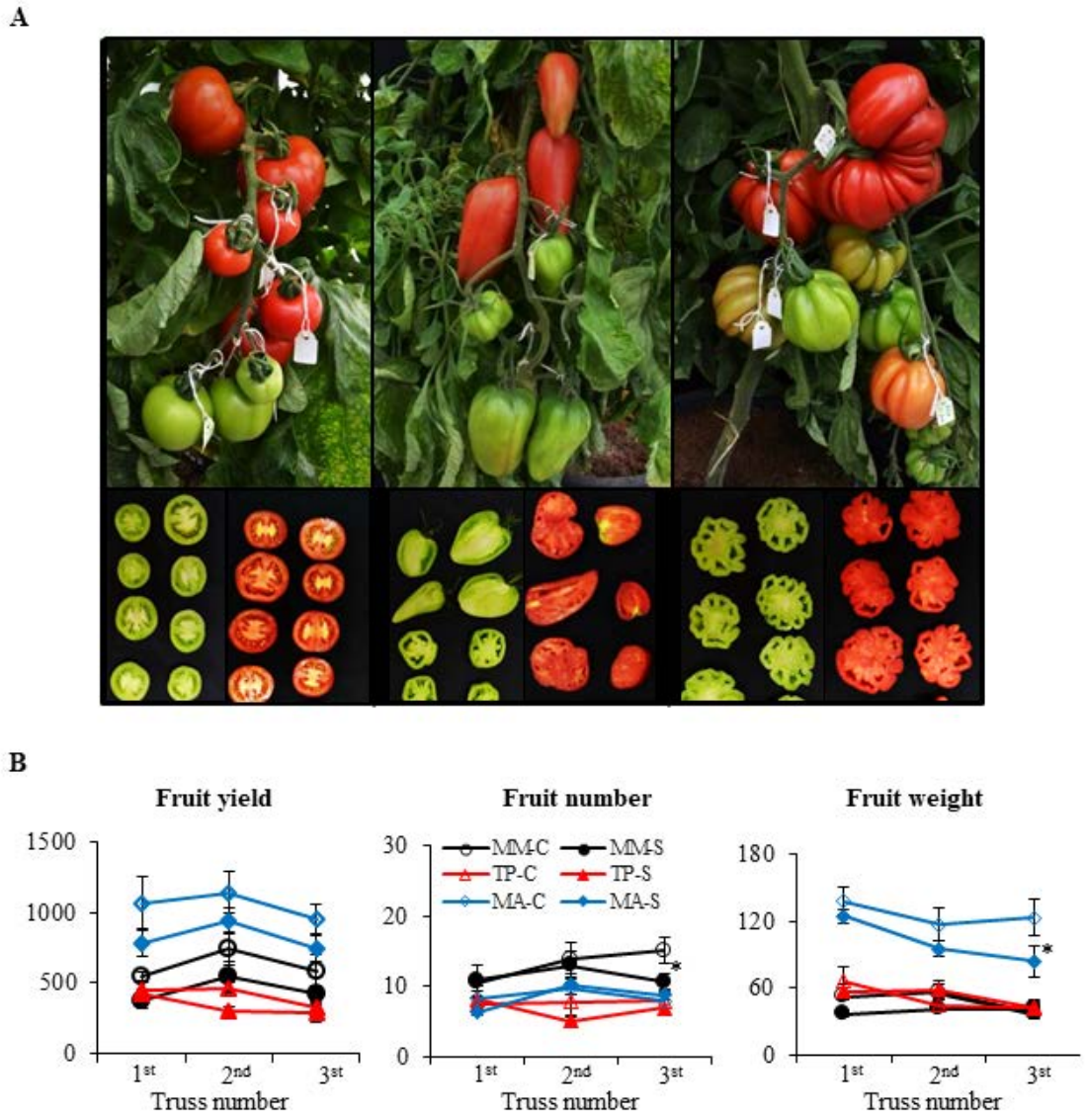


Figure 1. Agronomic response of 'Moneymaker' (MM) and two traditional varieties, 'Tomate Pimiento' (TP) and 'Muchamiel Aperado' (MA). (A) Representative images of the fruits from the first truss showing the different fruit morphological characteristics of both landraces with respect to MM. (B) Fruit yield (g FW), fruit number and fruit weight (g FW) from the first three trusses of plants grown in control (C) and salt (S) for 60, 70 and 80 days of 50 mM NaCl treatment. Values are expressed as means ± SE of seven plants per variety. Asterisks indicate significant differences between mean values of control and salt treatment for the same genotype at each truss (Student's *t*-test, $p<0.05$).

Regarding fruit quality parameters, SSC was significantly higher in RR fruits of 'TP' but significantly lower in RR fruits of 'MA' compared to MM under control treatment (Figure 2C). Salt stress increased SSC in RR fruits of both traditional varieties relative to the control treatment, whereas in 'MM' SSC values were similar under both conditions. TA was lower in RR fruits of both traditional varieties compared to the value in 'MM', with the difference being even greater under salt stress (Figure 2D). Interestingly, the maturation index (MI; i.e., SCC/TA ratio) was higher in both traditional varieties than in 'MM' under control as well as salt treatment (Figure 2E). To better quantify fruit quality we calculated BY, a composite index representing the weight of SSC per plant that has been used as a measure of commercial quality. BY was strikingly high in 'MA' (Figure 2F), with similar values in fruits obtained from control and salt-stressed plants. Thus, both traditional tomato varieties have similar fruit characteristics including a high MI, but differ in SSC and BY (elevated in 'TP' and 'MA', respectively).

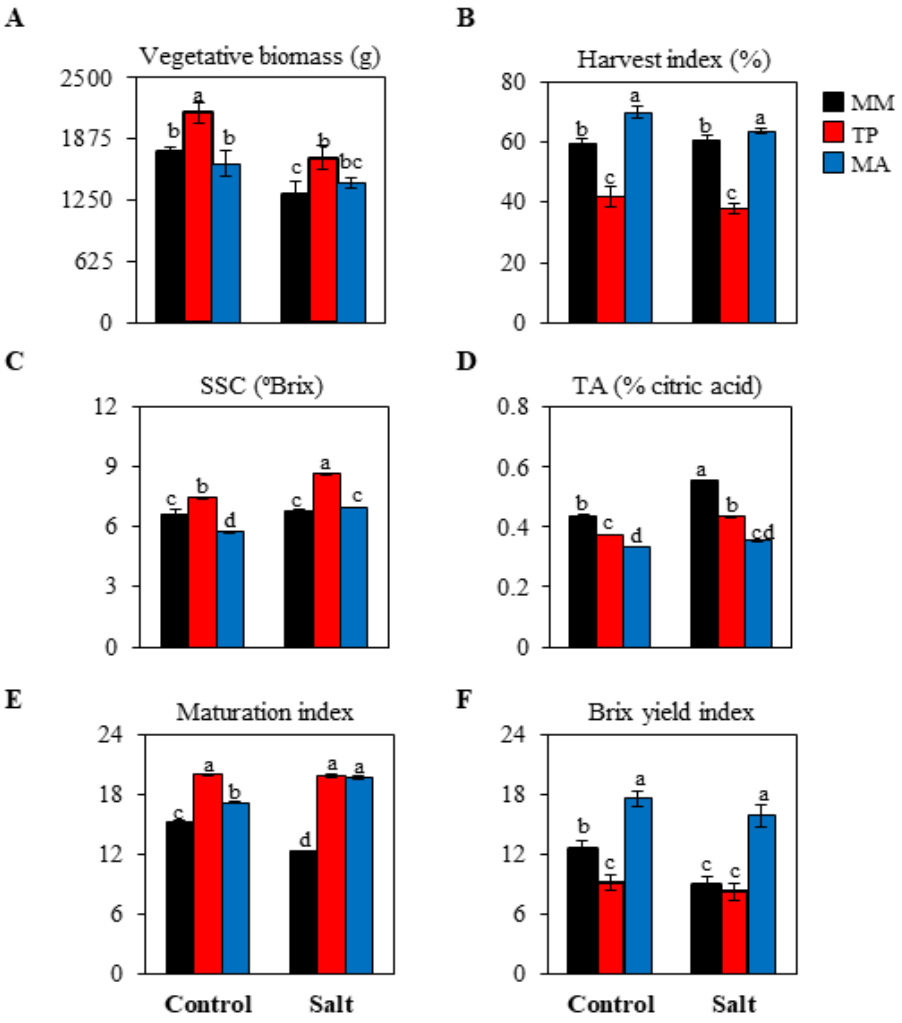


Figure 2. Agronomic and organoleptic quality parameters in red ripe fruits of tomato 'Moneymaker' (MM) and two traditional varieties, 'Tomate Pimiento' (TP) and 'Muchamiel Aperado' (MA), from plants grown in control and salt (50 mM NaCl). (A) Vegetative biomass, (B) harvest index (C) soluble solids content (SSC), (D) titratable acidity (TA), (E) Maturation Index (SSC/TA) (F) Brix yield index (SSC * fruit yield). For agronomic data seven plants were individually harvested; for quality traits three biological replicates of 10 fruits each were used. Values are expressed as means ± SE. Different letters indicate statistically significant differences according to Tukey´s test (p<0.05).

5.2.2 Physiologic changes induced by salt stress in tomato fruit

Given that physiologic and metabolic changes can vary during ripening, fruits were analyzed at two developmental stages, namely MG and RR. Notably, water content was lower under salt stress in all genotypes at both fruit stages (Supplementary Table S1). 'MA' showed the smallest reduction in water content with moderate salinity in RR fruits; at the MG stage, 'TP' had the lowest water content under the control treatment and its reduction under salt stress was also smaller than for 'MA' and 'MM'.

As increases in solute levels reflect both active solute accumulation and the concentration effect due to dehydration when values are determined based on fresh weight or water basis, we expressed cations and metabolites contents based on dry weight in order to avoid the effect of solutes increase exclusively due to a concentration effect. K^+ contents were similar between the two landraces and 'MM' at both stages of ripening, and did not change with salt stress (Supplementary Table S1). As expected, Na^+ significantly increased with salinity in the three genotypes, but the accumulation was significantly higher in MG and RR fruits of 'MA' than in the fruits of TP and MM, suggesting that this traditional variety accumulates more solutes in order to avoid a reduction in water content. Ca^{2+} and Mg^{2+}contents were similar in the three genotypes and were unaffected by salinity. The most important differences were observed for the Ca^{2+}/Mg^{2+} ratio in RR fruits, which was significantly higher in 'MM' than in the two traditional varieties, especially under salt stress (Supplementary Table S1).

5.2.3 Primary metabolites in MG and RR fruits

The levels of sugars and organic acids (Figure 3) as well as amino acids (Figure 4) were analyzed in MG and RR fruits. Sucrose significantly increased with salinity in MG fruits in the three genotypes, with 'MM' showing the greatest increase. The same

trend was observed in RR fruits, but at this fruit stage the increases were much higher in both traditional varieties than in 'MM' (up to 150% for 'MA' fruits) (Figure 3A). The most obvious changes regarding hexoses (glucose and fructose) were the high levels in RR fruits of 'TP' when plants were grown in under the control treatment, with the differences between genotypes disappearing under salt stress. The total sugars content reflected changes in the most abundant sugars (hexoses) (Figure 3A). The organic acids malate and citrate showed opposite trends between the two landraces and the commercial cultivar at the MG stage: malate levels were much lower in both traditional varieties than 'MM', especially under salt treatment, whereas citrate levels were significantly higher. Succinate showed a similar trend to malate (Figure 3B). Total organic acid contents in fruits at the MG stage were comparable across the three genotypes under the control condition, likely because any differences in the levels of individual organic acids were abolished through compensatory mechanisms; this did not occur under salt stress, as organic acids level was significantly lower in both traditional varieties compared to 'MM' due to the extremely high malate content of MG fruits in the latter. Organic acid levels were higher in RR fruits of both traditional varieties compared to those of 'MM' from control plants and the opposite was true in RR fruits from salt-treated plants, with the exception of succinate levels (Figure 3B).

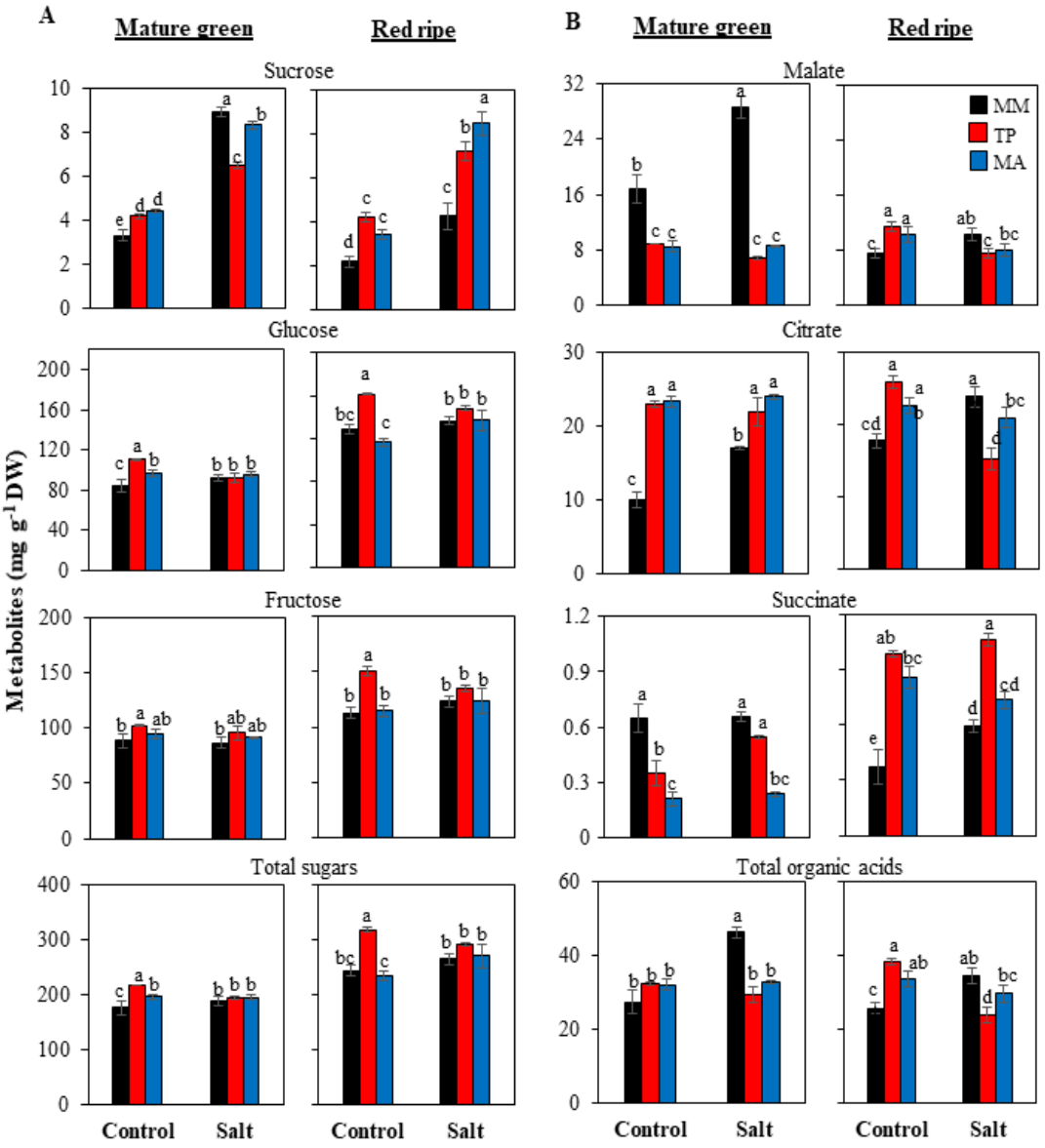


Figure 3. Accumulation of sugars (A) and organic acids (B) in mature green and red ripe fruits of 'Moneymaker' (MM) and two traditional varieties, 'Tomate Pimiento' (TP) and 'Muchamiel Aperado' (MA), from plants grown in control and salt (50 mM NaCl). Three biological replicates of 10 fruits each were used. Values are expressed as means ± SE. Different letters indicate statistically significant differences according to Tukey's test (p<0.05).

The most remarkable difference across genotypes in terms of primary metabolites was the amino acids profile: 'TP' accumulated more amino acids in its fruits than either 'MA' or 'MM' (Figure 4). 'TP' fruits showed the highest accumulation not only of major free amino acids such as glutamate, glutamine, and γ-aminobutyric acid (GABA), but also of minor ones including aromatic amino acids involved in the shikimate pathway such as tyrosine and phenylalanine. In 'MA' fruits, total amino acids content was lower than in 'TP' fruits but higher than in 'MM' fruits at the MG stage, whereas at the RR stage the levels were similar (control treatment) or even lower (salt treatment) than in 'MM' fruits (Figure 4).

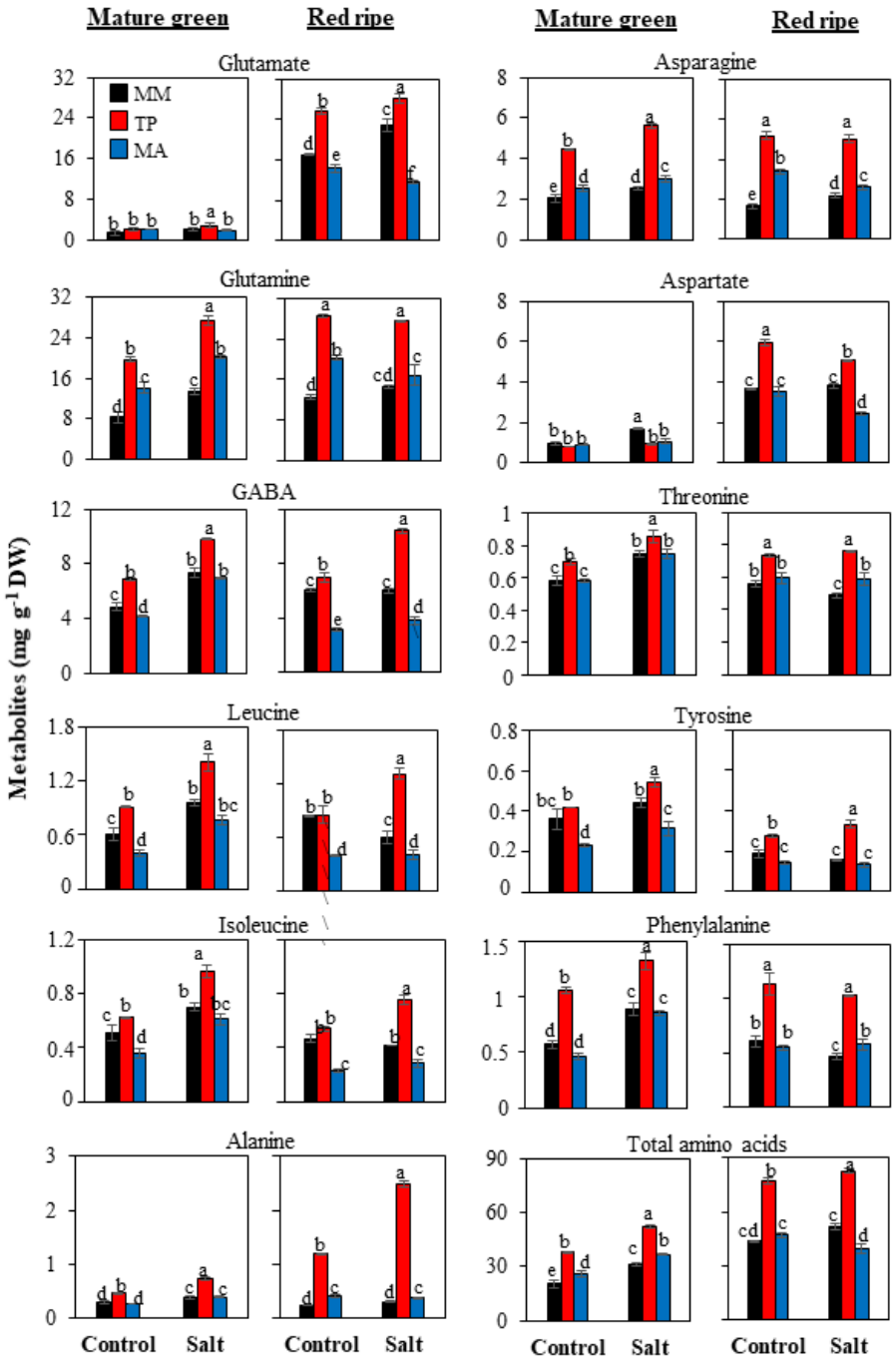


Figure 4. Accumulation of amino acids in mature green and red ripe fruits of 'Moneymaker' (MM) and two traditional varieties, 'Tomate Pimiento' (PT) and 'Muchamiel Aperado' (MA), from plants grown in control and salt (50 mM NaCl). Three biological replicates of 10 fruits each were used. Values are expressed as means ± SE. Different letters indicate statistically significant differences according to Tukey's test (p<0.05).

To determine whether separation in primary metabolism between genotypes and at what developmental stage this occurred, we carried out a principal component analysis (PCA) on all primary metabolites in MG and RR fruits (Figure 5). In the former, the PCA-biplot showed clear separation of and both traditional varieties from 'MM', with PC1 and PC2 accounting for 55% and 28% of the total variance, respectively (Figure 5A). Amino acids significantly contributed to the separation of samples by PC1 whereas organic acids were the most important metabolites for separation by PC2, with malate and succinate having higher coefficients in 'MM', and citrate having a higher coefficient in the two landraces. Salt stress had a marked effect on the metabolites profiles of the three genotypes: the metabolites contents increased but maintained their distinct metabolic signatures, as revealed by heatmap analysis (Figure 5A). PC1 accounted for a higher percentage of the total variation (63%) at the RR stage than at the MG stage and clearly separated the 'TP', which had higher positive coefficients for amino acids than the other genotypes (Figure 5B). Furthermore, RR fruits from control and salt-treated plants were closer in 'MM' and 'TP' and in the case of 'MA' no separation was observed, suggesting that the metabolic profiles of RR stage is influenced to a greater extent by genotype than by salt stress (Figure 5B). The PCA-biplot and heatmap analyses clearly indicated that sucrose was the main trait responsible for the separation of both traditional tomato varieties from 'MM'.

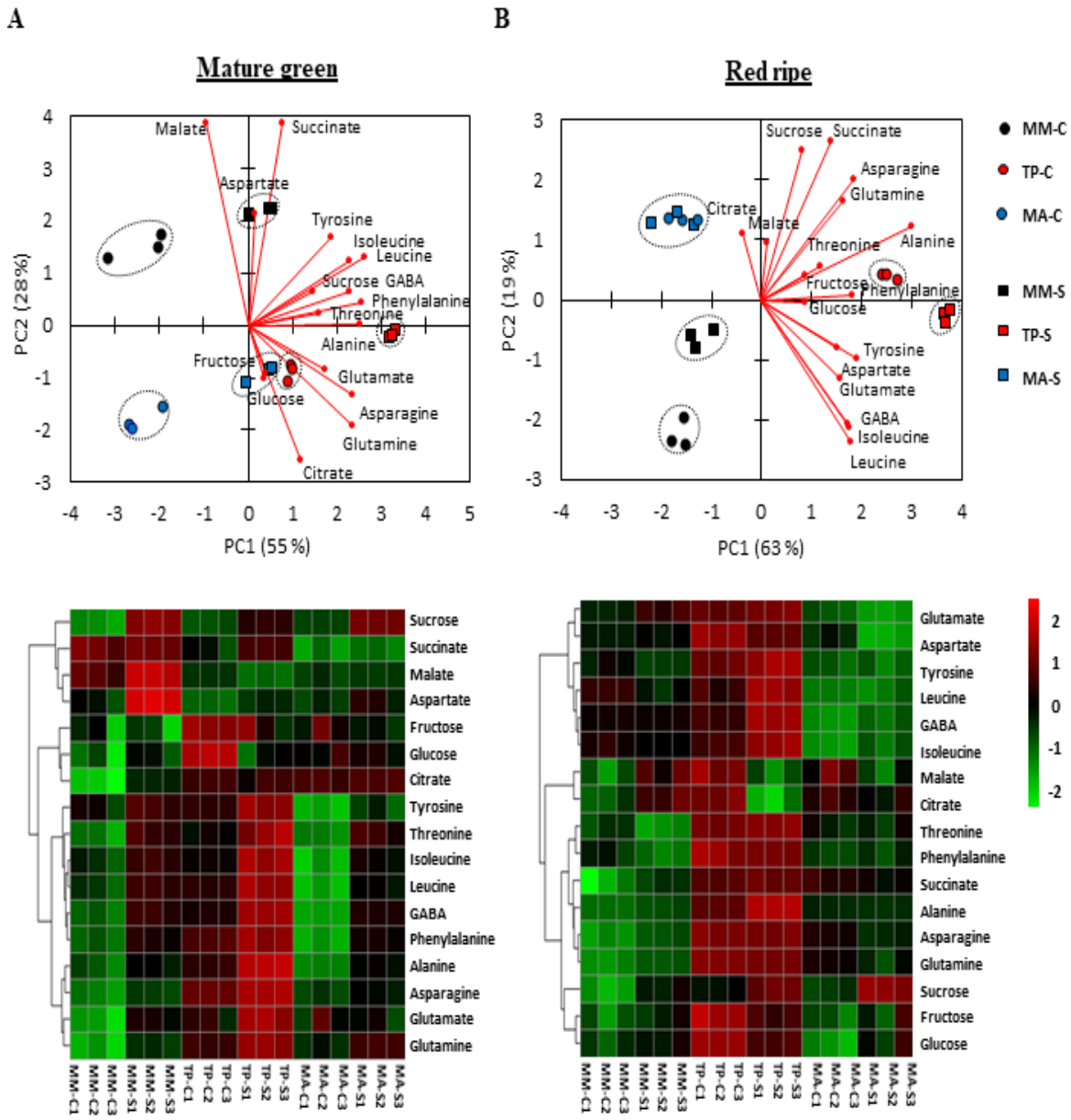


Figure 5. Relative primary metabolites in mature green and red ripe fruits of 'Moneymaker' (MM) and two traditional varieties, 'Tomate Pimiento' (TP) and 'Muchamiel Aperado' (MA), from plants grown in control (C) and salt (50 mM NaCl) (S). Non-supervised principal component analysis (PCA-biplot) and heatmap analysis representing the major sources of variability in mature green (A) and red ripe fruits (B). Color scale represents the variation in the relative concentration of compounds, from high (red) to low (green) contents.

5.2.4 Secondary metabolites in MG and RR fruits

As carotenoids, tocopherols and chlorophylls share a common precursor (geranyl geranyl diphosphate [GGPP]), there is an inverse relationship among these metabolites, which is evident in the PCA-biplots of MG and RR fruits (Figure 6B), although the patterns differed according to developmental stage. The α-tocopherol content was high in MG fruits of 'MA' whereas the opposite trend was observed for carotenoids and chlorophylls, while in 'TP' only chlorophylls content was elevated, especially chlorophyll a level in MG fruits from salt-treated plants (Figure 6A and Supplementary Table S2). RR fruits of both landraces showed a greater than 50% increase in α-tocopherol content compared to 'MM' under moderate salinity, which was reflected by their high α-tocopherol/carotenoids ratios (Figure 6A). Total carotenoids levels were similar in 'MM' and 'TP' fruits but were comparatively lower in 'MA' fruits except at the MG stage in salt-treated plants. This was mainly attributable to the high β-carotene and lycopene levels in MG and RR fruits, respectively (Figure 6A and Supplementary Table S2).

