

Ripening Genetics of the Tomato Fruit

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ABSTRACT: Tomato is a model fruit that is used in research programs that seek to understand ripening genetics in succulent and dry fruits. Many novel events take place to initiate the ripening process and its sustenance till completion. Lignification, involvement of ethylene-genes that control its activity, transcriptional regulatory events and miRNAs play major roles during the ripening process. Chemistry of flavor has not been studied in detail and genes that regulate it, but it's also a critical part of ripening genetics. Understanding the genetics involved will go a long way in advancing fruit breeding and genetics to ensure a constant source of Vitamin C which is a major dietary supplement.

Key words: carotenoids, chromoplasts, climacteric, flavor, lignification, physiological

INTRODUCTION

Genetics involved in tomato fruit ripening is one of the most extensively studied ripening model. Tomato is classified as a climacteric fruit. Other fruits that belong to this category include; apple, avocados and bananas. Presence of ethylene is an absolute requirement for them to ripen. Non-climacteric fruits like citrus, grape and strawberry do not require ethylene for them to ripen. Exogenous application of ethylene cannot initiate or promote ripening in these fruits (Harry & James, 2011). Fruits are an important source of supplementary diets for human beings. Quality of a fruit is determined by its flavor, nutritive value, the duration it stays fresh i.e. to avoid economic losses, among others. Perishability is aided by ripening and tissue softening in climacteric fruits (Vishwas, et al., 2010). Economic losses result when fruits become perishable quickly. Biochemical and physiological activities involved in fruit ripening are irreversible once they have started. They can only be slowed down or delayed by external application of certain procedures. Its high coordinated and programmed to achieve specific objectives. (Prasanna, Prabha, & Taranathan, 2007 and Martinez-Romero, et al., 2007).

Numerous physiological changes occur that result in red ripe tomatoes. They are regulated by developmental, environmental and hormonal signals which cause various tissues to mature and differentiate. The changes affect how the tomato appears in terms of texture, color, and smell thereby making it more appealing to consumers, who can disperse its seeds easily (Glenn & Betty, 2003). To study and research on tomato fruit ripening effectively, it is necessary to align all measurements to certain scales of time. Information also from many fruits must be gathered in order to understand biological variations that contribute to genetic activity (M.L.A.T.M, 2002). This issue, in the tomato fruit, has been tackled by categorizing to classes of colors after harvesting. i.e. immature green to mature red or by using the number of days post anthesis to classify them (Osorio, et al., 2011). Instead of always relying on color and firmness of a fruit to classify them as ripe or unripe, developmental patterns can be applied by assuming that they express it based on age in all fruits. This type of classification has been used in a few cases. Their biological age is based on expression of these patterns (LMM, Tatjana, Stanislav, Janez, & Marjan, 2009 and A, et al., 2009).

Lignification and Tomato ripening

The degree to which lignification takes place in the