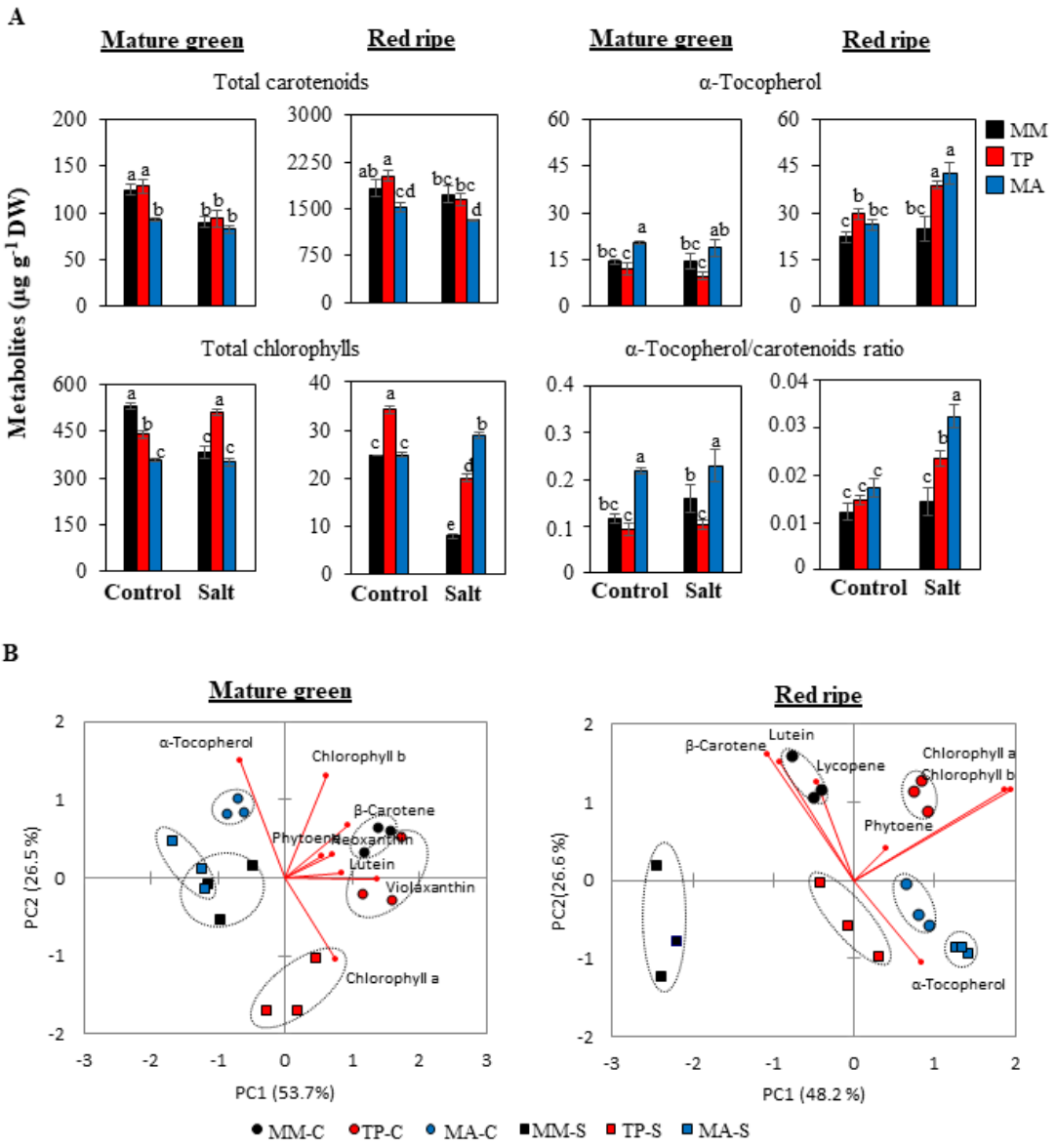


Figure 6. Secondary metabolites in mature green and red ripe fruits of 'Moneymaker' (MM) and two traditional varieties, 'Tomate Pimiento' (TP) and 'Muchamiel Aperado' (MA), from plants grown in control (C) and salt (50 mM NaCl) (S). (A) Accumulation of total carotenoids, total chlorophylls, and α-tocopherol, and α-tocopherol/carotenoids ratio. Three biological replicates of 10 fruits each were used. Values are expressed as means ± SE. Different letters indicate statistically significant differences according to Tukey's test (p<0.05). (B) Graphical representation of Principal

Component Analysis representing the major sources of variability for secondary metabolites in mature green and red ripe fruits.

There was a very small separation between control and salt treatments for 'MA' fruits at the RR stage while for 'MM' and 'TP' salt stress had a significant effect on the metabolite composition of RR fruits, similar to the changes observed in MG fruits (Figure 6B). This set of results suggests improvement of the metabolic profile occurs in both traditional varieties but the processes operating in each one seem to be different. Based on our observations, we present a model of global changes in primary and secondary metabolic profiles in MG and RR fruits of traditional tomato varieties compared to the commercial cultivar coming from control and salt treatments (Figure 7).

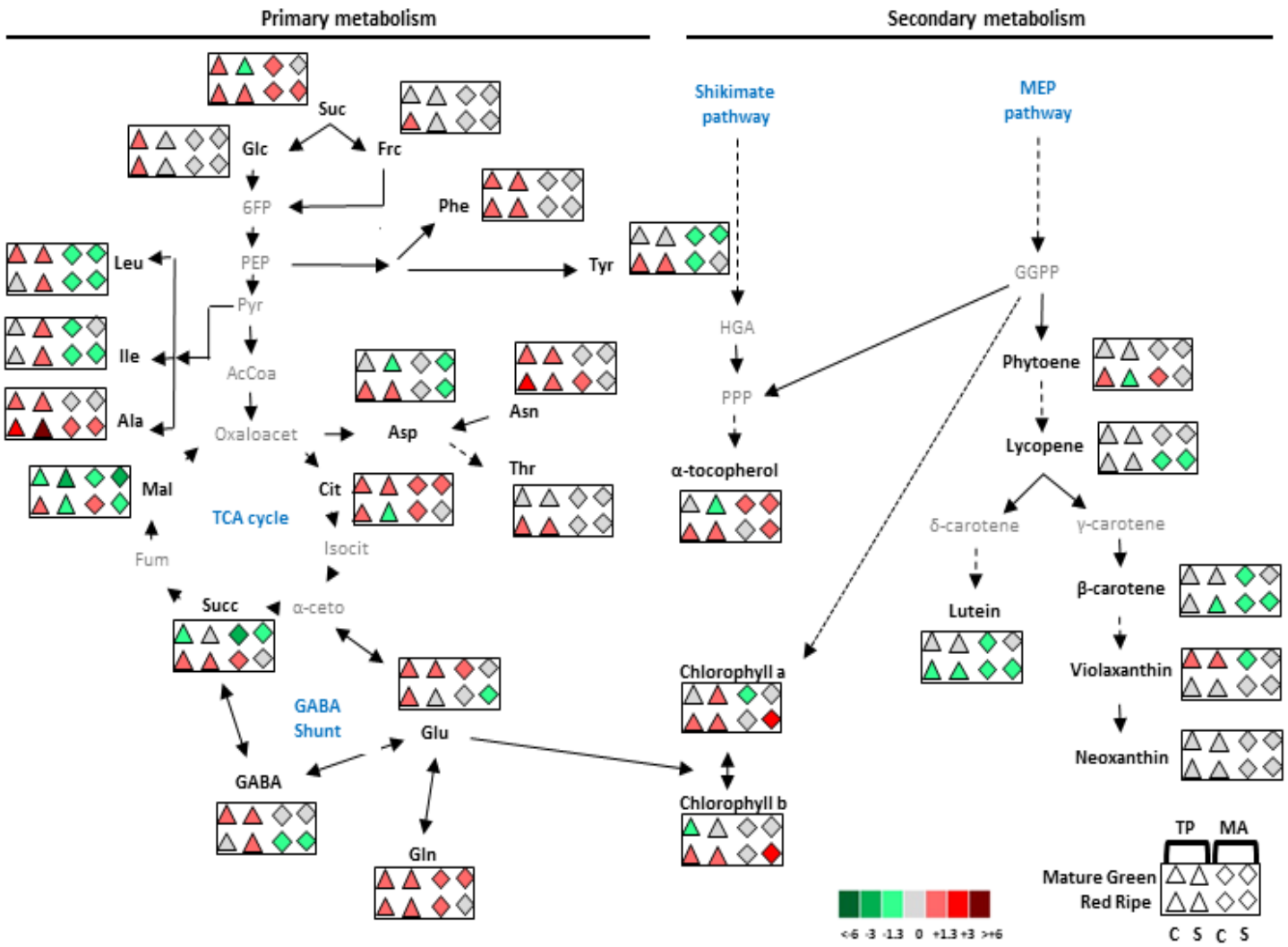


Figure 7. Global overview of metabolic changes occurring in mature green and red ripe fruits of 'Tomate Pimiento' (TP) and 'Muchamiel Aperado' (MA), from plants grown in control (C) and salt (50 mM NaCl) (S). Data were normalized to 'Moneymaker' (MM). Only those metabolites showing up-regulation or down-regulation in each traditional variety higher 1.3-fold than MM are showed. Color scale is used to display the different amount of metabolite in terms of fold-change relative to the level in the appropriate control. Suc, sucrose; Glc, glucose; Fru, fructose; 6FP, fructose-6-phosphate; PEP, phosphoenolpyruvate; Phe, phenylalanine; Tyr, tyrosine; Leu, leucine; Ile, isoleucine; Ala, alanine; Asp, aspartate; Asn, asparagine; Thr, threonine; Pyr, pyruvate; HGA, homogentisic acid; GGPP, geranylgeranyl diphosphate; PPP, phytyl diphosphate; AcCoA, acetylCoA; Oxaloacet, oxaloacetate; Cit, citrate; Isocit, isocitrate; α-ceto, α-cetoglutarate; Succ, succinate; Fum, fumarate; Mal, malate; Glu, glutamate; Gln, glutamine; GABA, gamma-aminobutyric acid.

5.3 Discussion

5.3.1 Moderate salt stress improves sensorial fruit quality of tomato without affecting fruit yield

The application of controlled abiotic stress can improve the sensory and nutritional value of fruits (Toscano *et al*., 2019). As changes in tomato fruit quality in response to abiotic stress are cultivar-dependent (Quinet *et al*., 2019), we compared two traditional varieties, 'TP' and 'MA' differing widely in terms of fruit shape and size between them and compared with the commercial cultivar 'MM' (Figure 1A) to determine whether fruit yield and/or quality are affected when plants were grown at moderate salt stress (50 mM NaCl). In tomato varieties with indeterminate growth, fruits at different positions on different trusses are always at different developmental stages (Ripoll *et al*., 2016); therefore, the effects of salt stress can vary for each truss. However, we observed similar fruit yields for the first and third trusses of all three tomato genotypes at moderate salinity, with a trend for slightly higher yield for the second truss (Figure 1B). It is worth noting that tomato varieties with small-to-medium fruit size exhibit greater improvements in fruit quality while maintaining the same fruit yield under abiotic stress (Albert *et al*., 2016), but this link is very rare to observe in varieties with a large fruit size such as 'MA'. This provides an opportunity to investigate the possibility of increasing the metabolites content of tomato fruits through irrigation with saline water without negatively affecting fruit yield.

Interestingly, both traditional varieties showed improved sensorial fruit quality compared to 'MM', although the quality characteristics differed. 'TP' is a variety of great interest because of its high SSC (Figure 2C), which is considered an important determinant of tomato fruit organoleptic quality (Hou *et al*., 2020). Meanwhile, 'MA' is

interesting because of its high BY index (Figure 2F), which is a measure of overall fruit quality (Rowland *et al*., 2020). One sensory feature that improved in both landraces was MI, although this index was independent of salinity in 'TP', and increased with salinity in 'MA', unlike in 'MM' (Figure 2E). In general, increases in SSC associated with salt stress reflect concentration effects resulting from a decreased amount of water in the fruit (Albert *et al*., 2016), but this was not the case in our study as the reductions in fruit water content induced by salinity were minimal and very similar between 'MM' and 'TP' (~2%), and even lower in 'MA' (Supplementary Table S1). This along with the fact that salinity reduced vegetative biomass by >20% in 'MM' and 'TP' (Figure 2A) suggests that the tomato plants buffered the osmotic effect of salinity by limiting their vegetative growth and reallocating water and solutes to the fruits (Osorio *et al*., 2014). Regarding inorganic solutes, the most significant change was in the Ca^{2+}/Mg^{2+}ratio, which is often correlated with organoleptic quality traits such as SSC and TA (Gerendás and Führs, 2013). In fact, our results suggest an inverse relationship between the Ca^{2+}/Mg^{2+} ratio and MI (SSC/TA ratio) in RR fruits (Figure 2E and Supplementary Table S1), since the decreases in the former (by ~40%) were accompanied by parallel increases in the latter in both traditional varieties compared to 'MM'.

5.3.2 TP fruits exhibit a remarkable accumulation of amino acids

Primary metabolism is essential for plant growth but is also a major contributor to fruit quality; thus, further advances in its understanding are needed in order to identify future strategies for manipulation of fruit metabolism (Beauvoit *et al*., 2018). The first major metabolic change in tomato fruit was sucrose content at the RR stage, as both traditional varieties significantly showed increased sucrose levels in under the control condition and especially under salt treatment (Figure 3A). A similar response has been

reported in two different tomato landraces, in which fruit yield was reduced when plants were irrigated with 100 mM NaCl (Massaretto *et al*., 2018); this implies that fruit quality is related to a greater ability to accumulate sucrose. ’MM’ fruits had a very high level of malate at the MG stage compared to 'TP' and 'MA' while the opposite was observed for citrate (Figure 3B), reflecting the “non-cyclic” partial tricarboxylic acid cycle in which one branch produces citrate while the other produces malate (Igamberdiev and Eprintsev, 2016). It was reported that changes in malate metabolism affect fruit quality, as tomato varieties with a high malate content had a low SSC at harvest (Centeno *et al*., (2011).

There was a clear separation between genotypes in the PCA of MG and especially RR fruits, with a greater abundance of metabolites in 'TP' than in 'MM' and 'MA' (Figure 5). The most remarkable difference was in total amino acids content, which was highest in ‘TP’ and increased with salt treatment (Figure 4). This included the free amino acids involved in the GABA shunt, which plays a major role in primary carbon and nitrogen metabolism and is especially important in certain physiologic situations such as plant stress and fruit ripening (Aghdam and Fard, 2017). The GABA shunt is involved in salt stress tolerance in tomato plants (Bao *et al*., 2014). Interestingly, we observed that GABA content showed the highest increase with salt stress in MG and RR fruits of 'TP' (Figure 4). GABA is a four carbon non-protein amino acid that has received much attention as a health-promoting functional compound (Takayama and Ezura, 2015; Zhao *et al*., 2018). Glutamate and glutamine—the other two amino acids involved in the GABA shunt—were the two major amino acids detected at the RR stage in every genotype, with especially high levels in 'TP' (Figure 4). Notably, glutamine was the only amino acid that showed similar developmental profiles in both landraces, with the levels higher compared to 'MM' (Figure 7). In sum, the roles of the GABA shunt and glutamine in the adaptation to salt stress and determination of tomato fruit quality warrant more detailed study.

5.3.3 α-Tocopherol content in tomato fruit is increased under salt stress in the two traditional varieties

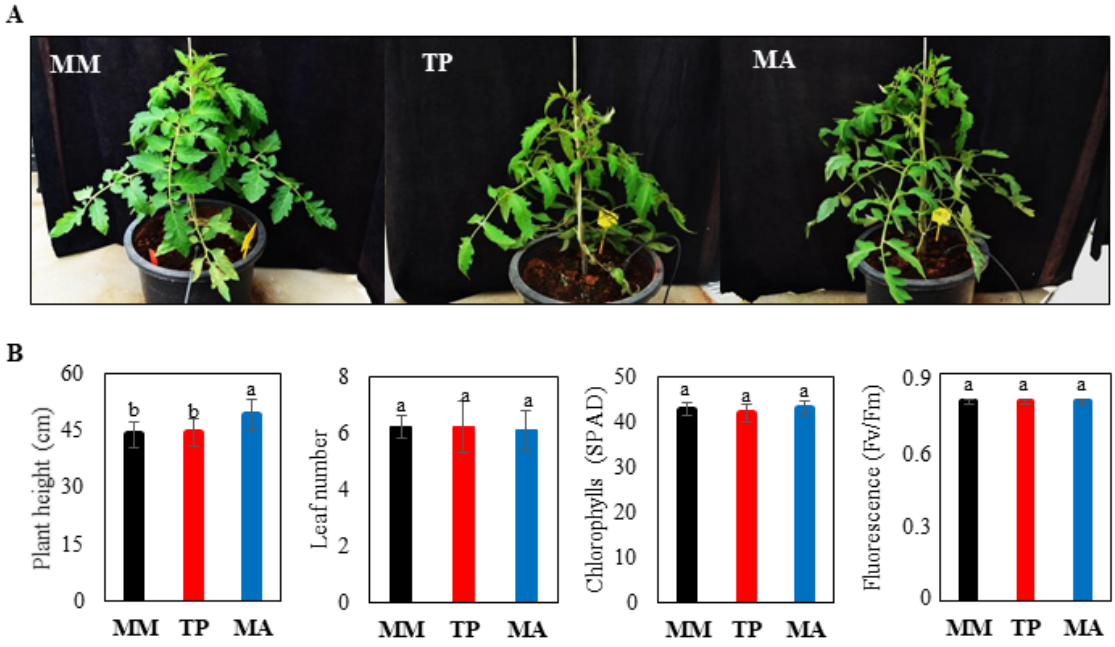
Carotenoids, tocopherols, and chlorophylls share a common precursor (GGPP) produced by the methylerythritol 4-phosphate pathway. Because of this metabolic cross-talk, changes in one of these compounds can affect the biosynthesis of the others (Almeida *et al*., 2015; Quadrana *et al*., 2013). We observed a marked increase in α-tocopherol content (>50%) in RR fruits of both traditional varieties under salt treatment compared to fruits from 'MM' plants under the same conditions (Figure 6A). However, the metabolic processes operating in the two landraces appear to differ, as the increase in α-tocopherol in 'MA' was accompanied by a decrease in carotenoids, whereas 'TP' showed similar carotenoid levels as 'MM' despite an increase in α-tocopherol (Figure 6 and Supplementary Table S2). Given that α-tocopherol is synthesized from phytyl diphosphate (PPP) generated by GGPP and homogentisate (HGA) from the shikimate pathway (Almeida *et al*., 2015), we speculate that the accumulation of α-tocopherol in RR fruits of 'TP' is related to that of amino acids such as tyrosine and phenylalanine from the shikimate pathway, which does not occur in 'MA' and 'MM' fruits (Figures 4, 7).

The mechanism of salt tolerance in plants subjected to salinity depends on the level of salt stress (Muñoz-Mayor *et al*., 2012), which along with genetic background determines the pathways that are induced and specific metabolites that accumulate as a result. In our previous study of two other tomato varieties subjected to a higher salt stress level (100 mM NaCl), the main secondary metabolites that were increased were carotenoids (Massaretto *et al*., 2018), whereas in the present work α-tocopherol showed the greatest change in tomato plants under a lower intensity of salt stress (50 mM NaCl). An unsolved question is whether this is only a result of differences in genotype or different salt stress levels may play a role.

5.4 Conclusion

In conclusion, the two traditional varieties used in this study are attractive plant materials for investigating production of metabolites of interest in response to abiotic stress from the standpoint of nutritional quality. Importantly, the fact that α-tocopherol level nearly doubled in ‘TP’ and ‘MA’ plants relative to ‘MM’ upon irrigation with 50 mM NaCl while fruit yield was unaffected demonstrates that cultivating these landraces under moderate salt stress is an effective agronomic strategy for improving the nutritional value of tomato fruit.

5.5 Supplementary files



Supplementary Figure S1. Phenotypes of 'Moneymaker' (MM) and both traditional varieties plants, 'Tomate Pimiento' (TP) and 'Muchamiel Aperado' (MA). (A) Representative images of plants and (B) traits of plant development (plant height and leaf number) and leaf chlorophyll content and fluorescence just before starting the salt treatment (50 mM NaCl). Values are expressed as means ± SE of fourteen plants per genotype. Different letters indicate statistically significant differences according to Tukey's test ($p< 0.05$).

Supplementary Table S1. Water percentage and cation contents (mg g^{-1} DW) in mature green and red ripe fruits of 'Moneymaker' and two traditional varieties, 'Tomate Pimiento' and 'Muchamiel Aperado', from plants grown in control and salt (50 mM NaCl).

	MoneyMaker		*Tomate Pimiento*		*Muchamiel Aperado*	
	Control	Salt	Control	Salt	Control	Salt
			Mature green			
Water	94.7±0.14^{a}	92.1±0.09^{c}	93.2±0.48^{b}	92.3±0.09^{c}	94.1±0.13^{a}	92.7±0.14^{bc}
Na^{+}	0.39±0.06^{c}	1.31±0.32^{b}	0.60±0.03^{c}	0.74±0.03^{c}	1.39±0.08^{b}	3.46±0.19^{a}
K^{+}	33.0±4.88^{a}	34.2±8.45^{a}	34.4±1.97^{a}	33.2±1.22^{a}	40.9±2.54^{a}	35.6±2.09^{a}
Ca^{2+}	1.09±0.17^{bc}	0.77±0.05^{c}	1.33±0.06^{ab}	1.07±0.07^{bc}	1.17±0.04^{bc}	1.57±0.26^{a}
Mg^{2+}	0.86±0.13^{b}	0.78±0.19^{b}	1.35±0.08^{a}	1.07±0.05^{ab}	1.36±0.09^{a}	1.26±0.07^{a}
Ca^{2+}/Mg^{2+}	1.27±0.01^{a}	0.99±0.06^{b}	0.99±0.01^{b}	1.00±0.03^{b}	0.86±0.04^{b}	1.24±0.14^{a}
Total cátions	35.4±5.24^{a}	36.9±9.14^{a}	37.7±2.15^{a}	36.1±1.36^{a}	44.9±2.77^{a}	41.9±2.59^{a}
			Red ripe			
Water	93.6±0.04^{ab}	91.7±0.46^{d}	93.0±0.08^{bc}	91.1±0.27^{d}	93.8±0.05^{a}	92.7±0.15^{c}
Na^{+}	0.44±0.05^{cd}	1.03±0.09^{b}	0.39±0.01^{d}	0.84±0.08^{bc}	1.08±0.23^{b}	3.14±0.26^{a}
K^{+}	35.7±2.01^{a}	35.4±2.91^{a}	35.4±0.85^{a}	31.1±3.10^{a}	38.2±3.08^{a}	36.2±2.57^{a}
Ca^{2+}	1.26±0.14^{b}	1.72±0.15^{a}	0.85±0.04^{c}	1.01±0.09^{bc}	1.17±0.24^{bc}	1.12±0.07^{bc}
Mg^{2+}	0.95±0.05^{a}	1.01±0.08^{a}	1.17±0.03^{a}	1.11±0.11^{a}	1.33±0.29^{a}	1.15±0.09^{a}
Ca^{2+}/Mg^{2+}	1.33±0.08^{b}	1.70±0.00^{a}	0.73±0.01^{e}	0.91±0.01^{cd}	0.88±0.00^{d}	0.97±0.01^{c}
Total cátions	38.4±2.24^{a}	39.1±3.24^{a}	37.8±0.94^{a}	34.0±3.39^{a}	41.8±3.85^{a}	41.6±3.01^{a}

Three biological replicates of 10 fruits each were used. Values are expressed as means ± SE. Different letters indicate statistically significant differences according to Tukey's test ($p< 0.05$).

Supplementary Table S2. Carotenoids and chlorophylls contents (μg g^{-1} DW) in mature green and red ripe fruits of 'Moneymaker' and two traditional varieties, 'Tomate Pimiento' and 'Muchamiel Aperado', from plants grown in control and salt (50 mM NaCl).

	MoneyMaker		*Tomate Pimiento*		*Muchamiel Aperado*	
	Control	Salt	Control	Salt	Control	Salt
Mature green						
Lycopene	ND	ND	ND	ND	ND	ND
β-Carotene	54.1±4.23^{a}	38.6±2.71^{b}	49.8±5.10^{a}	34.8±5.77^{b}	38.1±0.91^{b}	32.3±3.25^{b}
Lutein	20.2±2.91^{ab}	16.9±3.08^{abc}	22.8±2.09^{a}	15.7±1.96^{bc}	14.0±0.98^{c}	15.2±1.16^{bc}
Phytoene	20.4±1.07^{a}	17.1±0.97^{a}	19.4±2.15^{a}	18.7±1.54^{a}	18.3±2.36^{a}	17.0±1.05^{a}
Violaxanthin	21.7±1.91^{b}	11.2±1.70^{d}	28.8±2.80^{a}	17.3±1.18^{bc}	15.2±2.49^{cd}	11.9±1.94^{cd}
Neoxanthin	8.05±0.77^{a}	6.21±0.40^{b}	7.95±0.80^{a}	7.02±0.45^{ab}	7.29±0.85^{ab}	5.92±0.30^{b}
Chlorophyll a	377.7±12.8^{b}	279.2±11.5^{d}	319.1±2.90^{c}	429.2±5.18^{a}	234.4±6.89^{e}	252.4±5.40^{e}
Chlorophyll b	152.0±2.23^{a}	101.2±9.81^{cd}	119.8±10.8^{bc}	81.0±7.38^{e}	121.8±1.29^{b}	97.6±5.76^{de}
Red ripe						
Lycopene	1564.6±126.5^{ab}	1442.2±109.4^{bc}	1790.7±67.5^{a}	1467.7±64.4^{b}	1233.0±73.2^{cd}	1084.6±6.61^{d}
β-Carotene	110.0±9.08^{a}	97.7±13.3^{ab}	93.7±11.6^{ab}	72.9±16.8^{bc}	55.1±5.65^{c}	54.6±4.40^{c}
Lutein	12.9±1.16^{a}	10.4±3.75^{ab}	8.53±0.61^{ab}	8.20±1.56^{ab}	6.76±1.01^{b}	6.42±0.55^{b}
Phytoene	138.1±7.67^{cd}	173.5±18.1^{bc}	217.5±27.2^{ab}	102.2±22.5^{d}	234.3±26.2^{a}	171.8±4.50^{bc}
Violaxanthin	ND	ND	ND	ND	ND	ND
Neoxanthin	ND	ND	ND	ND	ND	ND
Chlorophyll a	9.47±0.03^{bc}	3.51±0.10^{d}	15.3±1.47^{a}	7.70±1.01^{c}	10.6±2.08^{bc}	11.1±0.20^{b}
Chlorophyll b	15.2±0.05^{bc}	4.34±0.43^{d}	19.0±1.30^{a}	12.3±0.05^{c}	14.2±2.58^{c}	17.8±0.32^{ab}

Three biological replicates of 10 fruits each were used. Values are expressed as means±SE. Different letters indicate statistically significant differences according to Tukey's test ($p< 0.05$).

6. SECTION 2: EFFECT OF EXOGENOUS ETHYLENE AND METHYL JASMONATE ON METABOLITE PROFILING OF TOMATO

Impact of hormonal treatment on tomato (Solanum lycopersicum L. cv. Grape) fruit quality: 1. Effects of exogenous methyl jasmonate and ethylene on primary and secondary metabolisms during ripening

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6.1 Objective

Although exogenous application of jasmonates modulates ethylene production and other processes related to ripening, the effects of jasmonates on metabolome during tomato ripening need to be better understood. For this reason, it is essential to comprehend the responses of tomato to treatments with ethylene and methyl jasmonate and how it acts in the accumulation of primary and secondary metabolites, in order to make the use of phytohormones as a tool to improve the fruit quality, as well as sensory and nutritional properties of crops. In this study, we investigated the response of tomato (*Solanum lycopersicum* L. cv. Grape) fruit to exogenous treatments of methyl jasmonate and ethylene, compared to the untreated tomato group, associated with changes in primary and secondary metabolites during fruit ripening.

6.2 Results and discussion

6.2.1 Effect of ethylene and jasmonate on the ethylene emission and fruit surface color

No treated Grape tomato fruits (CTRL) were used as reference of the assays. In the Figure 1 was possible to visualize the groups which hormonal treatment were applied: methyl jasmonate treated fruits (MeJA) and the ethylene treated fruits (ETHY) compared with CTRL group. The CTRL group achieved the breaker stage (Br) at 4 DAH, red stage at 10 DAH (Br+6) and final postharvest stage at 21 DAH (Br+17). Regarding treated fruits, MeJA and ETHY, breaker stage was achived at same day that CTRL (4 DAH) and red stage at 9 DAH (Br+5). The measures of ethylene emission and surface color of the Grape tomato fruit were conducted for characterization of ripening stages from the day of harvest to 21 DAH (Figure 1B and 1C). However, the analysis of metabolite profiling

were realized at 4, 10 and 21 DAH, aiming to observe the effect of treatments with respect to CTRL.

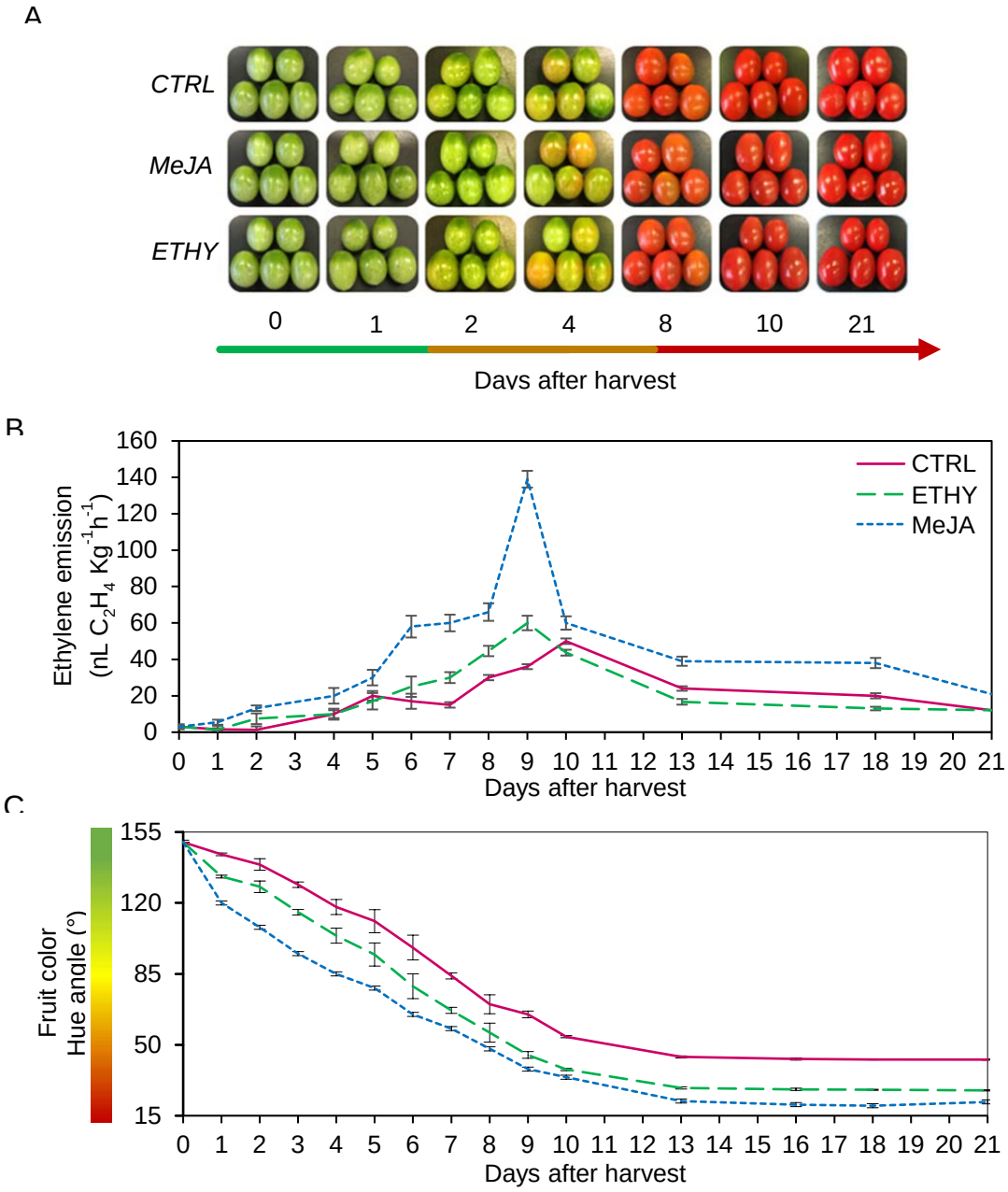

Figure 1. Characterization of tomato (*Solanum lycopersicum* L. cv. Grape) fruits treated with ethylene (ETHY) and methyl jasmonate (MeJA) hormones during ripening. Representative images of tomatoes (A). Effects of EHTY and MeJA on ethylene emission

(B) and fruit color (C) compared to the control group (CTRL). Values are means ± standard error of four biological replicates of at least 10 fruits each.

Treatments with ethylene and jasmonate anticipated fruit ripening changes when compared with no treated group. However, not only in the CTRL group was observed a typical behavior of climateric fruits in ethylene emission curves, but also in the treated groups. Peak of ethylene emission was observed at the ninth DAH in both ETHY and MeJA groups, and at tenth DAH in the CTRL group. However, the level of ethylene peak in MeJA-treated fruits at 9 DAH was 2 fold higher than observed in the other groups. This behavior can be related to stimulation of ethylene biosynthesis in climacteric fruits by methyl jasmonate hormone (Liu *et al.*, 2015).

Treatments induced changes in tomato fruit surface color slightly faster in comparison to no treated fruit. ETHY and MeJA firstly turned redness color, indicating that treated Grape tomatoes had completely ripened at ninth DAH, while CTRL fruits were full ripe at 10^{th} DAH. Ethylene hormone can improve the transcription of mRNAs that encode enzymes such as phytoene synthase, inducing the carotenoid metabolism. Thus, it is possible to observe acceleration in color changes in fruits exposed to treatment with exogenous ethylene (Liu *et al.*, 2015). In addition, previous studies had showed that methyl jasmonate contributed also to carotenoid production in tomatoes (Liu *et al*, 2015 and 2012). Ethylene emission and surface color analysis revealed and methyl jasmonate may play an important role in the regulation carotenoid pathway and lycopene accumulation anticipating these events in the ripening process.

6.2.2 Primary metabolite profiling affected by ethylene and jasmonate treatments

Primary metabolites are major components of fruit quality and related metabolisms are considered crucial for plant growth. For this fact, more advances in its comprehension can facilitate the finding of future strategies for manipulation of fruit metabolism (Beauvoit *et al.*, 2018). In this work, a total of 46 primary metabolites were identified by metabolite profiling analysis: 10 sugars (glucose, fructose, sucrose, allose, gulose, glucaric acid, myo-inositol, mannose, ribose, and arabinofuranse); 9 organic acids (oxaloacetic, citric, succinic, aconitic, malic, citraconic, fumaric, propanoic, and butanoic acids); 12 amino acids (proline, serine, valine, threonine, aspartic acid, glutamic acid, glutamine, γ-aminobutyric acid (GABA), asparagine, tryptophan, phenylalanine, and tyrosine); 12 saturated fatty acids (capric, lauric, myristic, palmitic, stearic, eicosanoic, docosanoic, tricosanoic, lignoceric, hyenic, cerotic, and montanic acids); and 3 unsaturated fatty acids (oleic, linoleic, and linolenic acids) at 4, 10 and 21 DAH (Table 1). Also, a global overview of the metabolic changes occurring in Grape tomato during ripening was performed to evaluate significant differences among accumulated metabolites in treated fruits compared with control group (Figure 2).

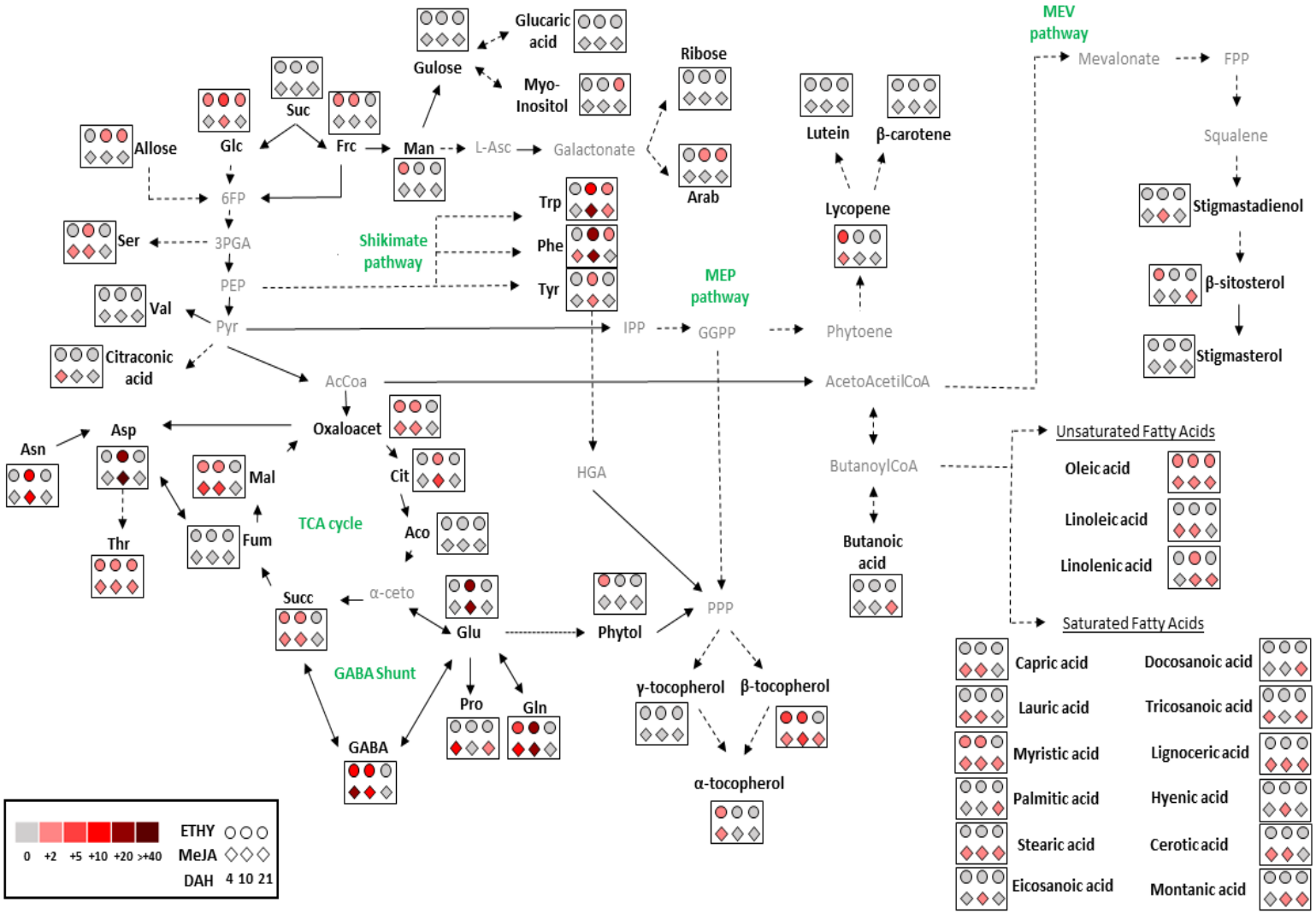

Figure 2. Global overview of metabolic changes occurring in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to ethylene (ETHY) and methyl jasmonate (MeJA) treatment at 04, 10 and 21 days after harvest (DAH). Data were normalized to the control group (CTRL). Only those metabolites showing up-regulation or down-regulation in each treatment higher 2.0 fold than control group are showed. Color scale is used to display the different amount of metabolite in terms of fold-change relative to the level in the appropriate control. Suc, sucrose; Glc, glucose; Frc, fructose; Man, Mannose; L-Asc, L-ascorbic acid; Arab, Arabinofuranose; 6FP, fructose-6-phosphate; 3-GPA, glyceraldehyde-3-phosphate; Ser, serine; PEP, phosphoenolpyruvate; Trp, tryptophan; Phe, phenylalanine; Tyr, Tyrosine; HGA, homogentisic acid; Pyr, pyruvic acid; IPP, isopentenyl pyrophosphate; GGPP, geranylgeranyl; PPP, phytyl pyrophosphate; Val, valine; AcCoA, acetyl-CoA; Oxaloacet, oxaloacetic acid; Cit, citric acid; Aco, aconitic acid; α-ceto, α-cetoglutaric acid; Succ, succinic acid; Fum, fumaric acid; Mal, malic acid; Glu, glutamic acid; GABA, γ-aminobutyric acid; Gln, glutamine; Pro, proline; Arg, arginine; Asp, aspartic acid; Thr, threonine; Asn, asparagine; FPP, farnesyl pyrophosphate.

A PCA was performed on primary metabolites at 4th, 10th and 21th ripening stages and confirmed the high reproducibility among the four biological replicates and groups analyzed. Also, a cleary separation of CTRL group and both treated groups was evidenced for the primary metabolites in the PCA-score. Heatmap analysis was used to analyze the differences between treated and no-treated groups regarding the metabolites changes at each day after harvest analyzed.

Highest levels of sugars were detected in ETHY at 21 DAH followed by 10 DAH, indicating that treated fruit with exogenous ethylene presented higher tendency to accumulate soluble sugars during ripening than CTRL and MeJA. The total sugars level reflected the changes in the most abundant hexoses (fructose and glucose) and sucrose (Table 1). Interestingly, ETHY showed an accumulation 2 fold higher of glucose than CTRL group during all ripening process (Table 1, Figure 2). In addition, ETHY showed a higher tendency to accumulate fructose, mannose, glucose, sucrose, glucaric acid, allose, gulose, ribose, myo-inositol and arabinofaranose at 4 and 10 DAH than other groups analyzed (Figure 3).

Highest levels of citric, succinic, malic, oxaloacetic and fumaric acids were detected in MeJA at 10 DAH (Table 1), which were confirmed by the strong tendency of methyl jasmonate accumulate these organic acids at 10 DAH (Figure 3). The main contribution for the total organic acids level was represented by citric, succinic and malic acids (Table 1). Some TCA cycle intermediates such as oxaloacetic, malic and succinic acids presented up-regulation 2 fold higher in treated fruits than CTRL at 4 and 10 DAH (Table 1, Figure 2). The ninth DAH was notable for increasing in ethylene emission (Figure 1) and consequently in respiration, characteristic of climacteric fruit, which may explain the rise in the level of organic acids, predominantly in intermediates of TCA cycle, detected mainly at 10 DAH.

Sugars (mainly fructose, sucrose and glucose) and organic acids (mainly citric and malic acids) contents are very important to define the quality of ripe fruits. Here, the most notable differences in sugars and organic acids were observed by ethylene and methyl jasmonate treatments, respectively.

Table 1. Primary metabolites in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to ethylene (ETHY) and methyl jasmonate (MeJA) treatment at 04, 10 and 21 days after harvest (DAH) detected by gas chromatography-mass spectrometry (GC-MS).

Metabolite	**04 DAH**			**10 DAH**			**21 DAH**		
	CTRL	***ETHY***	***MeJA***	***CTRL***	***ETHY***	***MeJA***	***CTRL***	***ETHY***	***MeJA***
A) Sugars									
Glucose	1534.5±76.0^{f}	4101.0±228.0^{d}	2225.7±107.5^{e}	1977.6±11.4^{ef}	9991.1±130.2^{b}	4489.9±24.7^{d}	4352.0±281.0^{d}	11543.0±359.0^{a}	8335.5±176.6^{c}
Fructose	27474.0±4039.0^{c}	95020.0±3675.0^{g}	45970.0±3735.0^{f}	59266.0±16310.0^{e}	115707.0±714.0^{b}	73375.0±1137.0^{d}	101194.0±5662.0^{c}	136003.0±5835.0^{a}	95526.0±11592.0^{c}
Sucrose	38205.0±596.0^{g}	69829.0±4129.0^{d}	49135.0±2493.0^{f}	54654.0±716.0^{ef}	93085.0±881.0^{b}	60830.0±367.0^{e}	84839.0±4545.0^{c}	123867.0±4068.0^{a}	81355.0±2080.0^{c}
Allose	1098.6±42.2^{e}	1536.5±33.8^{d}	1164.2±44.6^{e}	1563.4±21.5^{d}	4212.4±103.8^{b}	1397.8±35.4^{de}	3309.0±380.0^{c}	6571.0±202.0^{a}	4243.5±106.5^{b}
Gulose	221.5±9.78^{g}	397.2±11.9^{g}	244.6±10.7^{h}	790.4±38.3^{e}	1095.4±7.74^{b}	712.9±25.5^{f}	1017.0±60.4^{c}	1339.4±42.8^{a}	911.8±23.5^{d}
Glucaric acid	42.2±1.61^{e}	81.1±2.24^{cd}	46.5±3.67^{e}	71.9±1.30^{d}	128.7±1.63^{b}	89.9±2.39^{c}	124.6±8.70^{b}	206.4±6.98^{a}	133.0±3.25^{b}
Myo-inositol	77.9±2.91^{ef}	112.6±1.72^{e}	73.4±2.82^{f}	169.6±2.04^{d}	229.3±9.72^{c}	165.8±3.44^{d}	340.1±3.44^{b}	673.8±11.4^{a}	367.4±39.7^{b}
Mannose	42.2±3.10^{f}	86.2±5.01^{d}	58.6±4.34^{e}	100.0±2.30^{c}	139.4±2.03^{b}	97.9±1.51^{c}	142.4±11.0^{b}	249.6±2.33^{a}	146.6±4.90^{b}
Ribose	174.6±7.42^{f}	217.4±6.14^{e}	165.9±6.8^{f}	249.4±3.91^{d}	307.8±10.00^{c}	221.2±2.16^{e}	386.4±27.0^{b}	530.8±11.7^{a}	372.5±7.75^{b}
Arabinofuranose	15.1±0.74^{f}	21.7±0.62^{e}	14.0±0.45^{f}	25.5±0.84^{e}	68.2±3.77^{b}	27.3±0.49^{e}	45.2±3.28^{d}	104.1±2.88^{a}	60.8±5.20^{c}
Total	68885.0±4082.0^{h}	171403.0±6682.0^{d}	99098.0±5695.0^{g}	118868.0±5993.0^{f}	224964.0±1220.0^{b}	141408.0±1197.0^{e}	195750.0±9973.0^{c}	281088.0±10338.0^{a}	191152.0±2920.0^{c}
B) Organic acids									
Oxaloacetic acid	573.3±24.3^{g}	1321.4±32.5^{de}	1352.7±64.4^{d}	2380.4±56.5^{c}	6070.9±28.1^{b}	9559.7±56.9^{a}	1241.9±80.7^{de}	1203.3±64.1^{e}	911.5±25.1^{f}
Citric acid	6517.0±413.0^{g}	11256.0±290.0^{ef}	10808.0±505.0^{ef}	7878.0±457.0^{f}	38455.0±3291.0^{b}	74269.0±6427.0^{a}	18901.0±1208.0^{d}	16520.0±1124.0^{de}	25977.0±655.0^{c}
Succinic acid	2646.0±360.0^{e}	6886.0±519.0^{d}	8590.0±386.0^{d}	12894.0±485.0^{c}	26896.0±1390.0^{b}	55839.0±2291.0^{a}	2862.0±254.0^{e}	4309.2±183.6^{e}	4391.9±106.3^{e}
Aconitic acid	61.1±2.90^{e}	100.3±4.02^{c}	64.9±2.47^{e}	83.1±0.86^{g}	111.7±5.75^{b}	74.7±4.14^{d}	101.4±5.70^{c}	137.9±5.37^{a}	100.0±1.73^{c}
Malic acid	2537.5±101.3^{g}	9927.0±280.0^{e}	13126.0±502.0^{d}	6653.7±174.0^{f}	22582.0±316.0^{b}	53604.3±103.0^{a}	16800.0±1014.0^{c}	16511.0±460.0^{c}	17357.0±308.0^{c}
Citraconic acid	17.4±0.74^{d}	19.8±2.00^{d}	71.1±6.39^{c}	104.5±12.4^{a}	85.3±3.45^{b}	79.3±5.44^{bc}	101.9±6.07^{a}	105.4±3.44^{a}	110.3±1.75^{a}
Fumaric acid	167.9±6.66^{e}	170.8±3.76^{e}	178.4±2.07^{e}	181.8±1.17^{de}	217.9±9.13^{b}	234.7±4.53^{a}	237.8±10.4^{a}	196.9±8.40^{cd}	201.±4.80^{c}
Propanoic acid	111.7±6.77^{c}	114.5±5.59^{c}	99.5±6.91^{c}	145.6±1.50^{c}	138.3±0.86^{c}	132.9±0.51^{c}	458.6±79.1^{b}	412.5±37.0^{b}	2062.1±193.0^{a}
Butanoic acid	284.5±18.9^{fg}	268.5±18.6^{g}	460.6±34.1^{de}	393.4±10.9^{ef}	369.7±2.77^{efg}	540.7±4.82^{cd}	641.8±96.9^{bc}	690.9±56.8^{b}	1879.7±92.2^{a}
Total	12917.0±804.0^{f}	30065.0±567.0^{e}	34752.0±1341.0^{de}	30715.0±907.0^{e}	94927.0±3789.0^{b}	194334.0±8679.0^{a}	41346.0±2535.0^{d}	40087.0±1720.0^{d}	52991.0±1021.0^{c}

Continued Table 1

Metabolite	04 DAH			10 DAH			21 DAH		
	CTRL	*ETHY*	*MeJA*	*CTRL*	*ETHY*	*MeJA*	*CTRL*	*ETHY*	*MeJA*
C) Amino acids									
Proline	501.±14.8^{f}	974.5±62.4^{ef}	5207.0±259.0^{b}	1167.2±26.8^{e}	2266.1±135.6^{d}	2155.6±106.1^{d}	2863.0±483.0^{c}	5481.0±386.0^{b}	11972.0±243.0^{a}
Serine	78.0±5.68^{d}	145.6±65.6^{c}	361.6±221.0^{a}	93.6±2.26^{d}	264.8±1.33^{b}	254.0±1.00^{b}	49.0±1.56^{e}	50.1±1.84^{e}	53.7±1.10^{e}
Valine	6.23±0.19^{h}	10.1±0.26^{ef}	11.5±0.45^{d}	17.6±0.25^{b}	24.7±0.25^{a}	16.0±0.14^{c}	7.93±0.40^{g}	9.6±0.42^{f}	10.6±0.25^{e}
Threonine	6.01±0.45^{f}	17.5±0.24^{e}	27.8±2.41^{d}	25.9±0.44^{d}	79.4±1.77^{a}	59.5±0.33^{b}	25.2±0.85^{h}	54.0±1.17^{c}	55.4±1.12^{c}
Aspartic acid	1523.20±54.20^{c}	1528.3±48.0^{c}	2661.9±58.7^{c}	2166.8±58.7^{c}	60460.0±776.0^{b}	119398.0±2555.0^{a}	1540.8±161.0^{c}	2817.0±246.0^{c}	2649.3±55.7^{c}
Glutamic acid	1744.3±75.6^{b}	1848.4±61.0^{b}	2952.7±143.5^{b}	4906.7±42.6^{b}	134180.0±12596.0^{a}	130476.0±628.0^{a}	3957.0±424.0^{b}	7511.0±224.0^{b}	6848.0±148.1^{b}
Glutamine	185.1±7.71^{f}	1837.0±63.8^{d}	2928.8±122.1^{c}	519.3±9.24^{e}	10775.5±100.4^{b}	15189.7±38.1^{a}	475.8±38.7^{e}	613.2±10.4^{e}	290.9±26.0^{f}
GABA	207.6±17.2^{f}	3013.8±75.1^{d}	5553.0±415.0^{c}	1060.8±45.9^{e}	14633.7±97.0^{b}	15172.5±79.6^{a}	216.9±20.6^{f}	332.8±8.86^{f}	301.4±11.4^{f}
Asparagine	139.±6.22^{e}	152.9±4.24^{e}	239.6±11.5^{de}	260.2±6.88^{d}	4599.6±93.0^{a}	3160.6±90.4^{b}	235.6±11.7^{de}	440.2±14.0^{c}	408.2±10.9^{c}
Tryptophan	173.±7.10^{cd}	204.7±6.39^{cd}	279.5±11.4^{cd}	321.4±8.65^{c}	5987.0±328.0^{b}	9517.8±65.3^{a}	27.1±0.94^{d}	53.6±3.28^{d}	58.9±2.04^{cd}
Phenylalanine	7.64±0.47^{f}	13.7±0.18^{f}	18.8±0.73^{f}	26.4±1.32^{f}	606.8±27.9^{d}	717.4±4.43^{a}	50.9±2.67^{e}	147.9±4.86^{c}	77.6±1.87^{d}
Tyrosine	21.1±0.77^{e}	23.7±0.90^{de}	26.2±1.39^{de}	27.8±2.37^{de}	109.1±4.05^{a}	83.6±3.37^{b}	62.3±3.70^{c}	86.4±6.21^{b}	28.9±0.70^{d}
Total	4593.8±170.8^{f}	9770.0±289.0^{ef}	20268.0±1025.0^{cd}	10593.8±94.5^{def}	233987.0±11964.0^{b}	296201.0±2412.0^{a}	9512.0±940.0^{ef}	17597.0±548.0^{cde}	22755.0±435.0^{c}
D) Saturated fatty acids									
Capric acid	61.9±1.89^{c}	93.8±4.32^{b}	133.4±0.79^{a}	14.9±0.41^{f}	28.9±0.14^{e}	43.0±1.41^{d}	6.77±0.04^{g}	7.93±0.26^{g}	10.0±0.15^{g}
Lauric acid	40.5±2.10^{c}	56.4±2.24^{b}	83.1±1.02^{a}	19.7±1.16^{e}	30.3±0.94^{d}	42.2±1.44^{c}	6.82±0.04^{g}	8.34±8.27^{fg}	10.0±0.17^{f}
Myristic acid	18.9±0.83^{d}	42.1±1.52^{b}	55.5±1.60^{a}	15.0±0.49^{e}	38.4±0.30^{c}	56.1±1.96^{a}	6.13±0.07^{g}	8.73±0.29^{f}	13.5±0.24^{e}
Palmitic acid	436.7±10.4^{cd}	470.6±20.2^{b}	570.1±16.2^{a}	413.1±10.8^{d}	459.7±7.05^{bc}	541.2±22.8^{a}	159.6±22.8^{f}	264.1±13.1^{e}	451.2±10.0^{bc}
Stearic acid	16.5±0.49^{e}	20.1±0.76^{d}	43.5±10.9^{a}	14.6±10.3^{f}	21.6±0.18^{d}	31.6±1.16^{b}	9.94±0.18^{g}	14.7±0.66^{ef}	27.2±1.36^{c}
Eicosanoic acid	93.9±1.59^{b}	90.6±2.52^{b}	128.8±8.70^{a}	10.2±0.30^{de}	16.3±0.35^{d}	24.7±0.99^{c}	7.16±0.17^{e}	11.0±0.34^{de}	12.6±0.34^{de}
Docosanoic acid	43.7±1.04^{cd}	47.1±2.02^{b}	57.0±1.62^{a}	41.3±1.09^{d}	45.9±0.71^{bc}	54.1±2.28^{a}	15.9±0.15^{f}	26.4±1.31^{e}	45.1±1.00^{bc}
Tricosanoic acid	27.6±1.15^{d}	30.3±0.54^{c}	56.1±2.65^{a}	20.3±0.28^{f}	24.2±0.22^{e}	36.4±1.22^{b}	7.08±0.17^{h}	13.8±0.73^{g}	18.3±0.43^{f}
Lignoceric acid	22.1±1.82^{e}	32.6±1.28^{c}	49.9±1.05^{a}	13.9±0.19^{g}	19.1±0.50^{f}	38.5±2.54^{b}	7.17±0.24^{h}	13.3±0.66^{g}	26.5±0.67^{d}
Hyenic acid	32.5±1.05^{c}	45.5±1.67^{b}	53.9±1.01^{a}	8.83±0.21^{e}	17.2±0.54^{d}	32.3±1.32^{c}	6.58±0.08^{f}	8.91±0.32^{e}	10.0±0.45^{e}

Continued Table 1

Metabolite	04 DAH			10 DAH			21 DAH		
	CTRL	***ETHY***	***MeJA***	***CTRL***	***ETHY***	***MeJA***	***CTRL***	***ETHY***	***MeJA***
Cerotic acid	10.0±0.71^{d}	15.9±0.68^{c}	48.4±1.06^{a}	10.2±0.31^{d}	16.3±0.35^{c}	24.7±1.00^{b}	7.29±0.16^{e}	8.75±0.39^{de}	10.1±0.18^{d}
Montanic acid	34.4±1.33^{b}	35.0±1.35^{b}	41.7±0.93^{a}	9.49±0.22^{f}	14.8±0.15^{e}	23.9±1.28^{c}	9.85±0.16^{f}	16.9±0.69^{d}	23.9±0.44^{c}
Total	838.8±20.6^{c}	979.8±32.7^{b}	1321.4±17.9^{a}	591.6±14.5^{f}	732.6±9.80^{d}	948.9±38.3^{b}	250.4±2.03^{h}	403.0±18.7^{g}	658.7±15.0^{e}
E) Unsaturated fatty acids									
Oleic acid	610.5±26.0^{e}	1734.8±99.8^{b}	2076.7±43.4^{a}	339.4±7.63^{g}	714.1±22.9^{d}	1089.9±45.7^{c}	213.0±7.05^{h}	441.0±26.0^{f}	581.2±20.5^{e}
Linoleic acid	1079.5±56.5^{d}	1570.6±87.8^{c}	2704.4±51.1^{a}	749.6±21.4^{f}	922.6±10.5^{e}	2173.2±71.6^{b}	593.1±5.06^{g}	706.4±19.5^{f}	701.4±20.3^{fg}
Linolenic acid	94.9±4.36^{b}	99.9±3.82^{b}	152.4±2.46^{a}	24.0±0.33^{f}	52.5±0.89^{d}	70.4±2.25^{c}	17.3±2.32^{g}	31.9±1.65^{e}	47.9±1.04^{d}
Total	1784.9±78.7^{c}	3405.3±188.8^{b}	4933.6±96.7^{a}	1113.1±29.0^{e}	1689.3±30.0^{c}	3333.5±116.0^{b}	823.4±11.1^{f}	1179.2±46.0^{de}	1330.5±41.2^{d}

Values were presented as normalized area by ribitol or n-tridecane (internal polar and non-polar standards, respectively). CTRL: Control fruits. Different superscript letters indicate statistical significance ($p < 0.05$) at the same line (mean ± standard deviation, n = 4). GABA, γ-aminobutyric acid.

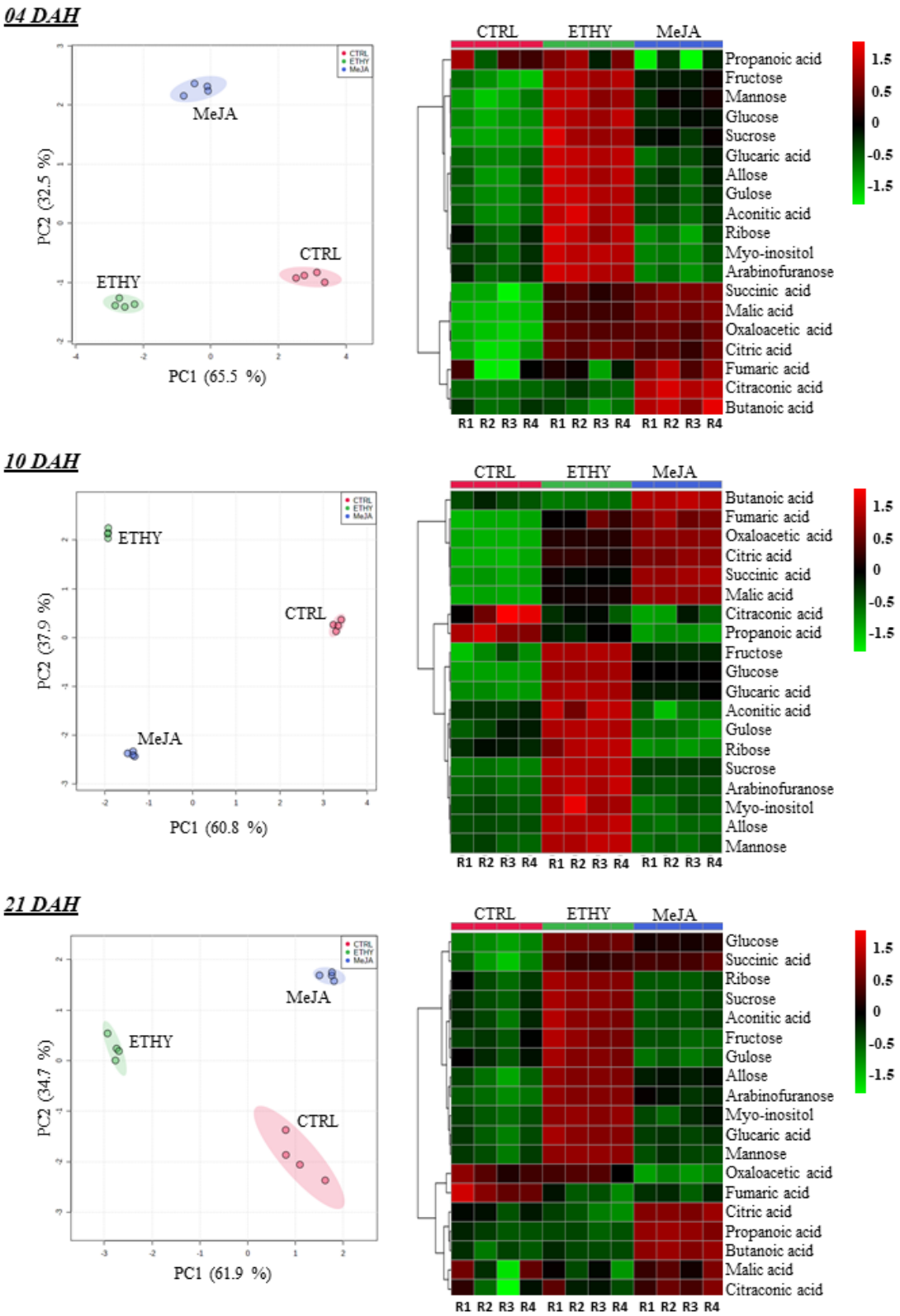

Figure 3. Relative contents of sugars and organic acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to ethylene (ETHY) and methyl jasmonate (MeJA) treatment compared to the control group (CTRL). Non-supervised principal

component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color scale represents the variation in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

As observed in organic acids, notable changes in amino acids metabolism were detected either by methyl jasmonate hormone. Highest accumulation was observed not only of major free amino acids such as glutamic acid, glutamine and GABA (involved in GABA shunt) and aspartic acid, but also of minor ones including aromatic amino acids involved in the shikimate pathway as tryptophan and phenyalanine in MeJA at 10 DAH (Table 1). However, methyl jasmonate showed a tendency to accumulate aspartic acid, glutamic acid, asparagine, serine, proline, and tryptophan in its fruits at 4 DAH (Figure 4). The total amino acids level was represented mostly by glutamic and aspartic acids (Table 1), which is interesting as they are considered a high-valued nutrient and important to fruit quality (Massaretto *et al.*, 2018). Glutamic acid (derivate from oxaloacetic acid and asparagine) and aspartic acid (derivate from α-cetoglutaric acid) levels were 30-fold increased by ethylene and methyl jasmonate hormones when compared with CTRL at 10 DAH. Also, both phytohormones positively regulated glutamine and GABA levels at least 15-fold higher than CTRL at 4 and 10 DAH (Figure 2). Glutamine is known to be an important form of nitrogen transport in tomato plants, and GABA is a four carbon non-protein amino acid that has received much attention as a health-promoting functional compound (Takayama & Ezura, 2015). From that, the role of free amino acids involved in GABA shunt in front of exogenous jasmonate hormone deserve more attention due to their importance in fruit ripening and nutritional quality.

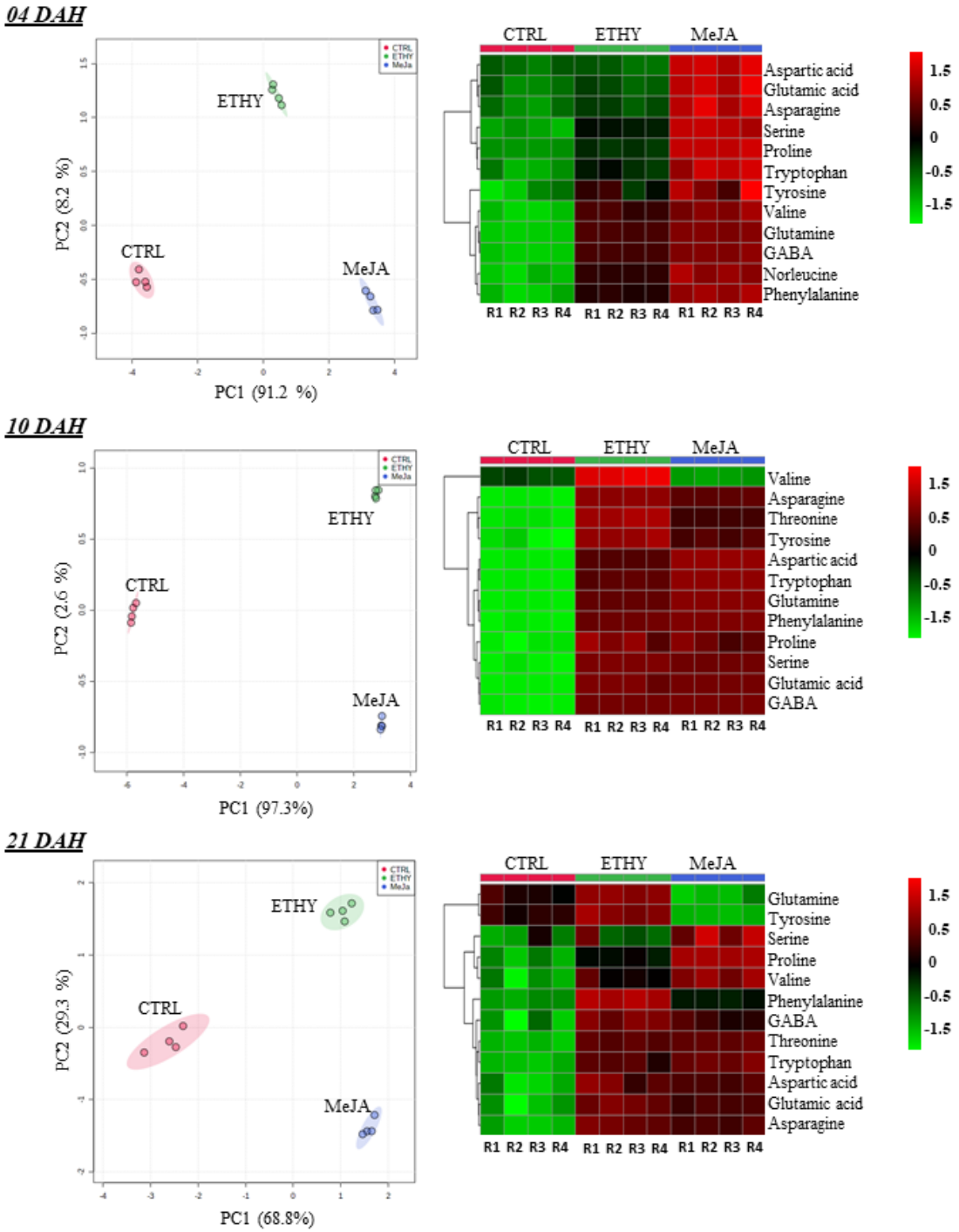

Figure 4. Relative contents of amino acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to ethylene (ETHY) and methyl jasmonate (MeJA) treatment compared to the control group (CTRL). Non-supervised principal component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color

scale represents the variation in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

The most remarkable difference among treatments regarding primary metabolism was found in the fatty acids profiles, since fruits treated with methyl jasmonate presented a stronger tendency to accumulate fatty acids in their fruits than CTRL and ETHY during ripening (Figure 5). Methyl jasmonate induced 2-fold higher the production of saturated and unsaturated fatty acids than CTRL in at least one maturation stage analyzed (Figure 2). Highest levels of fatty acids were detected in MeJA at 4 DAH (Table 1). Palmitic, capric and eicosanoic acids contributed essentialy with the total of saturated fatty acids level, while linoleic and oleic acids with the total of unsaturated fatty acids level. Linoleic and linolenic acids plays an important role as precursors of aroma compounds in tomato fruits, which is critical for the full ripe fruit quality.

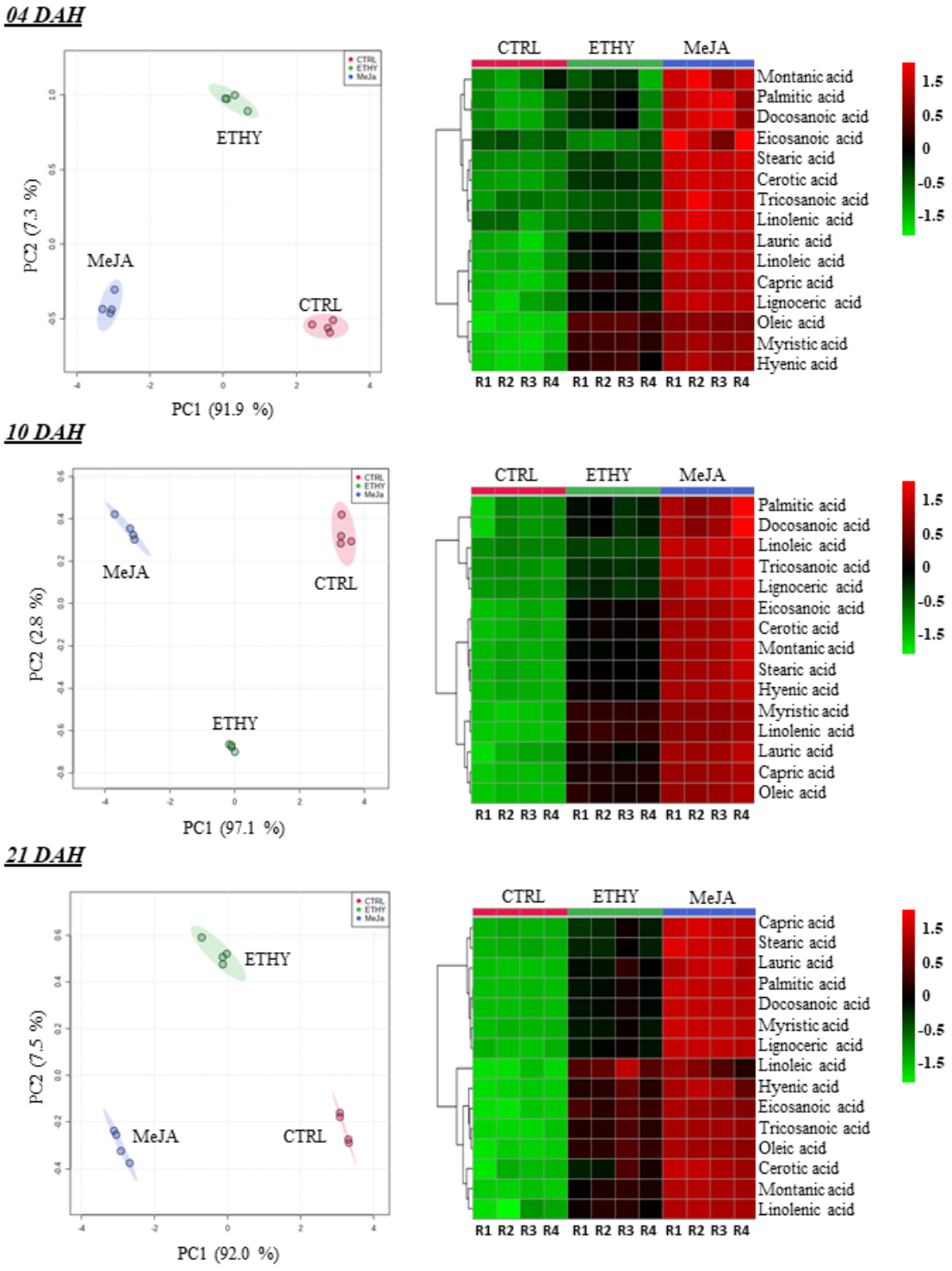

Figure 5. Relative contents of fatty acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to ethylene (ETHY) and methyl jasmonate (MeJA) treatment compared to the control group (CTRL). Non-supervised principal component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color

scale represents the variation in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

6.2.3 Secondary metabolite profiling affected by postharvest hormonal treatment

The secondary metabolites idenfied in Grape tomato fruits at, 10 and 21 DAH were: lycopene, β-carotene, and lutein by HPLC analysis; and α-tocopherol, β-tocopherol, γ-tocopherol, phytol, β-sitosterol, stigmasterol, and stigmastadienol by GC-MS analysis.

The most interesting difference in carotenoids accumulation was observe at 4 DAH when ethylene and methyl jasmonate improved total carotenoids levels 6- and 3-fold (Figure 2), respectively, compared with CTRL, resulting in major contents of lycopene, β-carotene, and lutein in treated tomato fruits during ripening (Figure 6A and Supplementary Figure S1). The antecipation in ripening process comproved by fruit surface color in ETHY and MeJA (Figure 1) can be related to the higher accumulation of these pigments when compared with CTRL during fruit ripening. The lycopene highlighted among the carotenoids due to the highest content found in Grape tomato fruits, representing mostly the total carotenoids levels (Figure 6A and Supplementary Figure S1). Major contents of lycopene, β-carotene, and lutein (1115.3; 10.16 and 5.25 $\mu g.g^{-1}$ FW, respectively) were detected at 21 DAH in ETHY followed by MeJA. However, an opposite behavior is observed at 10 DAH when higher lycopene, β-carotene and lutein levels were detected in MeJA (742.5; 7.25 and 4.43 $\mu g.g^{-1}$ FW, respectively) followed by ETHY.

Ethylene and methyl jasmonate induced significantly the production of α-tocopherol, β-tocopherol, and γ-tocopherol levels, mainly at 10 DAH. However, the highest accumulation of tocopherols were detected in MeJA (Figure 6B and

Supplementary Figure S2). The major source of tocopherol found in treated and non-treated Grape tomato fruits was the α form. Tocopherols levels were increased at least 2-fold by ethylene and methyl jasmonate treatments at 4 DAH (α-tocopherol) and at all stages (β-tocopherol) when compared to CTRL (Figure 2).

The accumulation of tocopherols, synthesized from phytyl diphosphate (PPP) generated by GGPP and homogentisic acid (HGA) from the shikimate pathway (Almeida *et al.*, 2015) in ripe fruits treated with jasmonate may be related to the presence of aromatic amino acids involved in shikimate pathway as phenylalanine, tryptophan and tyrosin mostly at 10 DAH, or still associated with the presence of phytol, precursor of tocopherol. Phytol was induced predominantly at 4 DAH by ethylene (2-fold) followed by methyl jasmonate (1.6-fold) treatments when compared to CTRL (Figure 2), decreasing by 80% their levels from 4th to 21th DAH (Figure 6B and Supplementary S2),

Carotenoids and tocopherols share a common precursor, geranylgeranyl diphosphate (GGPP), produced by the MEP pathway (Figure 2). Almeida *et al.* (2015) suggested that this metabolic interaction point out that changes in one of them may affect the biosynthesis of other metabolite. Interestingly, in both treated groups were observed higher contents of tocopherol at 10th DAH and carotenoids at 21th DAH, suggesting that the decrease in tocopherols is matched with a increase in carotenoids.This metabolic interaction suggests that changes in carotenoid biosynthesis and chlorophyll metabolism might affect tocopherol content, but a better understanding of the accumulation of these metabolites depends on deciphering the complexity of the isoprenoid metabolic network and its branching (Vranová *et al.*, 2012).

A similar behavior analyzed in tocopherol levels was observed in the phytosterols production. Highest phytosterols levels were observed in MeJA followed by ETHY at 10 DAH. Hormonal treatments applied enhanced the contents of β-sitosterol,

stigmasterol and stigmastadienol compared with CTRL. β-sitosterol and stigmasterol were the essential source of phytosterols indentifed in Grape tomato fruits. An increase of 60% of total phytosterols level were observed from 4 to 10 DAH in treated fruits. On the other hand, in the same fruits a reduction in phytosterols of 13% from 10 to 21 DAH was also notified (Figure 6C and Figure Supplementary S2). Compring to CTRL group, methyl jasmonate hormone improved 2-fold the levels of stigmastadienol and β-sitosterol at 10^{th} and 21^{th} DAH, respectively (Figure 2).

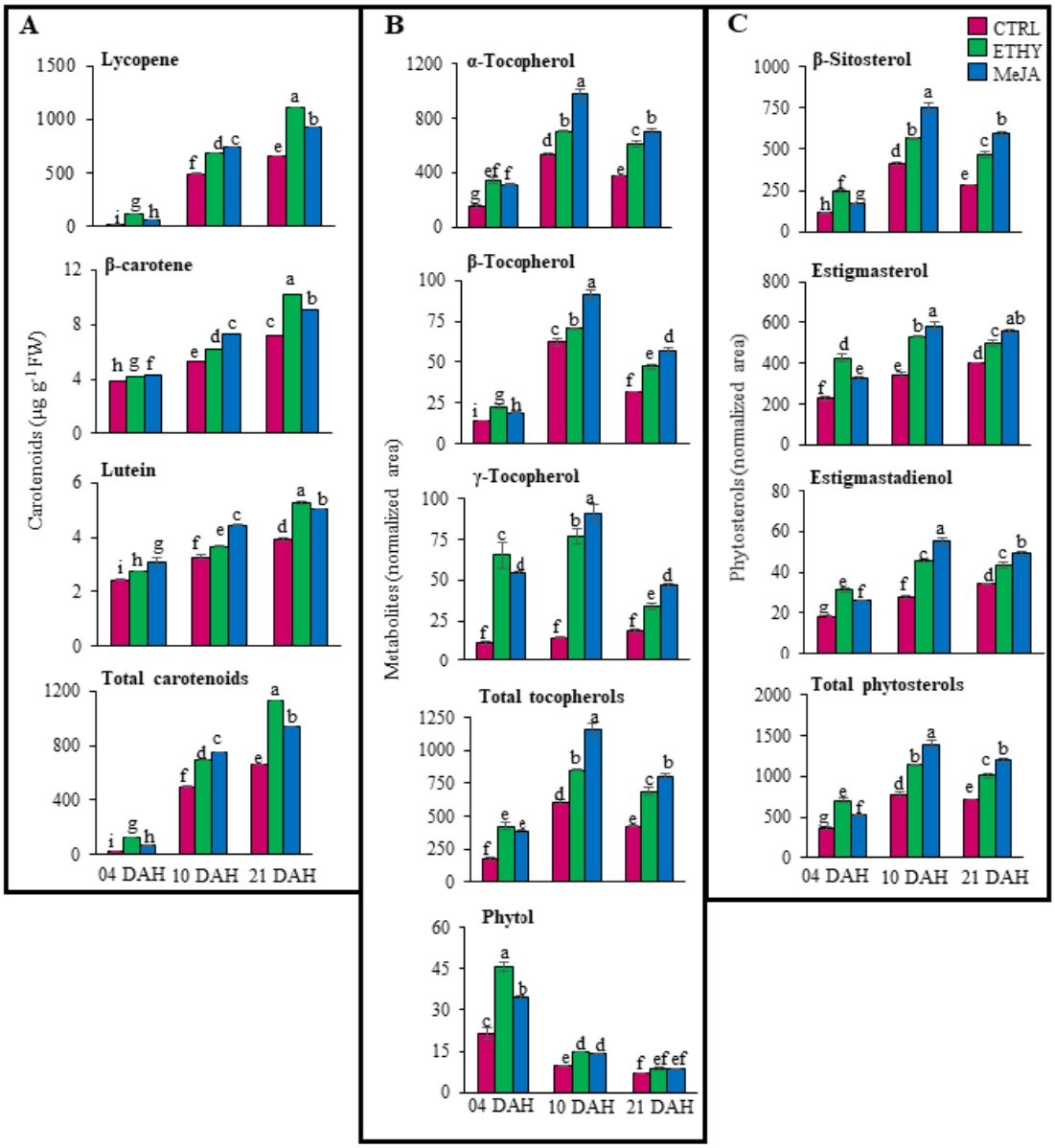

Figure 6. Secondary metabolites in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to ethylene (ETHY) and methyl jasmonate (MeJA) treatment compared to the control group (CTRL) at 04, 10 and 21 days after harvest (DAH). Contents of carotenoids (A), normalized area of tocopherols and phytol (B), and phytosterols (C). Values are means ± SE of four biological replicates of 10 fruits each. Different letters indicate statistically significant differences ($p < 0.05$).

6.2.4 Metabolite profiling of fruits treated with phytohormones

The metabolic profile of Grape tomato treated with phytohormones showed important impact to tomato fruit quality and nutritional value. Ethylene impacted predominantly sugars profile. Whereas the results of methyl jasmonate treatmentsuggest its role in organic acids (citric and malic acids) and fatty acids (mainly palmitic and linoleic acids) metabolisms, which are important to fruit quality. Amino acids, mainly with those involved in GABA shunt and shikimate pathway, responsible for plant growth and nutritional quality of fruit were positively affected by methyl jasmonate. Lycopene, α-tocopherol and β-sitosterol production were induced by methyl jasmonate, attributing a nutritional value of Grape tomato after hormonal treatment.

An global overview of metabolic pathway was built to observed the interaction and changes among sugars, organic acids, amino acids, fatty acids, carotenoids, tocopherols and phytosterols when compared to CTRL (Figure 2). Most of the metabolics interactions were observed by the action of methyl jasmonate in ripe fruits. For example, the increase of GABA level may be related to the largest contents of metabolites involved in GABA shunt as glutamic acid, succinic acid and glutamine at 10 DAH (Table 1), which were observed either by their higher accumulation compared to CTRL at same day (Figure 2). The production of asparagine and oxaloacetic acid mainly at 10 DAH can be responsible for the increase in the level of aspartic acid (Table 1), which refected in its accumulation of 40-fold increase when compared to CTRL (Figure 2). The presence of aromatic amino acids involved in shikimate pathway as phenylalanine, tryptophan and tyrosin at 10 DAH might be responsible for the great contents of tocopherols (Table 1 and Figure 6B), impacting also in the higher accumulation of these compounds compared to CTRL at this day (Figure 2). Finally, a metabolic interaction between tocopherols and

carotenoids was observed by concomitant deacrease in tocopherol and increase in carotenoids (Figure 6A and 6B, Supplementary Figure S1 and S2).

All these metabolites changes are relevant to study of fruit quality and nutritional value and may explain same metabolic interaction. However is important to point ou that detailed study related to integration of omics approaches such as genomic, transcriptomic, proteomic and metabolomic is essential to give better understanding about the metabolic profile of Grape tomato exposed to phytohormones.

6.3 Conclusion

Most of the detected metabolites changes were associated to the organoleptic and nutritional value of Grape tomato fruit. The ethylene exogenous treatment positively impacted soluble sugars (fructose, sucrose, and glucose) during the ripening. However, the most notable primary metabolites changes caused by methyl jasmonate treatment were the increased in the production of citric acid, succinic acid, malic acid, oxaloacetic acid, fumaric acid, glutamic acid, glutamine, GABA, aspartic acid, tryptophan and phenylalanine at 10 DAH; and fatty acids at 4 DAH. Changing in balance in sugars, organic acids and taste compounds, as glutamic acids, can straight impact taste perception. At the same time that secondary metabolites such as carotenoids, tocopherols, phytol and phytosterols directly related to the tomato nutritional value were also affected by the hormonal treatments. Methyl jasmonate significantly induced the accumulation of lycopene, α-tocopherol and β-sitosterol in full ripe fruits. Peculiarly, these phytochemicals had a greater impact at 4 DAH, showing at least 2 fold-increase compared with non-treated fruits. Taken together, our results suggest that methyl jasmonate and ethylene exogenous treatments can be used as tools to improve sensorial and nutritional

value of fruits, since important changes related to metabolome of tomato fruit were observed in metabolites responsible for the fruit quality.

6.4 Supplementary files

Supplementary Table S1. Carotenoids contents (μg.g^{-1} FW) in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to ethylene (ETHY) and methyl jasmonate (MeJA) treatment at 04, 10 and 21 days after harvest (DAH) detected by high performance liquid chromatography (HPLC).

Metabolite	**04 DAH**			**10 DAH**			**21 DAH**		
	CTRL	***ETHY***	***MeJA***	***CTRL***	***ETHY***	***MeJA***	***CTRL***	***ETHY***	***MeJA***
Lycopene	15.4±0.43^{i}	114.9±1.86^{g}	56.5±1.16^{h}	487.1±4.10^{f}	684.5±2.21^{d}	742.5±0.63^{c}	653.6±0.82^{e}	1115.3±0.54^{a}	923.5±0.99^{b}
β-carotene	3.87±0.02^{h}	4.16±0.03^{g}	4.26±0.03^{f}	5.32±0.10^{e}	6.23±0.02^{d}	7.25±0.03^{c}	7.23±0.01^{c}	10.2±0.03^{a}	9.13±0.01^{b}
Lutein	2.43±0.02^{i}	2.75±0.01^{h}	3.09±0.13^{g}	3.27±0.06^{f}	3.66±0.02^{e}	4.44±0.03^{c}	3.93±0.05^{d}	5.25±0.03^{a}	5.03±0.01^{b}
Total	21.7±0.42^{i}	121.8±2.18^{g}	63.8±1.22^{h}	495.6±4.10^{f}	694.4±2.18^{d}	754.1±0.63^{c}	664.7±0.85^{e}	1130.7±0.55^{a}	937.6±1.00^{b}

CTRL: Control fruits. Different superscript letters indicate statistical significance ($p < 0.05$) at the same line (mean ± standard deviation, n = 4).

Supplementary Table S2. Secondary metabolites in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to ethylene (ETHY) and methyl jasmonate (MeJA) treatment at 04, 10 and 21 days after harvest (DAH) detected by gas chromatography-mass spectrometry (GC-MS).

Metabolite	**04 DAH**			**10 DAH**			**21 DAH**		
	CTRL	***ETHY***	***MeJA***	***CTRL***	***ETHY***	***MeJA***	***CTRL***	***ETHY***	***MeJA***
A) Tocopherols									
α-tocopherol	152.7±14.5^{g}	337.4±21.7^{ef}	313.3±7.98^{f}	531.9±12.1^{d}	704.5±7.05^{b}	974.4±43.8^{a}	375.8±1.57^{e}	608.6±25.5^{c}	701.2±15.1^{b}
β-tocopherol	14.1±0.27^{i}	22.8±1.03^{g}	18.9±0.41^{h}	62.3±2.15^{c}	70.7±0.56^{b}	91.6±2.94^{a}	31.6±0.23^{f}	47.2±1.13^{e}	57.1±1.36^{d}
γ-tocopherol	11.6±0.49^{f}	65.3±7.87^{c}	54.8±0.87^{d}	14.6±0.38^{f}	76.6±4.67^{b}	91.1±5.03^{a}	18.9±0.72^{f}	33.9±1.96^{e}	46.6±1.09^{d}
Total	178.4±15.1^{f}	425.5±25.0^{e}	387.0±9.19^{e}	608.9±14.6^{d}	851.8±8.11^{b}	1157.1±45.3^{a}	426.4±1.45^{e}	689.6±26.5^{c}	804.9±17.5^{b}
B) Acyclic diterpenoids									
Phytol	21.3±2.25^{c}	45.7±1.80^{a}	34.7±0.58^{b}	9.54±0.18^{e}	14.6±0.10^{d}	14.1±0.29^{d}	6.75±0.04^{f}	8.73±0.41^{ef}	8.54±0.31^{ef}
C) Phytosterols									
β-sitosterol	113.2±7.14^{h}	244.8±9.71^{f}	244.8±4.04^{g}	410.2±10.9^{d}	566.1±4.20^{b}	750.6±27.1^{a}	282.7±1.32^{e}	466.7±14.6^{c}	594.4±14.1^{b}
Stigmasterol	227.3±10.5^{f}	427.1±10.7^{d}	326.6±6.49^{e}	344.1±8.75^{e}	528.2±3.64^{b}	581.4±21.5^{a}	400.8±1.71^{d}	497.1±15.6^{c}	554.8±12.3^{ab}
Stigmastadienol	18.2±0.26^{g}	31.2±1.09^{e}	26.0±0.50^{f}	27.8±0.68^{f}	45.4±0.72^{c}	55.1±1.79^{a}	34.2±0.16^{d}	43.7±1.36^{c}	49.2±1.18^{b}
Total	358.7±14.3^{g}	703.1±26.4^{e}	523.1±10.9^{f}	782.0±20.2^{d}	1139.7±2.59^{b}	1387.1±50.4^{a}	717.6±2.80^{e}	1007.5±31.5^{c}	1198.4±27.6^{b}

Values were presented as normalized area by n-tridecane (internal non-polar standard). CTRL: Control fruits. Different superscript letters indicate statistical significance ($p < 0.05$) at the same line (mean ± standard deviation, n = 4).

7. SECTION 3: EFFECT OF 1-METHYLCICLOPROPENE AND METHYL-JASMONATE ON METABOLITE PROFILING OF TOMATO

Postharvest Treatment with Methyl Jasmonate Impacts Lipid Metabolism in Tomatoes (Solanum lycopersicum L. cv. Grape) at Different Ripening Stages

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7.1 Objective

The 1-methylcyclopropene is an important inhibitor of ethylene action and is successfully employed for climacteric fruits. The high responsiveness of climacteric fruits to 1-methylcyclopropene is definitely due to the obligatory requirement for ethylene perception for the initiation and progression of fruit ripening (Zhang *et al*., 2009). It has been applied to prolong the shel-life of tomato throught retaining firmness, delaying lycopene production and consequently color development. Also, 1-methylcyclopropene reduces ethylene production and respiration rate of climacteric fruits (Su & Gubler, 2012; Cliff *et al*., 2009). In this study, we investigated the metabolic response to methyl jasmonate in Grape tomato fruits with ethylene inhibited by 1-methylcyclopropene during fruit ripening.

7.2 Results and Discussion

7.2.1 Effect of methyl jasmonate on the ethylene emission and fruit surface color in fruits with inhibited ethylene

In a previously study of our research group metabolic responses were observed in Grape tomato fruits treated with ethylene and methyl jasmonate hormones. Notable changes not only in primary metabolites such as linoleic acid, citric acid, malic acid, glutamic acid, aspartic acid, GABA, tryptophan, phenylalanine, but also in secondary metabolites such as lycopene, α-tocopherol and β-sitosterol were detected in fruits treated with methyl jasmonate hormone, improving fruit quality and nutritional value of the Grape tomatoes. From that, in this study we observed if the metabolic changes identified in fruits treated with methyl jasmonate are or not related to ethylene production. Therefore, one group of fruits with ethylene inhibited by 1-methylcyclopropene were

exposed to methy jasmonate hormone (MCP+MeJA), other group of fruit were treated only with 1-methylcyclopropene (MCP) and the no treated Grape tomato fruits (CTRL) were used as reference of the assays. The three groups of fruits were possible to visualize in the Figure 1A.

The CTRL group fruits achieved the breaker stage at 4 DAH and ripe stage at 10 DAH. Regading treated fruits, breaker and red stages were achieved by MCP at 13 and 21 DAH, respectively, while MCP+MeJA at 10 and 13 DAH, respectively. For the characterization of ripening stages of the CTRL group, measures of ethylene emission and surface color of the Grape tomato fruits were realized from the day of harvest to 21 DAH (Figure 1B and 1C). While the analysis of metabolite profiling were realized at 4, 10 and 21 DAH, aiming to observe the effect of treatments with respect to CTRL.

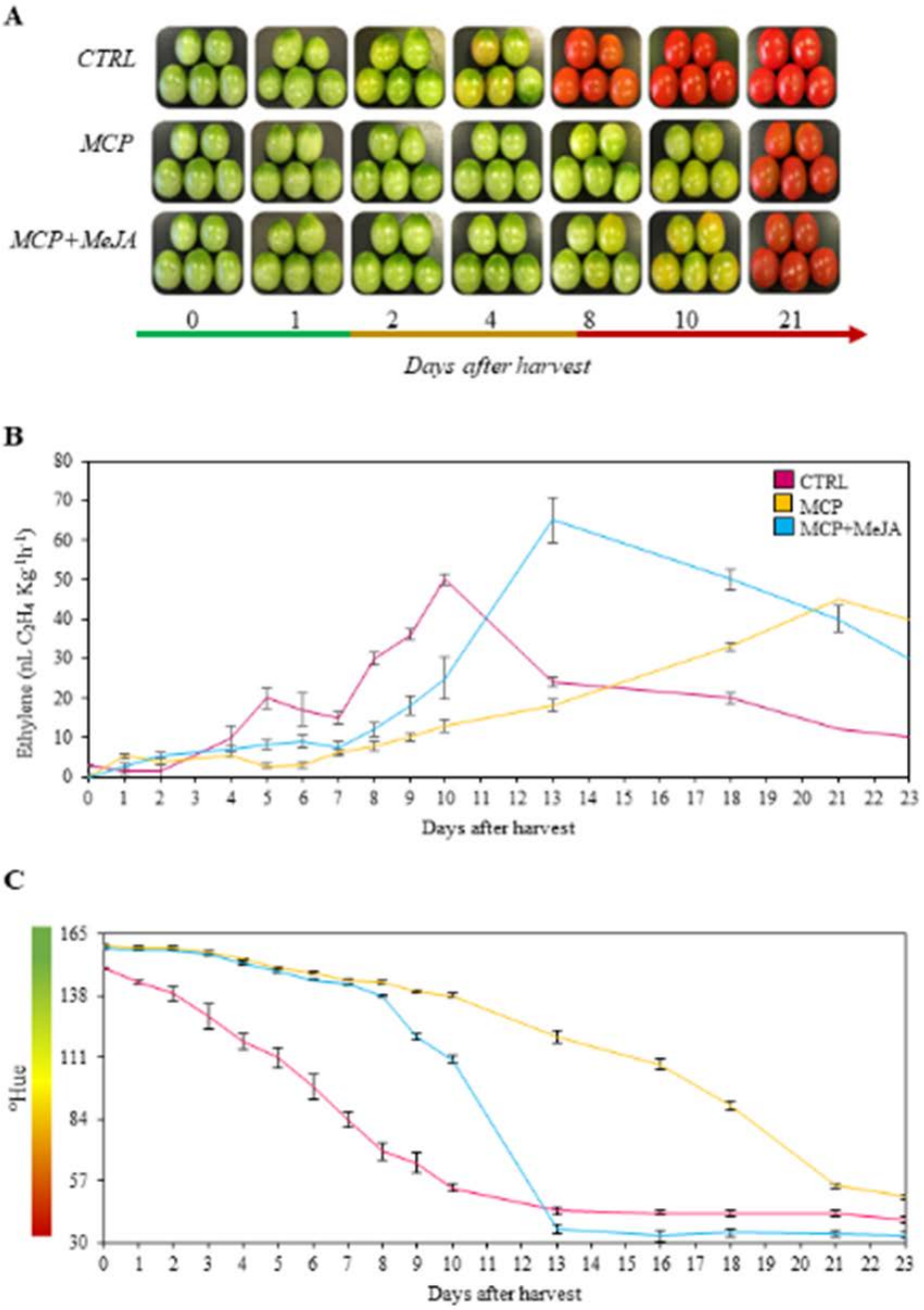

Figure 1. Characterization of tomato (*Solanum lycopersicum* L. cv. Grape) fruits treated with 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MCP+MeJA) during ripening. Representative images of tomatoes (A). Effects of MCP and MCP+MeJA on ethylene emission (B) and fruit color (C) compared to the control group (CTRL). Values are means ± standard error of four biological replicates of at least 10 fruits each.

Treatments with both 1-methylcyclopropene and methyl jasmonate, and only 1-methylcyclopropene showed a delay in fruit ripening by the reduction of ethylene emission and fruit surface color, when compared with CTRL group. Similar results were observed by Guillén *et al*. (2007), when treated tomato with 1-methylcyclopropene and reported a reduction in ethylene emission and respiration rate. Both groups MCP and MCP+MeJA presented the characteristics curves of ethylene emission of climacteric fruits. Fruits treated only with 1-methylcyclopropene showed the longest delay in fruit ripening, which were characterized by its peak ethylene and redness color at 21 DAH. While, tomatoes treated with both 1-methylcyclopropene and methyl jasmonate showed an ethylene peak at 13 DAH, when they acquired a reddish color.

It was possible to observe that the use of exogenous methyl jasmonate hormone in fruits with ethylene receptors blocked by 1-methylcyclopropene stimulated the ripening process when compared with those fruits treated only with 1-methylcyclopropene. This behavior induce that 1-methylcyclopropene is efficient to block ethylene receptors and consequently may avoid the interaction of ethylene with others phytohormones related to ripening processes such as the endogenous methyl jasmonate, occasioning the delay of fruit ripening. However, when dosis of exogenous methyl jasmonate hormone was applied in these fruits an acceleration in ripening was observed by the accumulation of pigments and anticipation of ethylene peak from 21 to 13 DAH. In addition, the highest peak of ethylene emission was observed in MCP+MeJA group which may be related to stimulation of ethylene biosynthesis in climacteric fruits by methyl jamosnate hormone. From that, our results suggest that the exogenous methyl jasmonate can act independently of ethylene or the blockage of ethylene receptors were reversed after some period. Therefore, for the treatment with MCP a synthesis of new receptors in Grape tomato could be possible as related in several fruits by Blankenship

and Dole (2003) and Guillén *et al*. (2007). This behavior may be responsible for the increase of ethylene production after some period, as it was obseverd after 10 DAH.

7.2.2 Effect of methyl jasmonate on the primary metabolite profiling in fruits with inhibited ethylene

Primary metabolites are major components of fruit quality and related metabolisms are considered crucial for plant growth. For this fact, more advances in its comprehension can facilitate the finding of future strategies for manipulation of fruit metabolism (Beauvoit *et al.*, 2018). In this work, a total of 46 primary metabolites were identified by metabolomic analysis: 10 sugars (glucose, fructose, sucrose, allose, gulose, glucaric acid, myo-inositol, mannose, ribose, and arabinofuranse); 9 organic acids (oxaloacetic, citric, succinic, aconitic, malic, citraconic, fumaric, propanoic, and butanoic acids); 12 amino acids (proline, serine, valine, threonine, aspartic acid, glutamic acid, glutamine, γ-aminobutyric acid (GABA), asparagine, tryptophan, phenylalanine, and tyrosine); 12 saturated fatty acids (capric, lauric, myristic, palmitic, stearic, eicosanoic, docosanoic, tricosanoic, lignoceric, hyenic, cerotic, and montanic acids); and 3 unsaturated fatty acids (oleic, linoleic, and linolenic acids) at 4, 10 and 21 DAH (Table 1). Also, a global overview of the metabolic changes occurring in Grape tomato during ripening was performed to evaluate significant differences among accumulated metabolites in treated fruits compared with control group (Figure 2).

Table 1. Primary metabolites in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MeJA+MCP) treatments at 04, 10 and 21 days after harvest (DAH) detected by gas chromatography-mass spectrometry (GC-MS).

Metabolite	**04 DAH**			**10 DAH**			**21 DAH**		
	CTRL	***MCP***	***MCP+MeJA***	***CTRL***	***MCP***	***MCP+MeJA***	***CTRL***	***MCP***	***MCP+MeJA***
A) Sugars									
Glucose	1534,5±76,0^{d}	70,6±4,67^{g}	598,7±37,9^{f}	1977,6±11,4^{c}	156,5±7,5^{g}	2977,0±253,0^{b}	4352,0±281,0^{a}	189,4±15,2^{g}	1112,6±140,1^{e}
Fructose	27474,0±4039,0^{d}	3935,0±489,0^{e}	37418,0±5231,0^{c}	59266,0±6310,0^{b}	4858,0±544,0^{e}	26343,0±3352,0^{d}	101194,0±5662,0^{a}	25944,0±1592,0^{d}	24324,0±1808,0^{d}
Sucrose	38205,0±569,0^{c}	5105,0±559,0^{f}	20507,0±1161,0^{d}	54654,0±716,0^{b}	3661,0±356,0^{f}	11961,0±469,0^{e}	84839,0±4545,0^{a}	10885,0±358,0^{e}	11248,0±284,0^{e}
Allose	1098,6±44,2^{c}	172,6±12,6^{ef}	115,1±4,78^{f}	1563,4±21,5^{b}	630,6±39,0^{d}	687,0±56,9^{d}	3309,0±380,0^{a}	469,1±49,6^{de}	584,1±27,8^{d}
Gulose	221,5±9,78^{d}	196,9±15,1^{d}	229,8±10,9^{d}	790,4±38,3^{b}	92,6±4,47^{e}	228,8±10,4^{d}	1017,0±60,4^{a}	183,5±17,6^{d}	404,0±12,9^{c}
Glucaric acid	42,2±1,61^{d}	21,9±2,08^{e}	23,1±0,44^{e}	72,0±1,30^{c}	93,4±6,25^{b}	126,7±8,50^{a}	124,6±8,69^{a}	15,1±1,33^{e}	21,2±0,83^{e}
Myo-inositol	77,9±2,91^{e}	33,1±3,20^{f}	91,3±4,53^{e}	169,6±2,04^{d}	178,5±8,05^{d}	156,6±4,09^{d}	340,1±20,6^{c}	412,4±23,9^{b}	481,0±9,67^{a}
Mannose	42,2±3,10^{d}	3,37±0,22^{f}	6,00±0,26^{f}	100,0±2,30^{b}	69,5±3,46^{c}	148,3±4,68^{a}	142,4±11,04^{a}	28,9±2,42^{e}	66,9±9,67c
Ribose	174,6±7,42^{c}	5,89±0,40^{g}	14,35±0,57^{g}	249,4±3,91^{b}	26,2±1,29^{g}	128,5±3,75^{d}	386,4±27^{a}	60,7±6,44^{f}	89,8±3,46^{f}
Arabinofuranose	15,1±0,74^{c}	2,12±0,28^{e}	4,74±0,13^{e}	25,5±0,84^{b}	9,33±0,62^{d}	8,06±0,54^{d}	45,2±3,28^{a}	17,17±1,44^{c}	17,61±1,61^{c}
Total	68885,0±4082,0^{c}	9546,0±771,0^{e}	59009,0±5831,0^{c}	118868,0±5993,0^{b}	9775,0±904,0^{e}	42764,0±3869,0^{d}	195750,0±9973,0^{a}	38206,0±1874,0^{d}	38348,0±2196,0^{d}
B) Organic acids									
Oxaloacetic acid	573,3±24,3^{e}	173,3±18,6^{f}	214,5±6,23^{f}	2380,4±56,5^{a}	1234,0±59,1^{c}	1482,7±55,4^{b}	1241,9±80,7^{c}	703,7±72,1^{d}	823,0±24,2^{d}
Citric acid	6517,0±413,0^{c}	765,4±85,1^{e}	998,9±62,0^{e}	7878,0±457,0^{c}	889,9±79,5^{e}	4117,0±591,0^{d}	18901,0±1208,0^{a}	15494,0±1393,0^{b}	15948,0±747,0^{b}
Succinic acid	2646,0±360,0^{cd}	642,8±49,6^{f}	1909,3±81,4^{de}	12894,0±485,0^{a}	2826,0±650,0^{c}	3587,5±102,0^{b}	2862,0±254,0^{bc}	1851,1±135,1^{e}	2372,9±71,7^{cde}
Aconitic acid	61,1±2,90^{c}	2,91±0,31^{f}	52,9±2,63^{d}	83,1±0,86^{b}	4,22±0,63^{f}	51,0±1,06^{d}	101,4±5,70^{a}	18,2±1,57^{e}	67,1±2,84^{c}
Malic acid	2537,5±101,3^{d}	118,7±5,34^{f}	156,1±6,21^{f}	6653,7±174,0^{c}	1208,0±109,4^{ef}	2076,1±46,3^{de}	16800,0±1014,0^{a}	13024,0±1137,0^{b}	12285,0±385,0^{b}
Citraconic acid	17,3±0,74^{c}	4,15±0,34^{d}	3,73±0,24^{d}	104,5±12,4^{a}	3,87±0,18^{d}	2,74±0,30^{d}	101,9±6,07^{a}	20,9±1,86^{c}	49,0±2,18^{b}
Fumaric acid	167,9±6,66^{c}	61,0±5,29^{fg}	73,4±2,73^{f}	181,7±1,17^{b}	47,7±1,99^{h}	99,0±2,98^{e}	237,8±10,3^{a}	55,8±5,18^{gh}	128,6±5,42^{d}
Propanoic acid	111,7±6,77^{c}	14,6±2,06^{e}	19,0±1,17^{de}	145,6±1,5^{c}	15,4±0,76^{e}	15,4±0,50^{e}	458,6±79,1^{b}	90,7±5,02^{cd}	1318,0±51,3^{a}
Butanoic acid	284,5±18,9^{cd}	221,3±19,7^{d}	314,9±8,60^{cd}	393,4±10,9^{c}	179,5±8,62^{d}	274,4±5,89^{cd}	641,8±96,9^{b}	412,7±22,5^{c}	2404,7±175,3^{a}
Total	12917,0±804,0^{d}	2003,4±173,2^{f}	3743,0±130,7^{ef}	30715,0±907,0^{c}	6409,0±787,0^{e}	11706,0±691,0^{d}	41346,0±2535,0^{a}	31671,0±2748,0^{c}	35397,0±1239,0^{b}

Continued Table 1

Metabolite	04 DAH			10 DAH			21 DAH		
	CTRL	*MCP*	*MCP+MeJA*	*CTRL*	*MCP*	*MCP+MeJA*	*CTRL*	*MCP*	*MCP+MeJA*
C) Amino acids									
Proline	501,2±14,8^{d}	798,4±71,8^{bcd}	992,4±55,3^{bc}	1167,2±26,8^{b}	693,5±40,9^{cd}	470,9±38,8^{d}	2863,8±483,0^{a}	2546,8±166,2^{a}	2452,7±44,8^{a}
Serine	78,0±5,68^{b}	24,8±1,38^{e}	58,5±4,32^{c}	93,6±2,26^{a}	27,7±1,98^{e}	24,8±1,02^{e}	49,0±1,56^{d}	16,1±1,95^{f}	26,9±1,35^{e}
Valine	6,22±0,18^{e}	1,89±0,18^{f}	2,24±0,04^{f}	17,6±0,24^{a}	17,0±0,76^{a}	14,9±0,52^{b}	7,93±0,40^{d}	6,60±0,89^{e}	9,10±0,30^{c}
Threonine	6,01±0,45^{e}	4,29±0,59^{e}	6,42±0,11^{de}	25,9±0,44^{b}	9,88±0,48^{d}	14,9±0,54^{c}	25,2±0,85^{b}	26,4±2,71^{b}	50,5±3,51^{a}
Aspartic acid	1523,2±54,2^{b}	54,7±4,83^{e}	141,7±3,94^{e}	2166,8±58,7^{a}	823,2±42,5^{d}	1163,1±43,0^{c}	1540,8±161,0^{b}	1148,6±97,4^{c}	1116,9±43,0^{c}
Glutamic acid	1744,3±75,6^{d}	215,3±17,3^{e}	228,7±8,32^{e}	4906,7±42,6^{a}	1614,2±71,8^{d}	1530,1±50,3^{d}	3957,0±424,0^{b}	2729,0±208,0^{c}	2551,6±68,0^{c}
Glutamine	185,1±7,71^{c}	77,8±5,64^{e}	172,9±4,83^{c}	519,3±9,24^{a}	99,9±5,26^{de}	148,3±4,71^{cd}	475,8±38,7^{a}	351,8±38,2^{b}	314,9±24,4^{b}
GABA	207,6±17,2^{bc}	148,4±13,2^{de}	173,9±4,47^{cd}	1060,8±45,9^{a}	104,3±4,63^{ef}	76,9±4,36^{f}	216,9±20,6^{bc}	221,9±23,6^{bc}	242,5±12,0^{b}
Asparagine	139,7±6,22^{c}	19,8±1,72^{e}	22,6±0,52^{e}	260,2±6,88^{a}	117,6±5,58^{d}	142,8±4,84^{c}	235,6±11,7^{b}	134,2±10,9^{cd}	148,5±11,1^{c}
Tryptophan	173,7±7,10^{b}	21,9±1,96^{d}	22,8±0,87^{d}	321,4±8,65^{a}	154,6±7,93^{c}	154,2±4,93^{c}	27,1±0,94^{d}	25,0±1,78^{d}	31,9±2,85^{d}
Phenylalanine	7,64±0,47^{e}	76,2±9,84^{a}	69,1±2,14^{a}	26,4±1,32^{d}	5,90±0,30^{e}	8,33±0,38^{e}	50,9±2,67^{b}	36,3±4,96^{c}	36,4±1,61^{c}
Tyrosine	21,1±0,77^{e}	47,8±3,61^{c}	71,0±3,93^{a}	27,8±2,37^{de}	35,7±2,0^{d}	27,7±1,08^{de}	62,3±3,70^{b}	51,9±5,01^{c}	46,4±4,33^{c}
Total	4593,8±170,8^{d}	1491,4±125,1^{f}	1962,3±70,7^{f}	10593,8±94,5^{a}	3703,5±149,7^{e}	3777,0±148,3^{de}	9512,0±940,0^{b}	7294,0±482,0^{c}	35397,0±1239,0^{b}
D) Saturated fatty acids									
Capric acid	61,9±1,90^{a}	26,6±0,62^{c}	52,2±0,71^{b}	15,0±0,41^{d}	1,48±0,08^{g}	2,27±0,01^{g}	6,77±0,03^{e}	4,05±0,11^{f}	5,76±0,13^{ef}
Lauric acid	40,5±2,10^{a}	18,9±0,08^{c}	22,4±0,35^{b}	19,7±1,16^{c}	1,40±0,07^{e}	2,14±0,04^{e}	6,81±0,03^{d}	5,56±0,11^{d}	5,91±0,15^{d}
Myristic acid	19,0±0,83^{a}	0,76±0,03^{f}	2,08±0,02^{e}	15,0±0,50^{b}	1,25±0,05^{f}	2,56±0,03^{e}	6,13±0,07^{c}	4,89±0,12^{d}	5,94±0,13^{c}
Palmitic acid	436,7±10,4^{a}	148,7±5,32^{e}	285,9±23,0^{d}	413,1±10,9^{ab}	23,9±0,91^{f}	36,2±0,31^{f}	159,6±1,41^{e}	349,8±6,23^{c}	400,5±9,30^{b}
Stearic acid	16,5±0,49^{a}	9,20±0,52^{ef}	8,60±0,16^{f}	14,6±0,26^{b}	1,01±0,05^{h}	13,2±0,07^{c}	9,94±0,18^{d}	7,61±0,23^{g}	9,34±0,07^{de}
Eicosanoic acid	93,9±1,59^{b}	84,1±6,01^{c}	170,9±2,83^{a}	10,19±0,31^{d}	5,76±0,25^{de}	8,73±0,17^{d}	7,16±0,17^{d}	7,10±0,11^{d}	0,74±0,02^{de}
Docosanoic acid	43,7±1,04^{b}	23,6±2,36^{c}	53,8±1,60^{a}	41,3±1,09^{b}	6,72±0,29^{f}	10,9±0,13^{e}	16,0±0,14^{d}	8,22±0,19^{f}	9,24±0,42^{ef}
Tricosanoic acid	27,6±1,15^{b}	22,7±1,28^{c}	45,2±0,31^{a}	20,3±0,28^{d}	5,50±0,26^{g}	8,83±0,18^{e}	7,08±0,17^{f}	6,62±0,13^{fg}	7,68±0,27^{ef}
Lignoceric acid	22,1±1,82^{c}	163,6±5,06^{b}	618,9±23,1^{a}	13,9±0,19^{c}	5,27±0,21^{c}	11,1±0,48^{c}	7,17±0,23^{c}	6,84±0,12^{c}	7,79±0,39^{c}
Hyenic acid	32,5±1,05^{b}	25,8±0,23^{c}	37,2±1,73^{a}	8,82±0,21^{d}	3,27±0,13^{f}	6,92±0,02^{e}	6,58±0,08^{e}	5,66±0,10^{e}	7,28±0,15^{de}
Cerotic acid	10,0±0,70^{b}	7,86±0,38^{cd}	26,9±1,64^{a}	10,2±0,31^{b}	5,76±0,25^{e}	8,73±0,17^{bc}	7,29±0,16^{cd}	7,18±0,11^{de}	7,53±0,28^{cd}
Montanic acid	34,4±1,33^{a}	15,2±0,40^{c}	17,7±0,63^{b}	9,49±0,22^{d}	5,37±0,23^{f}	7,39±0,08^{e}	9,85±0,16^{d}	6,11±0,12^{f}	9,13±0,15^{d}
Total	838,8±20,6^{b}	547,0±6,96^{d}	1341,7±15,0^{a}	591,6±14,5^{c}	66,7±2,68^{i}	119,0±0,51^{h}	250,4±2,03^{g}	419,7±7,50^{f}	476,8±11,0^{e}

Continued Table 1

Metabolite	04 DAH			10 DAH			21 DAH		
	CTRL	***MCP***	***MCP+MeJA***	***CTRL***	***MCP***	***MCP+MeJA***	***CTRL***	***MCP***	***MCP+MeJA***
E) Unsaturated fatty acids									
Oleic acid	610,5±26,0^{b}	278,4±5,43^{d}	866,7±20,9^{a}	339,4±7,63^{c}	19,9±0,92^{g}	44,8±0,42^{g}	213,0±7,05^{e}	103,6±10,5^{f}	340,2±6,53^{c}
Linoleic acid	1079,5±56,5^{c}	8,77±0,51^{g}	46,9±0,31^{g}	749,6±21,4^{d}	285,0±14,9^{f}	588,5±16,7^{e}	593,1±5,06^{e}	5065,0±93,4^{b}	5880,9±144,9^{b}
α-Linolenic acid	94,9±4,36^{c}	354,6±6,55^{a}	292,1±1,54^{b}	24,0±0,33^{ef}	11,3±0,53^{g}	33,2±0,14^{d}	17,3±2,32^{fg}	14,1±0,44^{g}	25,3±1,10^{e}
Total	1784,9±78,7^{c}	641,8±10,3^{f}	1205,7±22,1^{d}	1113,1±29,0^{d}	316,2±15,8^{g}	666,5±16,4^{f}	823,4±11,1^{e}	5183,7±89,5^{b}	6246,4±147,8^{a}

Values were presented as normalized area by ribitol or n-tridecane (internal polar and non-polar standards, respectively). CTRL: Control fruits. Different superscript letters indicate statistical significance ($p < 0.05$) at the same line (mean ± standard deviation, n = 4). GABA, γ-aminobutyric acid.

A PCA was performed on primary metabolites at 4th, 10th and 21th ripening stages and confirmed the high reproducibility among the four biological replicates and groups analyzed. Also, a cleary separation of CTRL group and both treated groups was evidenced for the primary metabolites in the PCA-score. Heatmap analysis was used to analyze the differences between treated and no-treated groups regarding the metabolites changes at each day after harvest analyzed.

Definitly, treatment with 1-methylcyclopropene impacted sugar and organic acids, inhibiting their production during ripening. Fruits treated only with 1-methylcyclopropene were most affected, showing a greater delay in accumulate sugars and organic acids than those fruits treated with both 1-methylcyclopropene and methyl jasmonate (Figure 2). For instance, glucose showed a significantly reduction of 22, 13 and 23 fold at 4, 10 and 21 DAH, respectively, in MCP when compared with CTRL. Manose, ribose, malic and aconitic acids exhibited a decrease in their levels of 14, 30, 21 and 20 fold at 4 DAH, whereas fructose, sucrose and citraconic acid showed 12, 15 and 27 fold lower levels at 10 DAH when compared with CTRL (Table 1 and Figure 2).

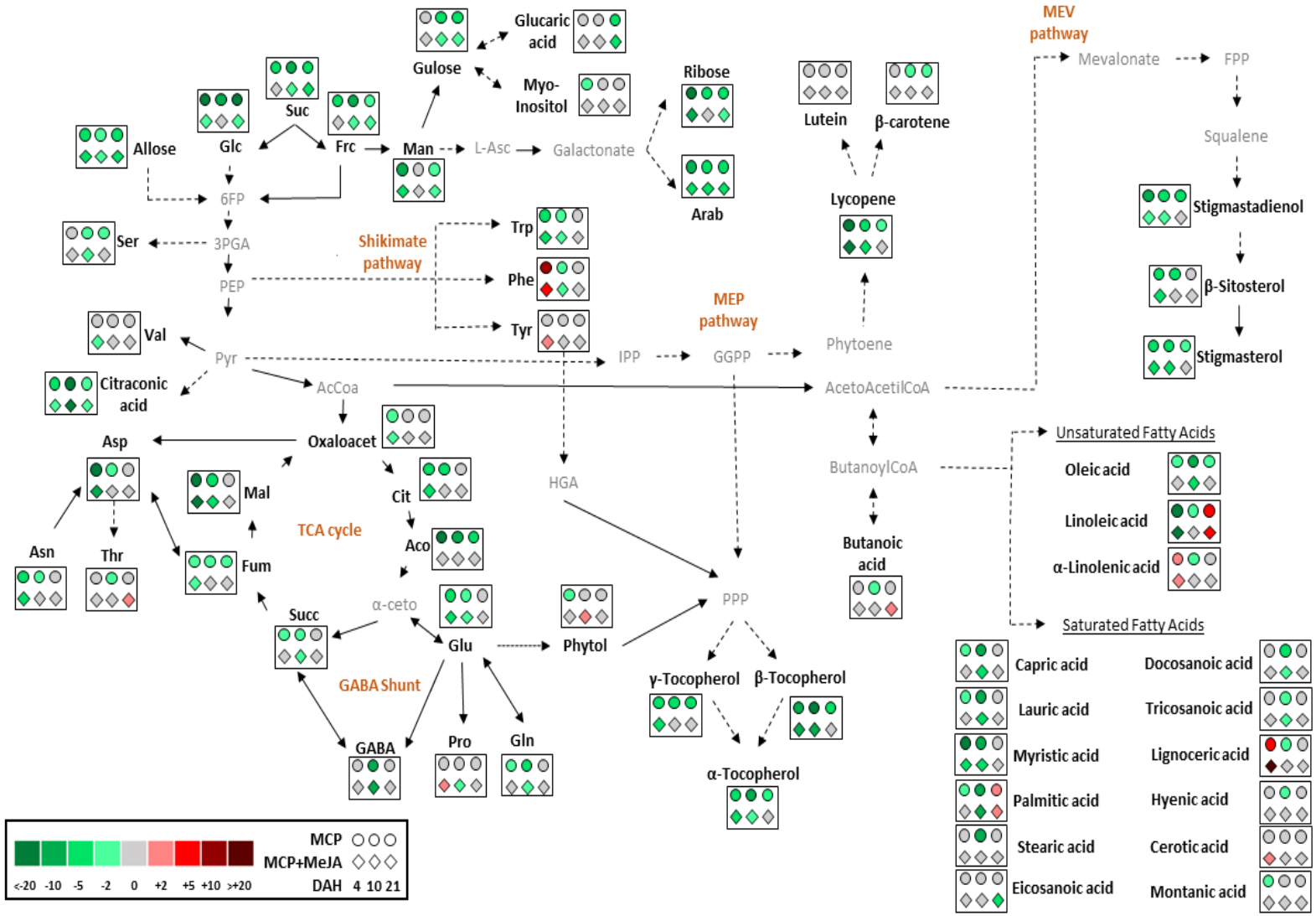

Figure 2. Global overview of metabolic changes occurring in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MCP+MeJA) treatments at 04, 10 and 21 days after harvest (DAH). Data were normalized to the control group (CTRL) . Only those metabolites showing up-regulation or down-regulation in each treatment higher 2.0 fold than control group are showed. Color scale is used to display the different amount of metabolite in terms of fold-change relative to the level in the appropriate control. Suc, sucrose; Glc, glucose; Frc, fructose; Man, Mannose; L-Asc, L-ascorbic acid; Arab, Arabinofuranose; 6FP, fructose-6-phosphate; 3-GPA, glyceraldehyde-3-phosphate; Ser, serine; PEP, phosphoenolpyruvate; Trp, tryptophan; Phe, phenylalanine; Tyr, Tyrosine; HGA, homogentisic acid; Pyr, pyruvic acid; IPP, isopentenyl pyrophosphate; GGPP, geranylgeranyl; PPP, phytyl pyrophosphate; Val, valine; AcCoA, acetyl-CoA; Oxaloacet, oxaloacetic acid; Cit, citric acid; Aco, aconitic acid; α-ceto, α-cetoglutaric acid; Succ, succinic acid; Fum, fumaric acid; Mal, malic acid; Glu, glutamic acid; GABA, γ-aminobutyric acid; Gln, glutamine; Pro, proline; Arg, arginine; Asp, aspartic acid; Thr, threonine; Asn, asparagine; FPP, farnesyl pyrophosphate.

Exceptionally, levels of glucose, glucaric acid and mannose showed an increase at 10 DAH in MCP+MeJA when compared to CTRL. Similar behavior was observed by myo-inositol, propanoic and butanoic acids at 21 DAH (Table 1, Figure 3). As observed by ethylene emission, the minor impact on the production sugars and organic acids observed by MCP+MeJA may suggest that methyl jasmonate play an important role in ripening process, which may act independently of endogenous ethylene, or a stimulation of the synthesis of new receptors, or the blockage of ethylene receptos were reversed after some period.

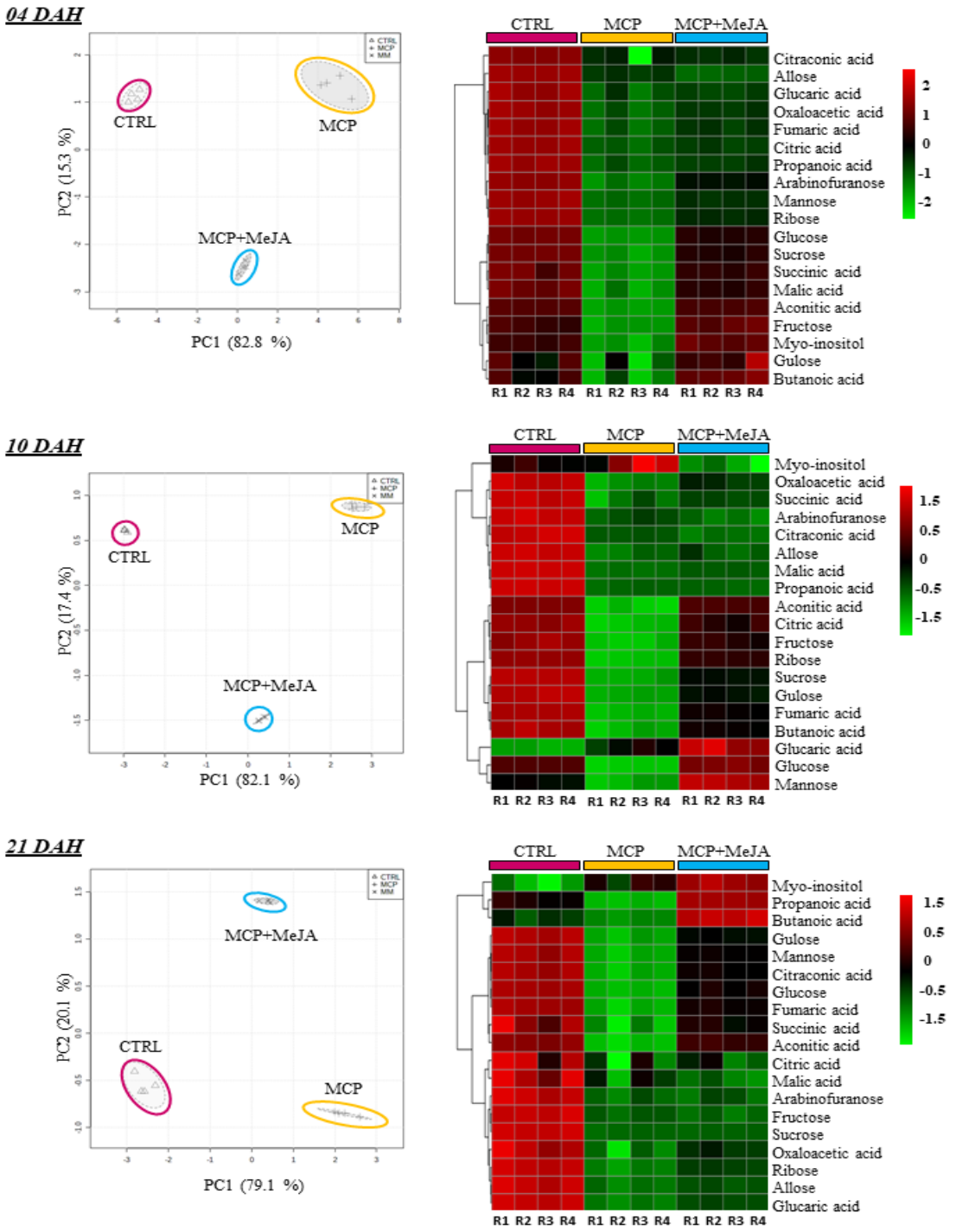

Figure 3. Relative contents of sugars and organic acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MCP+MeJA) treatments compared to the control group (CTRL). Non-supervised principal component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color

scale represents the variation in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

Amino acids profiling were also affected by the action of 1-methylcyclopropene. A inhibition in the production of amino acids during ripening were observed in both MPC and MCP+MeJA when compared with control (Figure 2 and 4). The most affected amino acids were aspartic acid at 4 DAH and GABA at 10 DAH, showing a reduction in their levels of 28 and 10 fold in MPC, respectively, while MCP+MeJA showed 11 and 14 fold decreased, respectively, when compared with CTRL. In contrast, tyrosine and phenylalanine showed levels 2 and 9 fold higher in MCP and MCP+MeJA at 4 DAH when compared with CTRL (Table 1, Figure 2). Phenylalanine and tyrosine are important aromatic amino acids which participate of shikimate pathway and are responsabile for aroma development of fruit. The total amino acids level was represented mostly by proline, glutamic and aspartic acids, which are important to fruit quality (Table 1).

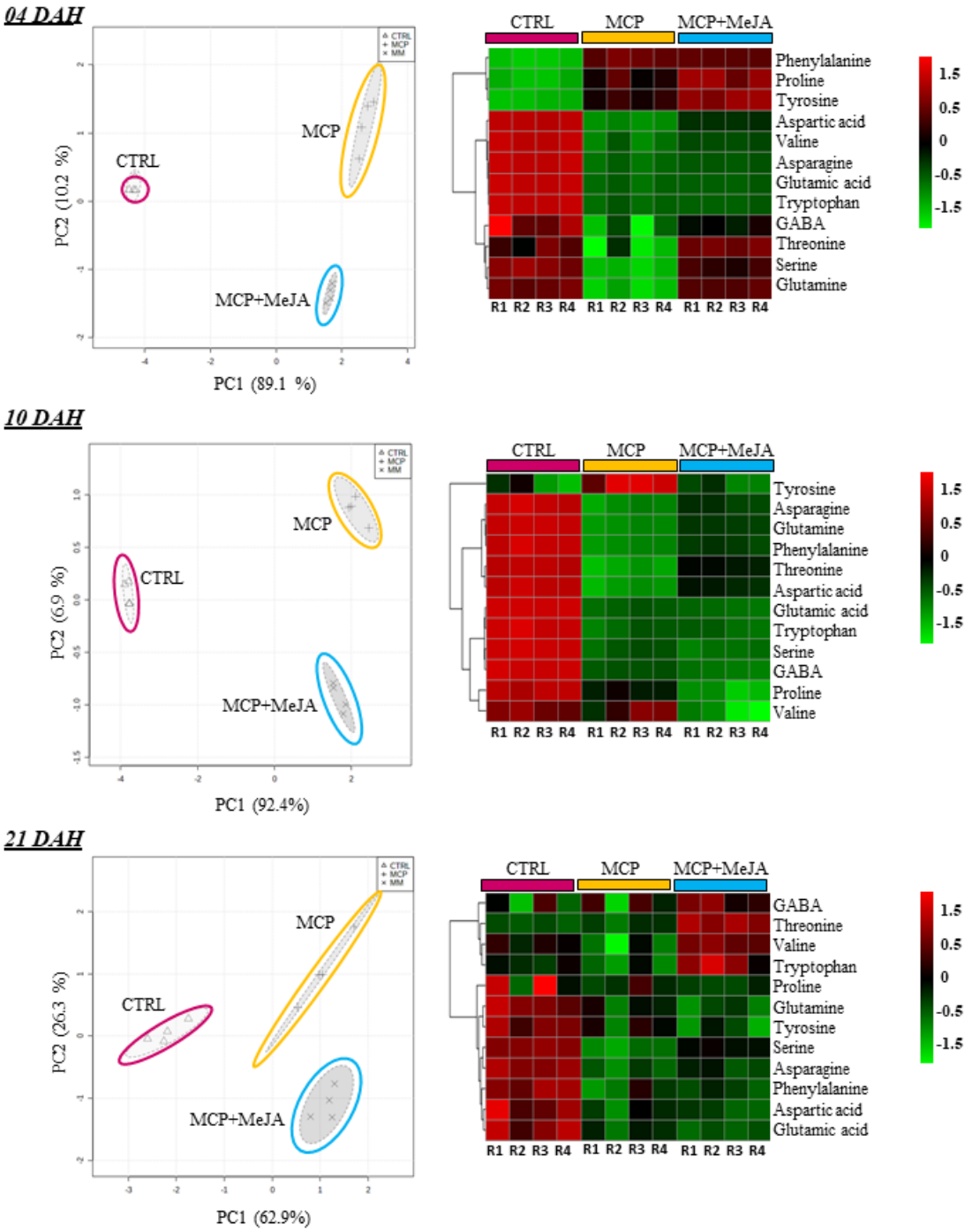

Figure 4. Relative contents of amino acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MCP+MeJA) treatments compared to the control group (CTRL). Non-supervised principal component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color scale represents the variation

in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

In addition, fatty acids profiling were also affected by the post-harvest treatments. The action of 1-methylciclopropene showed a greater impact on fatty acids such as oleic, capric, lauric, palmitic, stearic and myristic acids at 10 DAH, decreasing their levels 17, 10, 14, 17, 14 and 12 fold in MPC group, respectively, and 7, 6, 9, 11, 1, 7 fold in MCP+MeJA, repectively, when compared with CTRL. MCP+MeJA group also showed a reduction in fatty acids levels, but they were lesser impacted when compared with MPC group. However, the most impacted was the linoleic and myristic acids at 4 DAH with a reduction of 119 and 26 fold in MCP, respectively, and 23 and 9 in MCP+MeJA, repectively, when compared with CTRL (Table 1, Figure 2).

In contrast, an increase in the levels of some fatty acids was also detected as well as in lignoceric, cerotic, α-linolenic acids at 4 DAH, and palmitic and linoleic acids at 21 DAH by MCP and MCP+MeJA groups (Figure 2 and 5). In MCP group was detected an increase in the levels of lignoceric and α-linolenic acids at 4 DAH by 7 and 4 fold, respectively, while in MCP+MeJA the increase was 28 and 3 fold, respectively. Moreover, palmitic and linoleic acids was increased by 2 and 8 fold, respectively, in MCP, and 3 and 10, respectively, in MCP+MeJA at 21 DAH (Table 1). Interesting, MCP+MeJA group was lesser impacted when reductions were observed, and greater impacted when increases were observed comparing with MCP. This behavior may induce that methyl jasmonate can act as stimulator in the production of fatty acids. Palmitic and eicosanoic acids contributed essentialy with the total of saturated fatty acids level, whereas oleic and linoleic acids with the total of unsaturated fatty acids level.

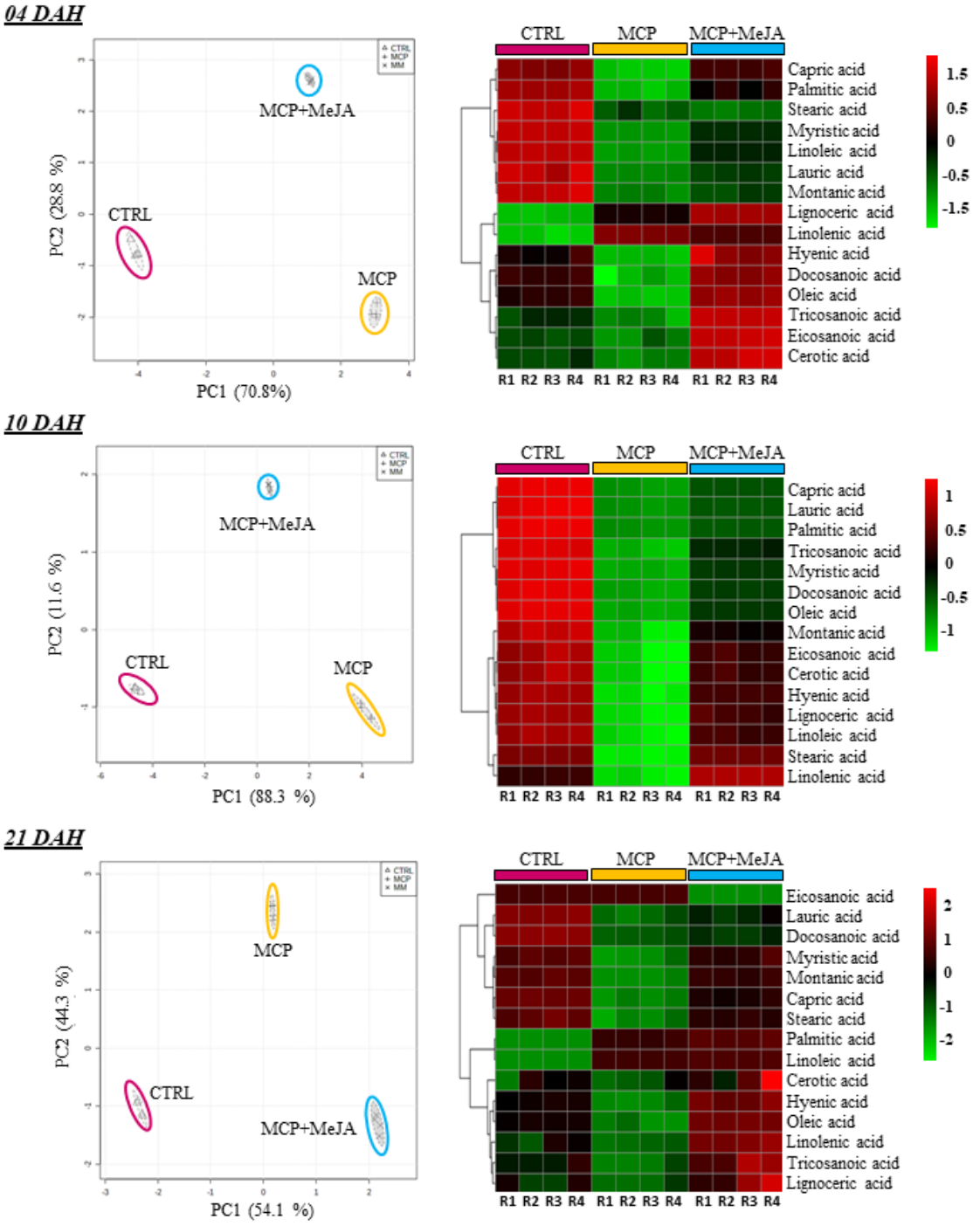

Figure 5. Relative contents of fatty acids in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MCP+MeJA) treatments compared to the control group (CTRL). Non-supervised principal component analysis (PCA-score) and heatmap analysis representing the major sources of variability. Color scale represents the variation

in the relative concentration of compounds, from low (green) to high (red) contents at 04, 10 and 21 days after harvest (DAH).

7.2.3 Secondary metabolite profiling affected by postharvest hormonal treatment

The secondary metabolites idenfied in Grape tomato fruits at 4, 10 and 21 DAH were: lycopene, β-carotene, and lutein by HPLC analysis; and α-tocopherol, β-tocopherol, γ-tocopherol, phytol, β-sitosterol, stigmasterol, and stigmastadienol by GC-MS analysis.

Lycopene was the most affected by the action of 1-methylciclopropene, reducing its level not only in MCP, but also in MCP+MeJA by 29 and 25 fold, respectively, at 4 DAH, while at 10 DAH the reduction was 8 and 6 fold, respectively, when compared with CTRL. At 21 DAH, lycopene suffer a reduction of 2.8 in MCP. The impact lower than 2 fold in the level of carotenoids of the both treatments compared with CTRL can be observed also in the Figure 2. A reduction lower than 2-fold was observed in β-carotene and lutein at 4, 10 and 21 DAH, expectionally for β-carotene which reduced 2.4 fold in MCP at 21 DAH (Figure 6A and Supplementary Figure S1). In contrast, an increase in carotenoids levels were detected at 21 DAH in MCP+MeJA. Lycopene and β-carotene showed an increase of 10 %, and lutein of 20% when compared with CTRL (Figure 6A and Supplementary Figure S1). Total carotenoids level was represented mainly by lycopene.

Tocopherol profiling showed a similar behavior that carotenoids during ripening, with decreasing in its levels in both treatments groups at 4 and 10 DAH (Figure 2), and at 21 DAH presented a descrease in MPC group and an increase in MCP+MeJA of tocopherols when compared with CTRL. Levels of α-tocopherol showed a reduction in MCP and MCP+MeJA of 5 and 4 fold, respectively, at 4 DAH, while at 10 DAH

decreased 12 and 3 fold, respectively. β-tocopherol levels suffer a reduction of 14 and 12 fold at 4 DAH, and 23 and 9 fold at 10 DAH in MCP and MCP+MeJA, respectively. In addition, γ-tocopherol was deacresed by 6 fold at 4 and 10 DAH in both treatments groups, exceptionally for the MCP+MeJA at 10 DAH which descreased 1.7 fold when compared with CTRL (Figure 6B and Supplementary Figure S2).

In contrast, at 21 DAH tocopherol profiling was lesser affected by 1-methylcicloproene, and impacted positively by the concomitant treatment of 1-methylciclopronene and methyl jasmonate, showing an improved of 40 % in the levels of α-tocopherol and β-tocopherol and 21 % in the levels of γ-tocopherol when compared with CTRL (Figure 6B and Supplementary Figure S2). Total tocopherols level was characterized mainly by the content of α-tocopherol. An acyclic diterpenoid identified was phytol, which presented a reduction of 2 fold in MPC at 4 DAH and increase of 2 fold in MCP+MeJA at 10 DAH (Figure 6B and Supplementary Figure S2). The impact of these treatments at 4 and 10 DAH can be observed also in the Figure 2.

Phytosterols were also affected by 1-methylciclopropene, showing reductions in β-sitosterol levels of 5 fold in MCP at 4 and 10 DAH, and 3 fold in MCP+MeJA at 4 and 10 DAH, comparing with CTRL. Stigmasterol exhibited reduction in MCP of 4 and 7 fold at 4 and 10 DAH, respectively, while MCP+MeJA showed a decrease of 3 and 5 fold at 4 and 10 DAH, respectively. Stigmastadienol was the most affected by 1-methylciclopropene, deacresing 9 fold at 4 DAH (Figure 6C and Supplementary Figure S2). β-sitosterol and stigmasterol was the major source of total phystosterols level. Besides, the down-regulation higher than 2 fold compared with CTRL can be observed by the phytosterols in the Figure 2. Divergently of behavior of phytosterols profiling at 4 and 10 DAH, β-sitosterol, stigmasterol and stigmastadienol showed an enhancement in their levels of 42, 34 and 32 %, respectively, in fruits treated with both 1-

methylciclopropene and methyl jasmonate at 21 DAH (Figure 6C and Supplementary Figure S2).

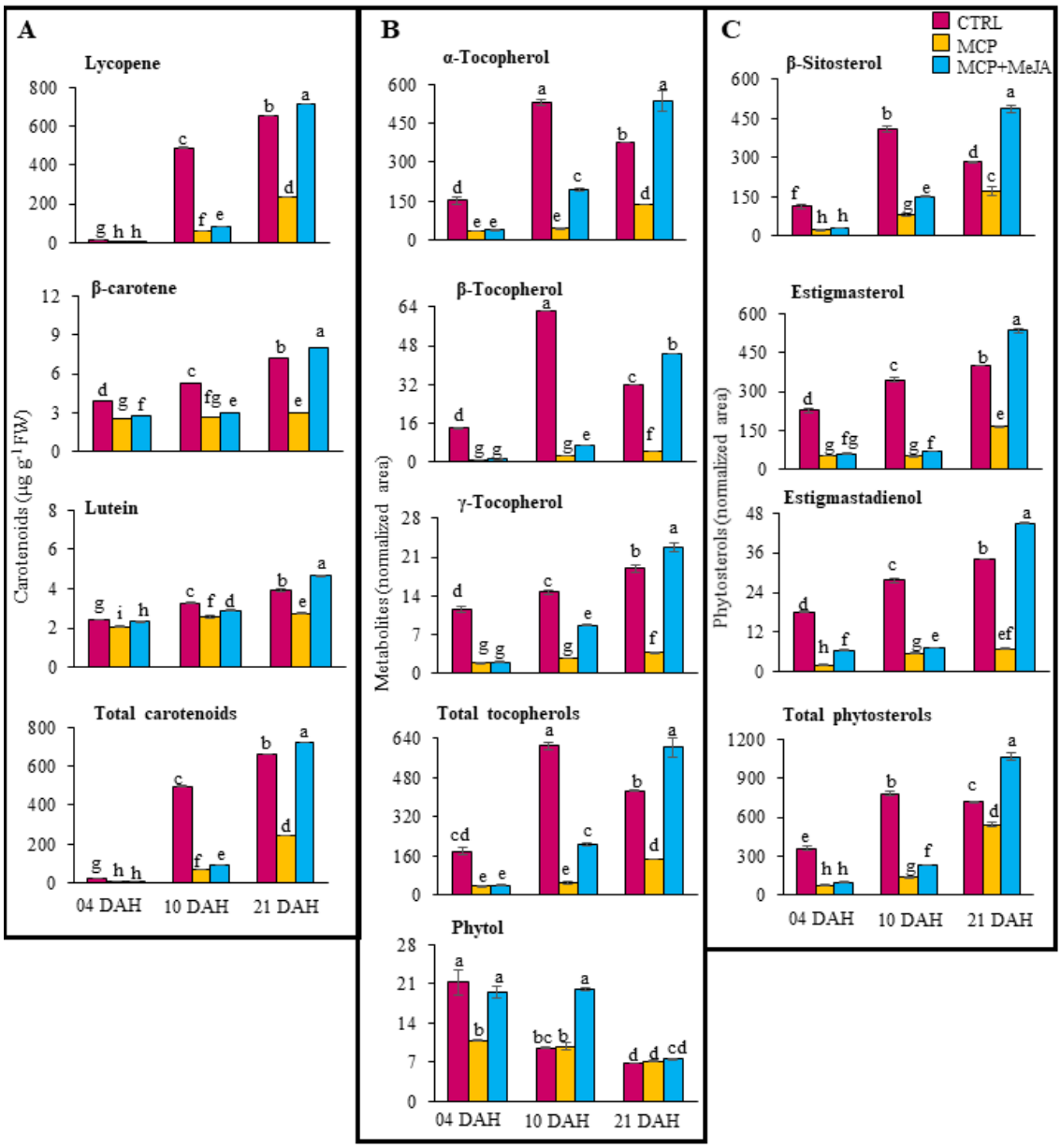

Figure 6. Secondary metabolites in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MCP+MeJA) treatments compared to the control group (CTRL) at 04, 10 and 21 days after harvest (DAH). Contents of carotenoids (A), normalized area of tocopherols and phytol (B), and phytosterols (C). Values are means ± SE of four

biological replicates of 10 fruits each. Different letters indicate statistically significant differences ($p < 0.05$).

7.2.4 Metabolite profiling of fruits treated with phytohormones

The metabolite profiling of Grape tomato fruits treated with only 1-methylcicloprope ne and with both 1-methylcicloprope ne and methyl jasmonate showed a significant impact to the fruit quality and, consequently, to the ripening process.

Sugars and organic acids were significantly affected by 1-methylcicloprope ne, showing a greater delay in their production during ripening mainly at 4 and 10 DAH. Levels of glucose, manose, ribose, malic acid and aconitic acid revealed reduction upper than 14 fold when compared with CTRL at 4 DAH. In addition, fructose, sucrose and citraconic acid also showed a significant reduction of 12, 16 and 27 fold comparing with CTRL (Table 1). However, fruits treated with both 1-methylcicloprope ne and methyl jasmonate showed a tendency to increase the levels of sugars and organic acids such as glucose, glucaric acid and manose at 10 DAH and myo-inositol, propanoic acid and butanoic acid at 21 DAH (Figure 3).

Amino acids and fatty acids demonstrated significant reduction in the both groups, MCP and MCP+MeJA, essencially at 4 and 10 DAH (Figure 4 and 5). Aspartic acid and GABA reached a reduction of 28 fold in MCP at 4 DAH and 14 fold in MCP+MeJA at 10 DAH, respectively. Linoleic and myristic acids showed decrease of 119 and 26 fold in MCP, respectively, and 23 and 9 in MCP+MeJA, respectively, at 4 DAH (Table 1).

Regarding to secondary metabolites, carotenoids, tocopherols and phytosterols, showed similar behavior in face of treatments. Important reductions in the levels of secondary metabolites were observed by both groups MCP and MCP+MeJA mainly at 4

and 10 DAH (Figure 5). However, at 21 DAH fruits treated with both MCP+MeJA reported an improvement in the levels of secondary metabolites. Increasing of 10 to 20 % in carotenoids, 21 to 42 % in tocopherols, and 32 to 42 % in phytosterols were observed in fruits treated with both 1-methylcicloprope ne and methyl jasmonate at 21 DAH (Supplementary Figure S2).

Fruits which were treated only with 1-methylcicloprope ne suffered major reducitons in the levels of primary and secondary metabolites indentified than fruits treated with both 1-methylcicloprope ne and methyl jasmonate (Figure 2). Demonstrating, that the addition of exogenous methyl jasmonate to the fruit with ethylene receptos blocked may support that methyl jasmonate can act indepently of endogenous ethylene or the blockage of ethylene receptors were reversed after 10 DAH or new receptors of ethylene was synthesized.

7.3 Conclusions

Treatment with 1-methylcyclopropene and methyl jasmonate showed lower effect in delay of production of metabolites than treatment with only 1-methylcyclopropene. An important reduction in the levels of sugars, organic acids, amino acids and fatty acids, such as glucose, manose, ribose, malic acid, aconitic acid and aspartic acid at 4 DAH; and fructose, sucrose, citraconic acid, GABA, oleic acid and palmitic acid at 10 DAH were detected in fruits treated with 1-methylcyclopropene when compared with CTRL. Fruits treated with both 1-methylcyclopropene and methyl jasmonate noticed also reduction such as in the levels of oleic and palmitic acids at 4, 10 and 21 DAH; and carotenoids, tocopherols and phytosterols at 4 and 10 DAH. On the other hand, secondary metabolites were improved by both 1-methylcyclopropene and methyl jasmonate at 21 DAH.

7.4 Supplementary files

Supplementary Table S1. Carotenoids contents (μg.g^{-1} FW) in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MeJA+MCP) treatments at 04, 10 and 21 days after harvest (DAH) detected by high performance liquid chromatography (HPLC).

Metabolite	**04 DAH**			**10 DAH**			**21 DAH**		
	CTRL	***MCP***	***MeJA+MCP***	***CTRL***	***MCP***	***MeJA+MCP***	***CTRL***	***MCP***	***MeJA+MCP***
Lycopene	15.4±0.43^{g}	0.52±0.01^{h}	0.63±0.01^{h}	487.1±4.10^{c}	61.1±0.59^{f}	81.9±0.19^{e}	653.6±0.82^{b}	234.8±0.66^{d}	712.5±0.35^{a}
β-carotene	3.87±0.02^{d}	2.57±0.02^{g}	2.72±0.01^{f}	5.32±0.10^{c}	2.68±0.07^{fg}	2.97±0.02^{e}	7.23±0.01^{b}	3.02±0.01^{c}	8.02±0.01^{a}
Lutein	2.43±0.02^{g}	2.07±0.01^{i}	2.31±0.01^{h}	3.27±0.06^{c}	2.56±0.04^{f}	2.88±0.03^{d}	3.93±0.05^{b}	2.75±0.04^{e}	4.67±0.03^{a}
Total	21.7±0.42^{g}	5.17±0.01^{h}	5.66±0.03^{h}	495.6±4.10^{c}	66.3±0.63^{f}	87.8±0.22^{e}	664.7±0.85^{b}	240.8±0.68^{d}	725.1±0.35^{a}

CTRL: Control fruits. Different superscript letters indicate statistical significance ($p < 0.05$) at the same line (mean ± standard deviation. n = 4).

Supplementary Table S2. Secondary metabolites in tomato (*Solanum lycopersicum* L. cv. Grape) fruits exposed to 1-methylcyclopropene (MCP) and both hormones methyl jasmonate and 1-methylcyclopropene (MeJA+MCP) treatments at 04, 10 and 21 days after harvest (DAH) detected by gas chromatography-mass spectrometry (GC-MS).

Metabolite	**04 DAH**			**10 DAH**			**21 DAH**		
	CTRL	***MCP***	***MeJA+MCP***	***CTRL***	***MCP***	***MeJA+MCP***	***CTRL***	***MCP***	***MeJA+MCP***
A) Tocopherols									
α-tocopherol	152,7±14,5^{d}	32,1±1,41^{e}	37,1±6,01^{e}	531,9±12,1^{a}	42,7±4,84^{e}	193,4±9,80^{c}	375,8±1,57^{b}	137,2±1,41^{d}	535,8±40,2^{a}
β-tocopherol	14,1±0,27^{d}	0,98±0,13^{g}	1,15±0,18^{g}	62,3±2,15^{a}	2,66±0,12^{g}	6,85±0,10^{e}	31,6±0,23^{c}	4,70±0,02^{f}	44,8±0,03^{b}
γ-tocopherol	11,6±0,49^{d}	1,91±0,15^{g}	2,03±0,05^{g}	14,6±0,38^{c}	2,71±0,09^{g}	8,71±0,16^{e}	18,9±0,72^{b}	3,71±0,12^{f}	22,7±0,79^{a}
Total	178,4±15,1^{cd}	34,9±0,86^{e}	40,3±0,99^{e}	608,9±14,6^{a}	48,11±4,77^{e}	208,9±6,07^{c}	426,4±1,45^{b}	145,6±1,67^{e}	603,2±39,8^{a}
B) Acyclic diterpenoids									
Phytol	21,3±2,25^{a}	10,9±0,21^{b}	19,5±1,05^{a}	9,54±0,18^{bc}	9,80±0,59^{b}	19,9±0,18^{a}	6,75±0,04^{d}	7,19±0,14^{d}	7,52±0,16^{cd}
C) Phytosterols									
β-sitosterol	113,2±7,14^{f}	21,9±0,61^{h}	29,7±0,89^{h}	410,2±10,9^{b}	79,5±4,59^{g}	151,9±2,45^{e}	282,7±1,32^{d}	171,1±16,5^{c}	487,6±15,3^{a}
Stigmasterol	227,3±10,5^{f}	50,9±1,04^{d}	60,5±1,38^{e}	344,1±8,75^{e}	49,6±1,76^{b}	68,9±0,19^{a}	400,8±1,71^{d}	164,5±4,68^{c}	535,6±10,6^{ab}
Stigmastadienol	18,2±0,26^{d}	2,10±0,13^{h}	6,47±0,21^{f}	27,8±0,68^{c}	5,62±0,11^{g}	7,31±10,18^{e}	34,2±0,16^{b}	7,03±0,13^{ef}	44,9±0,21^{a}
Total	358,7±14,3^{e}	74,9±1,63^{h}	96,7±2,14^{h}	782,0±20,2^{b}	134,8±6,05^{g}	228,2±2,39^{f}	717,6±2,80^{c}	542,7±19,9^{d}	1068,0±25,3^{a}

Values were presented as normalized area by n-tridecane (internal non-polar standard). CTRL: Control fruits. Different superscript letters indicate statistical significance ($p < 0.05$) at the same line (mean ± standard deviation, $n = 4$).

8. CONCLUDING REMARKS AND PERSPECTIVE

The knowledge of pre- and post-harvest treatments applied in tomato fruits is important to achieved better quality and nutritional value of tomato, improving the metabolites profiling as well as the primary and secondary metabolites. Regarding to pre-harvest treatment, the two traditional varieties (TP and MA), as described in the **Section 1**, may be very attractive plant materials in order to attain a better understanding on the production of metabolites of interest from the nutritional quality perspective. Furthermore, it is evident the interest of plant culture in moderate saline conditions as an agronomic tool to improve the nutritional value of fruit, since α-tocopherol levels increase in such culture condition as much as twice in these landraces compared with 'MM' and, moreover, without fruit yield penalty.

As described in **Section 2 and 3**, post-harvest treatment with phytohormones directly affected the metabolites profiling of Grape tomato. Most of the detected metabolites changes were associated to the organoleptic and nutritional value of Grape tomato fruit. In the **Section 2**, the exogenous ethylene treatment positively impacted soluble sugars (fructose, sucrose, and glucose) during the ripening. However, the most notable primary metabolites changes caused by methyl jasmonate treatment were the increased in the production of citric acid, succinic acid, malic acid, oxaloacetic acid, fumaric acid, glutamic acid, glutamine, GABA, aspartic acid, tryptophan and phenylalanine at 10 DAH; and fatty acids at 4 DAH. Changing in balance in sugars, organic acids and taste compounds, as glutamic acids, can straight impact taste perception. At the same time that secondary metabolites such as carotenoids, tocopherols, phytol and phytosterols directly related to the tomato nutritional value were also affected by the hormonal treatments. Methyl jasmonate significantly induced the accumulation of lycopene, α-tocopherol and β-sitosterol in full ripe fruits. Peculiarly, these

phytochemicals had a greater impact at 4 DAH, showing at least 2 fold-increase compared with non-treated fruits.

In the **Section 3**, Grape tomatoes treated with 1-methylcyclopropene and methyl jasmonate showed lower effect in delay of production of metabolites than treatment with only 1-methylcyclopropene. An important reduction in the levels of sugars, organic acids, amino acids and fatty acids, such as glucose, manose, ribose, malic acid, aconitic acid and aspartic acid at 4 DAH; and fructose, sucrose, citraconic acid, GABA, oleic acid and palmitic acid at 10 DAH were detected in fruits treated with 1-methylcyclopropene when compared with CTRL. Fruits treated with both 1-methylcyclopropene and methyl jasmonate noticed also reduction such as in the levels of oleic and palmitic acids at 4, 10 and 21 DAH; and carotenoids, tocopherols and phytosterols at 4 and 10 DAH. On the other hand, secondary metabolites were improved by both 1-methylcyclopropene and methyl jasmonate at 21 DAH. Taken together, our results suggest that the pre-harvest treatment such as salinity and post-harvest treatment such as phytohormones can be used as tools to improve sensorial and nutritional value of tomato fruits, since important changes related to metabolome of tomato fruit were observed in metabolites responsible for the fruit quality.

All these metabolites changes are relevant to the study of fruit quality and nutritional value and may explain same metabolic interaction. However, is important to point out that detailed study related to integration of omics approaches such as genomic, transcriptomic, proteomic and metabolomic is essential to give better understanding about the metabolic profile of tomato exposed to salinity and phytohormones.

9. REFERENCES

1. Aghdam, M. S., and Fard, J. R. (2017). Melatonin treatment attenuates postharvest decay and maintains nutritional quality of strawberry fruits (*Fragaria ananassa* cv. Selva) by enhancing GABA shunt activity. *Food Chem.* 221, 1650–1657.doi: 10.1016/j.foodchem.2016.10.123
2. Agius, C., Tucher, S., Poppenberger, B. & Rozhon, W. (2018). Quantification of sugars and organic acids in tomato fruits. MethodsX, 5, 10. doi: 1016/j.mex.2018.05.014.
3. Albaladejo, I., Meco, V., Plasencia, F., Flores, F.B., Bolarin, M.C., and Egea, I. (2017). Unravelling the strategies used by the wild tomato species *Solanum pennellii* to confront salt stress: from leaf anatomical adaptations to molecular responses. *Environ. Exp. Bot.* 135, 1–12. doi: 10.1016/j.envexpbot.2016.12.003
4. Albert, E., Segura, V., Gricourt, J., Bonnefoi, J., Derivot, L., andCausse, M. (2016). Association mapping reveals the genetic architecture of tomato response to water deficit: focus on major fruit quality traits. *J. Exp. Bot.* 67, 6413–6430.doi: 10.1093/jxb/erw411
5. Almeida, J., Quadrana, L, Asís, R., Setta, N., De Godoy, F., Bermúdez, L., *et al*. (2011). Genetic dissection of vitamin E biosynthesis in tomato. *J. Exp. Bot.* 62, 3781-3798. doi:10.1093/jxb/err055
6. Almeida, J., Asís, R., Molineri, V.N., Sestari, I., Lira, B.S., Carrari, F., *et al*. (2015). Fruits from ripening impaired, chlorophyll degraded and jasmonate insensitive tomato mutants have altered tocopherol content and composition. *Phytochemistry* 111, 72–83.doi: 10.1016/j.phytochem.2014.11.007
7. Bao, H., Chen, X., Lv, S., Jiang, P., Feng, J., Fan, P., *et al*. (2014). Virus-induced gene silencing reveals control of reactive oxygen species accumulation and salt

tolerance in tomato by γ-aminobutyric acid metabolic pathway. *Plant Cell Environ.* 38, 600–613. doi:10.1111/pce.12419

8. Bapat, V. A., Trivedi, P. K., Ghosh, A., Sane, V. A, Ganapathi, T. R., & Nath, P. (2010). Ripening of fleshy fruit: Molecular insight and the role of ethylene. Biotechnology Advances, 28, 94–107. doi: 10.1016/j.biotechadv.2009.10.002
9. Beauvoit, B., Belouah, I., Bertin, N., Cakpo, C. B., Colombié, S., Dai, Z., *et al*. (2018). Putting primary metabolism into perspective to obtain better fruits. *Annals Bot.* 122, 1–21.doi: 10.1093/aob/mcy057
10. Blankenship, S. & Dole, J. (2003). 1-Methylcyclopropene: A review. Postharvest Biology and Technology, 28, 1-25. doi: 10.1016/S0925-5214(02)00246-6
11. Bligh, E. G., & Dyer, W. J. (1959). A rapid method of total lipid extraction and purification. Canadian Journal of Biochemistry and Physiology, 37.
12. Bramley, B. M. (2002). Regulation of carotenoid formation during tomato fruit ripening and development. Journal of Experimental Botany, 53, 2107-2113. doi: 10.1093/jxb/erf026
13. Cahoon, E. & Li-Beisson, Y. (2020). Plant unusual fatty acids: learning from the less common. Current Opinion in Plant Biology. 55, 66-73. doi: 10.1016/j.pbi.2020.03.007
14. Centeno, D.C., Osorio, S., Nunes-Nesi, A., Bertolo, A.L.F., Carneiro, R.T., Araujo, W.L., *et al*. (2011). Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. *Plant Cell* 23, 162-184. doi: 10.1105/tpc.109.072231
15. Chen, Q., Steinhauer, L., Hammerlindl, J., Keller, W. & Zou, J. (2007). Biosynthesis of Phytosterol Esters: Identification of a Sterol O-Acyltransferase in Arabidopsis. Plant physiology, 145, 974-84. 10.1104/pp.107.106278.

16. Choi, H. K., Choi, Y. H., Verberne, M., Lefeber, A.W., Erkelens, C., and Verpoorte, R. (2004). Metabolic fingerprinting of wild type and transgenic tobacco plants by 1H NMR and multivariate analysis technique. *Phytochemistry* 65, 857-864. doi: 10.1016/j.phytochem.2004.01.019
17. Choi, Y.H., Kim, H.K., Linthorst, H.J.M., Hollander, J.G., Lefeber, A.W.M., Erkelens, C., *et al*. (2006). NMR metabolomics to revisit the tobacco mosaic virus infection in *Nicotiana tabacum* leaves. *J. Nat. Prod.* 69, 742-748. doi: 10.1021/np050535b
18. Choi, S., Lee, S., Kim, H., Lee, I., Kozukue, N., Levin, C. & Friedman, M. (2010). Changes in Free Amino Acid, Phenolic, Chlorophyll, Carotenoid, and Glycoalkaloid Contents in Tomatoes during 11 Stages of Growth and Inhibition of Cervical and Lung Human Cancer Cells by Green Tomato Extracts. Journal of agricultural and food chemistry, 58, 7547-7556. doi: 10.1021/jf100162j.
19. Chong, J., Soufan, O., Li, C., Caraus, I., Li, S., Bourque, G., *et al*. (2018). MetaboAnalyst 4,0: towards more transparent and integrative metabolomics analysis. *Nucl. Acids Res.* 46: W486-W494. doi: 10.1093/nar/gky310
20. Deewatthanawong, R., Rowell, P., & Watkins, C. B. (2010). γ-Aminobutyric acid (GABA) metabolism in CO_2 treated tomatoes, Postharvest Biology and Technology, 57, 97-105. doi: 10.1016/j.postharvbio.2010.03.007
21. D'Esposito, D., Ferriello, F., Dal Molin, A., Diretto, G., Sacco, A., Minio, A., *et al*. (2017). Unraveling the complexity of transcriptomic, metabolomic and quality environmental response of tomato fruit. *BMC Plant Biol.* 17:66. doi: 10.1186/s12870-017-1008-4
22. Dwivedi, S.L., van Bueren, E.T.L., Ceccarelli, S., Grando, S., Upadhyaya, H.D., and Ortiz, R. (2017). Diversifying food systems in the pursuit of sustainable food

production and healthy diets. *Trends Plant Sci.* 22, 843-856. doi: 10.1016/j.tplants.2017.06.011

23. Etienne, A., Génard, M., Lobit, P., Mbeguié-A-Mbéguié, D. & Bugaud, C. (2013). What controls fleshy fruit acidity? A review of malate and citrate accumulation in fruit cells. Journal of Experimental Botany, 64, 1451-1469. doi: 10.1093/jxb/ert035
24. Fabi, J. P., Cordenunsi, B. R., Barreto, G. P de M., Mercadante, A. Z., Lajolo, F. M., & Nascimento, J. R. O. (2007). Papaya fruit ripening: response to ethylene and 1-methylciclopropene. Journal of Agricultural and Food Chemistry, 55, 6118-6123. doi: 10.1021/jf070903c
25. Famiani, F., Battistelli, A., Moscatello, S., Cruz-Castillo, J. G. & Walker, R. (2015). The organic acids that are accumulated in the flesh of fruits: occurrence, metabolism and factors a ffecting their contents – a review Ácidos orgánicos acumulados en la pulpa de los frutos: ocurrencia, metabolismo y factores que afectan sus contenidos-una revisión. Revista Chapingo Serie Horticultura, 21, 97-128. doi: 10.5154/r.rchsh.2015.01.004
26. Fan, X., Mattheis, J. P., & Fellman, J. K. (1998). A role for jasmonates in climacteric fruit ripening. Planta, 204, 444-449. doi: 10.1007/s004250050278
27. Fanciullino, A.L., Bidel, L.P.R., and Urban, L. (2014). Carotenoid responses to environmental stimuli: integrating redox and carbon controls into a fruit model. *Plant Cell Environ.* 37, 273-289. doi: 10.1111/pce.12153
28. FAOSTAT, UN Food and Agriculture Organization statistics. (2018). http://www.fao.org/faostat/en [Accessed July 9, 2020]
29. Farcuh, M., Rivero, R. M., Sadka, A. & Blumwald, E. (2018). Ethylene regulation of sugar metabolism in climacteric and non-climacteric plums. Postharvest Biol. Technol., 139, 20–30. doi: 10.1016/j.postharvbio.2018.01.012

30. Fiehn, O., Kopka, J., Dörmann, P. *et al.* (2000). Metabolite profiling for plant functional genomics. *Nat Biotechnol*, 18, 1157–1161. doi: 10.1038/81137

31. Filiz, E., Cetin, D. & Akbudak, M. A. (2019). Aromatic amino acids biosynthesis genes identification and expression analysis under salt and drought stresses in Solanum lycopersicum L. Scientia Horticulturae, 250, 127-137. doi: 10.1016/j.scienta.2019.02.044

32. Garcia-Abellan, J.O., Fernandez-Garcia, N., Lopez-Berenguer, C., Egea, I., Flores, F. B., Angosto, T., *et al*. (2015). The tomato *res* mutant which accumulates JA in roots in non-stressed conditions restores cell structure alterations under salinity. *Physiol. Plant.* 155, 296–314. doi: 10.1111/ppl.12320

33. Gascuel, Q., Diretto, G., Monforte, A.J., Fortes, A.M., and Granell, A. (2017). Use of natural diversity and biotechnology to increase the quality and nutritional content of tomato and grape. *Front. Plant Sci.* 8:652. doi: 10.3389/fpls.2017.00652

34. Gerendás, J., and Führs. H. (2013). The significance of magnesium for crop quality. *Plant and Soil* 368, 101–128.doi: 10.1007/s11104-012-1555-2

35. Guillen, F., Castillo, S., Zapata, P. J., Martinez-Romero, D., Vallero, D., & Serrano, M. (2006). Efficacy of 1-MCP treatment in tomato fruit. 2. Effect of cultivar and ripening stage at harvest. Postharvest Biology and Technology, 42, 235–242.

36. Handa, A. K., Tiznado-Hernández, M. E., & Mattoo A. K. (2012). Fruit development and ripening: A molecular perspective. Plant Biotechnology and Agriculture, 26, 405-424. doi: 10.1016/B978-0-12-381466-1.00026-2

37. Hoagland, D. R., and Arnon, D.I. (1950). The water-culture method for growing plants without soil, College of Agriculture, UC: Berkeley, CA, USA. *Calif. Agric. Exp. Stn.*347, 1–39.

38. Hou, X., Zhang, W., Du, T., Kang, S., and Davies, W. J. (2020). Responses of water accumulation and solute metabolism in tomato fruit to water scarcity and implications for main fruit quality variables. *J. Exp. Bot.* 71, 1249–1264.doi: 10.1093/jxb/erz526

39. Ichihara, K.I., & Fukubayashi, Y. (2010). Preparation of fatty acid methyl esters for gas-liquid chromatography. Journal of Lipid Research, 51, 635-40. doi: 10.1194/jlr.D001065

40. Igamberdiev, A.U., and Eprintsev, A.T. (2016). Organic acids: the pools of fixed carbon involved in redox regulation and energy balance in higher plants. *Front. Plant Sci.* 7:1042. doi: 10.3389/fpls.2016.01042

41. Ito, H., Ueno, H. & Kikuzaki, H. (2017). Free Amino Acid Compositions for Fruits. Journal of Nutrition and Dietetic Practice, 1.

42. Jia, H., Jiu, S., Zhang, C., Wang, C., Tariq, P., Liu, Z., Wang, B., Cui, L., Fang, J. (2016). Abscisic acid and sucrose regulate tomato and strawberry fruit ripening through the abscisic acid-stress-ripening transcription factor. Plant Biotechnol. J., 14, 2045–2065. doi: 10.1111/pbi.12563

43. Jia, H., Wang, Y., Sun, M., Li, B., Han, Y., Zhao, Y., Li, X., Ding, N., Li, C., Ji, W., *et al*. (2013). Sucrose functions as a signal involved in the regulation of strawberry fruit development and ripening. New Phytol., 198, 453–465. doi: 10.1111/nph.12176

44. Johanningsmeiner, S. D., Mcfeeters, R. F., & Drake, M. (2005). A hypothesis for the chemical basis for perception of sour taste. Journal for the Food Science, 70, 44-48. doi: 10.1111/j.1365-2621.2005.tb07111.x

45. Kim, S., Schlicke, H., Ree, K., Karvonen, K., Subramaniam, A., Richter, A., Grimm, B. & Braam, J. (2013). Arabidopsis Chlorophyll Biosynthesis: An Essential Balance

between the Methylerythritol Phosphate and Tetrapyrrole Pathways. The Plant cell, 25. doi: 10.1105/tpc.113.119172

46. Kombrink, E. (2012). Chemical and genetic exploration of jasmonate biosynthesis and signaling paths. Planta, 236, 1351-66. 10.1007/s00425-012-1705-z.
47. Kumar, V., Sharma, A., Kaur, R., Thukral, A., Bhardwaj, R. & Ahmad, P. (2017). Differential distribution of amino acids in plants. Amino Acids, 49. doi: 10.1007/s00726-017-2401-x
48. Lashbrooke, J., Young, P., Dockrall, S., Vasanth, K. & Vivier, M. (2013). Functional characterisation of three members of the Vitis vinifera L. carotenoid cleavage dioxygenase gene family. BMC plant biology, 13, 156. doi: 10.1186/1471-2229-13-156
49. Li, J., Avila, C., Tieman, D., Klee, H., & Goggin, L. (2018). A Comparison of the Effects of FATTY ACID DESATURASE 7 and HYDROPEROXIDE LYASE on Plant-Aphid Interactions. International journal of molecular sciences, 19. doi: 10.3390/ijms19041077
50. Li, J., Yan, J., Ritenour, M., Wang, J., Cao, J. & Jiang, W. (2016). Effects of 1-methylcyclopropene on the physiological response of Yali pears to bruise damage. Scientia Horticulturae, 200, 137-142. doi: 10.1016/j.scienta.2016.01.018
51. Lin, Y. P. & Charng, Y. Y. (2021). Chlorophyll dephytylation in chlorophyll metabolism: a simple reaction catalyzed by various enzymes. Plant Science, 302, 110682. doi: 10.1016/j.plantsci.2020.110682.
52. Liu, L., Wei, J., Zhang, M., Zhang, L., Li, C., &Wang, Q. (2012). Ethylene independent induction of lycopene biosynthesis in tomato fruits by jasmonates. Journal of Experimental Botany, 63, 5751–5761. doi: 10.1093/jxb/ers224

53. Liu, M., Pirrello, J., Chervin, C., Roustan, J. P. & Bouzayen, M. (2015). Ethylene control of fruit ripening: revisiting the complex network of transcriptional regulation. Plant Physiology, 169, 2380–2390. doi: 10.1104/pp.15.01361

54. Liu, Y. H., Offler, C. E. & Ruan, Y. L. (2013). Regulation of fruit and seed response to heat and drought by sugars as nutrients and signals. Frontiers in plant science. 4, 282. doi: 10.3389/fpls.2013.00282.

55. Lisec, J., Schauer, N., Kopka, J., Willmitzer, L., & Fernie, A.R. (2006). Gas chromatography mass spectrometry-based metabolite profiling in plants. Nature Protocols, 1, 387-396. doi: 10.1038/nprot.2006.59

56. Maeda, H., Sage, T. L., Isaac, G., Welti, R. & Dellapenna, D. (2008). Tocopherols modulate extraplastidic polyunsaturated fatty acid metabolism in Arabidopsis at low temperature. Plant Cell, 20, 452–470. doi: 10.1105/tpc.107.054718

57. Marangoni, F. & Poli, A. (2010). Phytosterols and cardiovascular health. Pharmacological research : the official journal of the Italian Pharmacological Society, 61, 193-199. doi: 10.1016/j.phrs.2010.01.001

58. Massaretto, I. L., Albaladejo, I., Purgatto, E., Flores, F. B., Plasencia, F., Egea-Fernández, J. M., *et al.* (2018) Recovering Tomato Landraces to Simultaneously Improve Fruit Yield and Nutritional Quality Against Salt Stress. *Front. Plant Sci.* 9:1778. doi: 10.3389/fpls.2018.01778

59. Moles, T. M., de Brito, F. R., Mariotti, L., Pompeiano, A., Lupini, A., Incrocci, L., *et al*. (2019) Salinity in Autumn-Winter Season and Fruit Quality of Tomato Landraces. *Front. Plant Sci.* 10:1078. doi: 10.3389/fpls.2019.01078

60. Moreau, R. A, Nyström, L., Whitaker, B. D., Winkler-Moser, J. K., Baer, D. J., Gebauerf, S. K., & Hicks, K. B. (2018). Phytosterols and their derivatives: Structural

diversity, distribution, metabolism, analysis, and health-promoting uses. Progress in Lipid Research, 70, 35-61. doi: 10.1016/j.plipres.2018.04.001

61. Munns, R., Passioura, J. B., Colmer, T. D., and Byrt, C. S. (2020). Osmotic adjustment and energy limitations to plant growth in saline soil. *NewPhytologist* 225, 1091–1096. doi: 10.1111/nph.15862
62. Muñoz-Mayor, A., Pineda, B., Garcia-Abellán, J.O., Antón, T., Garcia-Sogo, B., Sanchez-Bel, P., *et al*. (2012). Overexpression of dehydrin*tas14* gene improves the osmotic stress imposed by drought and salinity in tomato. *J. Plant. Physiol.* 169, 459–468. doi: 10.1016/j.jplph.2011.11.018
63. Nagata, M., and Yamashita, I. (1992). Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruit. *J. Japan. Soc. Food Sci. Technol.* 39, 925-928. doi: 10.3136/nskkk1962.39.925
64. Odriozola-Serrano, I., Garde-Cerdán, T., Soliva-Fortuny, R. & Martín-Belloso, O. (2013). Differences in free amino acid profile of non-thermally treated tomato and strawberry juices, Journal of Food Composition and Analysis, 32, 51-58. doi: 10.1016/j.jfca.2013.07.002
65. Ohlrogge, J., Thrower, N., Mhaske, V., Stymne, S., Baxter, M., Yang, W., Liu, J., Shaw, K., Shorrosh, B., Zhang, M., Wilkerson, C. & Matthäus, B. (2018). PlantFAdb: A Resource for Exploring Hundreds of Plant Fatty Acid Structures Synthesized by Thousands of Plants and their Phylogenetic Relationships. The Plant Journal, 96. doi: 10.1111/tpj.14102
66. Oms-Oliu, G., Hertoga, M. L. A. T. M., Van de Poel, P., Ampofo-Asiamaa, J., Geeraerda, A. H., & Nicolaï, B. M. (2011). Metabolic characterization of tomato fruit during preharvest development, ripening, and postharvest shelf-life. Postharvest Biology and Technology, 11, 7-16. doi: 10.1016/j.postharvbio.2011.04.010

67. Osorio, S., Ruan, Y.L., and Fernie, A. (2014). An update on source-to-sink carbon partitioning in tomato. *Front. Plant Sci.* 5:516. doi: 10.3389/fpls.2014.00516

68. Patrick J. W., Botha F. C. & Birch R. G. (2013). Metabolic engineering of sugars and simple sugar derivatives in plants. Plant Biotechnol J., 11, 142-56. doi: 10.1111/pbi.12002.

69. Paul, V. & Pandey, R. (2013). Delaying tomato fruit ripening by 1-methylcyclopropene (1-MCP) for better postharvest management: Current status and prospects in India. Indian Journal of Plant Physiology, 18, 95-207. doi: 10.1007/s40502-013-0039-6

70. Paul, V., Pandey, R. & Srivastava, G. (2012). The fading distinctions between classical patterns of ripening in climacteric and non-climacteric fruit and the ubiquity of ethylene-An overview. Journal of food science and technology, 49, 1-21. doi: 10.1007/s13197-011-0293-4.

71. Per T. S., Khan M. I. R., Anjum N. A., Masood A., Hussain S. J. & Khan N. A. (2018) Jasmonates in plants under abiotic stresses: crosstalk with other phytohormones matters. Environ Exp Bot., 145, 104–120. doi: 10.1016/j.envexpbot.2017.11.004

72. Pérez-Gálvez A., Viera I. & Roca M. (2017). Chemistry in the bioactivity of chlorophylls: an overview. Curr Med Chem., 24, 4515-4536. doi: 10.2174/0929867324666170714102619

73. Piironen, V., Lindsay, D., Miettinen, T., Toivo, J. & Lampi, A. M. (2000). Plant sterols: Biosynthesis, biological function and their importance to human nutrition. Journal of the Science of Food and Agriculture, 80, 939 – 966. doi: 10.1002/(SICI)1097-0010(20000515)80:7<939::AID-JSFA644>3.0.CO;2-C

74. Pott, D., Vallarino, J. & Osorio, S. (2020). Metabolite Changes during Postharvest Storage: Effects on Fruit Quality Traits. Metabolites, 10, 187. doi: 10.3390/metabo10050187.

75. Quadrana, L., Almeida, J., Asís, R., Duffy, T., Dominguez, P.G., Bermúdez, L., Conti, G., da Silva, J. V. C., Colot, V., Asurmendi, S., Fernie, A. R., Rossi, M. & Carrari, F. (2014). Natural occurring epialleles determine vitamin E accumulation in tomato fruits. Nat. Commun., 26, 3027. doi: 10.1038/ncomms5027

76. Quadrana, L., Almeida, J., Otaiza, S.N., Duffy, T., Da Silva, J.V.C., de Godoy, F., *et al*. (2013). Transcriptional regulation of tocopherol biosynthesis in tomato. *Plant Mol. Biol.,* 81, 309-325. doi: 10.1007/s11103-012-0001-4

77. Quinet, M., Angosto, T., Yuste-Lisbona, F. J., Blanchard-Gros, R., Bigot, S., Martinez, J. P., *et al*. (2019). Tomato Fruit Development and Metabolism. *Front. Plant Sci.* 10:1554. doi: 10.3389/fpls.2019.01554

78. Ripoll, J., Urban, L., Brunel, B., and Bertin, N. (2016). Water deficit effects on tomato quality depend on fruit developmental stage and genotype. *J. Plant Physiol.*190, 26–35.doi: 10.1016/j.jplph.2015.10.006

79. Rivera, S., and Canela, R. (2012). Influence of sample processing on the analysis of carotenoids in maize. *Molecules* 17, 11255-11268. doi: 10.3390/molecules170911255

80. Rivera, S.M., Vilaró, F.; Zhu, C., Bai, C., Farré, G., Christou, P. *et al*. (2013). Fast quantitative method for the analysis of carotenoids in transgenic maize. *J. Agric. Food Chem.* 61, 5279-5285. doi: 10.1021/jf400694z

81. Rouphael, Y., Petropoulos, S.A., Cardarelli, M., and Colla, G. (2018). Salinity as eustressor for enhancing quality of vegetables. *Sci. Hort.* 234, 361-369. doi: 10.1016/j.scienta.2018.02.048

82. Rowland, S. D., Zumstein, K., Nakayama, H., Cheng, Z., Flores, A. M., Chitwood, D. H., *et al*. (2020). Leaf shape is a predictor of fruit quality and cultivar performance in tomato. *New Phytologist* 226, 851–865 doi: 10.1111/nph.16403

83. Seo, Y., Kim, S. J., Harn, C. & Kim, W. T. (2011). Ectopic expression of apple fruit homogentisate phytyltransferase gene (MdHPT1) increases tocopherol in transgenic tomato (*Solanum lycopersicum* Cv. Micro-Tom) leaves and fruits. Phytochemistry, 72, 321-329. 10.1016/j.phytochem.2010.12.013

84. Sérino, S., Gomez, L., Costagliola, G., & Gautier, H. (2009). HPLC assay of tomato carotenoids: validation of a rapid microextraction technique. Journal of Agricultural and Food Chemistry, 57, p.8753-8760. doi: 10.1021/jf902113n

85. Souza, M. A. S. *et al*. (2019). Changes in flavonoid and carotenoid profiles alter volatile organic compounds in purple and orange cherry tomatoes obtained by allele introgression. Journal of the Science of Food and Agriculture, 100, 1662-1670. doi: 10.1002/jsfa.10180

86. Sorrequieta, A., Ferraro, G., Boggio, S. & Valle, E. (2009). Free amino acid production during tomato fruit ripening: A focus on L-glutamate. Amino acids, 38, 1523-1532. doi: 10.1007/s00726-009-0373-1

87. Takayama, M., and Ezura, H. (2015). How and why does tomato accumulate a large amount of GABA in the fruit? *Front. Plant Sci.* 6: 612.doi: 10.3389/fpls.2015.00612

88. Takizawa, A., Hyodo, H., Wada, K., Ishii, T., Satoh, S, & Iwai, H. (2014). Regulatory Specialization of Xyloglucan (XG) and Glucuronoarabinoxylan (GAX) in Pericarp Cell Walls during Fruit Ripening in Tomato (Solanum lycopersicum). PloS one, 9, e89871. doi: 10.1371/journal.pone.0089871

89. Tieman, D., Zhu, G., Resende Jr. F. R., Lin T., Nguyen, C., *et al.* (2017). A chemical genetic roadmap to improved tomato flavor. Science, 355, 391-394. doi: 10.1126/science.aal1556

90. Tieman, D., Taylor, M., Schauer, N.. Fernie, A., Hanson, A. & Klee, H. (2006). Tomato aromatic amino acid decarboxylases participate in synthesis of the flavor volatiles 2-phenylethanol and 2-phenylacetaldehyde. Proceedings of the National Academy of Sciences of the United States of America, 103, 8287-8292. doi: 10.1073/pnas.0602469103

91. Tang, N., An, J., Deng, W., Gao, Y., Chen, Z. & Li, Z. (2020). Metabolic and transcriptional regulatory mechanism associated with postharvest fruit ripening and senescence in cherry tomatoes. Postharvest Biology and Technology, 168, 111274. doi: 10.1016/j.postharvbio.2020.111274

92. Tang, N., Deng, W., Hu, N., Chen, N. & Li, Z. (2016). Metabolite and transcriptomic analysis reveals metabolic and regulatory features associated with Powell orange pulp deterioration during room temperature and cold storage. Postharvest Biol. Technol., 112, 75–86. doi: 10.1016/j.postharvbio.2015.10.008

93. Thelen, J. & Ohlrogge, J. (2002). Metabolic Engineering of Fatty Acid Biosynthesis in Plants. Metabolic engineering. 4. 12-21. doi: 10.1006/mben.2001.0204

94. Thompson, G. & Grundy, S. (2005). History and Development of Plant Sterol and Stanol Esters for Cholesterol-Lowering Purposes. The American journal of cardiology, 96, 3D-9D. doi: 10.1016/j.amjcard.2005.03.013

95. Toscano, S., Trivellini, A., Cocetta, G., Bulgari, R., Francini, A., Romano, D., *et al*. (2019).Effect of Preharvest Abiotic Stresses on the Accumulation of Bioactive Compounds in Horticultural Produce. *Front. Plant Sci.* 10:1212. doi: 10.3389/fpls.2019.01212

96. Tripathi, K., Pandey, S., Malik, M. & Kaul, T. (2016). Fruit ripening of climacteric and non climacteric fruit. Journal of Environmental and Applied Bioresearch, 4, 27-34.

97. Valente, J., Almeida, R. & Kooistra, L. (2019). A Comprehensive Study of the Potential Application of Flying Ethylene-Sensitive Sensors for Ripeness Detection in Apple Orchards. Sensors, 19, 372. doi: 10.3390/s19020372

98. Valitova, J., Sulkarnayeva, A. & Minibayeva, F (2016). Plant Sterols: Diversity, Biosynthesis, and Physiological Functions. Biochemistry (Moscow), 81, 819-834. doi: 10.1134/S0006297916080046

99. Vallarino, J. G., Erban, A., Fehrle, I., Fernie, A. R., Kopka, J. & Osorio, S. (2018). Acquisition of Volatiles Compounds by Gas Chromatography–Mass Spectrometry (GC-MS). In Plant Metabolomics: Methods and Protocols; Antonio, C., Ed.; Humana Press: New York, NY, USA, 1778, 225–239. doi: 10.1007/978-1-4939-7819-9_16

100. Vranová, E., Coman, D. & Gruissem, W. (2013). Network Analysis of the MVA and MEP Pathways for Isoprenoid Synthesis. Annual review of plant biology., 64. doi: 10.1146/annurev-arplant-050312-120116

101. Vranová, E., Coman, D., & Gruissem, W. (2012). Structure and dynamics of the isoprenoid pathway network. Mol. Plant, 5 318-333. doi: 10.1093/mp/sss015

102. Wang, C., Xing, J., Chin, C., Ho, C. & Martin, C.. (2001). Modification of fatty acids changes the flavor volatiles in tomato leaves. Phytochemistry, 58, 227-32. doi: 10.1016/S0031-9422(01)00233-3.

103. Wang, M., Zhang, L., Boo, K. H., Park, E., Drakakaki, G., and Zakharov, F. (2019). PDC1, a pyruvate/α-ketoacid decarboxylase, is involved in acetaldehyde, propanal and pentanal biosynthesis in melon (Cucumis melo L.) fruit. Plant J. 98, 112–125. doi: 10.1111/tpj.14204

104. Watkins, C. (2006). The use of 1-methlcyclopropene (1-MCP) on fruits and vegetables. Biotechnology advances, 24, 389-409. doi: 10.1016/j.biotechadv.2006.01.005

105. Wu, X., Jiang, L., Yu, M., An, X., Ma, R., Yu, Z. (2016). Proteomic analysis of changes in mitochondrial protein expression during peach fruit ripening and senescence. J. Proteomics, 147, 197–211. doi: 10.1016/j.jprot.2016.06.005

106. Yoshimura, M., Toyoshi, T., Sano, A, Izumi, T., Fujii, T., Inai, S., Matsukura, C., Fukuda, N., Ezura, H. & Obata, A. (2010). Antihypertensive Effect of a γ-Aminobutyric Acid Rich Tomato Cultivar 'DG03-9' in Spontaneously Hypertensive Rats. Journal of agricultural and food chemistry, 58, 615-619. doi: 10.1021/jf903008t

107. Zhai, Q., Yan, C., Li, L., Xie, D. & Li, C. (2017). Jasmonates. doi: 10.1016/B978-0-12-811562-6.00007-4

108. Zhang, Y., Pan, Z., Venkitasamy, C., Ma, H. & Li, Y. (2015). Umami taste amino acids produced by hydrolyzing extracted protein from tomato seed meal, LWT - Food Science and Technology, 62, 1154-1161. doi: 10.1016/j.lwt.2015.02.003

109. Zhang, Z., Huber, D. J., & Rao, J. (2010). Short-term hypoxic hypobaria transiently decreases internal ethylene levels and increases sensitivity of tomato fruit to subsequent 1-methylcyclopropene treatments. Postharvest Biology and Technology, 56, 131-137. doi: 10.1016/j.postharvbio.2010.01.004.

110. Zhang, Z., Huber, D. J., & Rao, J. (2009). Delay of tomato fruit ripening in response to 1-methylcyclopropene is influenced by internal ethylene levels. Postharvest Biology and Technology, 54, 1-8. doi: 10.1016/j.postharvbio.2009.06.003

111. Zhao, X., Yuan, X., Chen, S., Meng, L., and Fu, D. (2018). Role of the tomato *TAGL1* gene in regulating fruit metabolites elucidated using RNA sequence and metabolomics analyses. PloS One 13, e0199083. doi: 10.1371/journal.pone.0199083

112. Zhu, G., Wang, S., Huang, Z., Zhang, S., Liao, Q., Zhang, C. *et al*. (2018). Rewiring of the fruit metabolome in tomato breeding. *Cell* 172, 249-261. doi: 10.1016/j.cell.2017.12.019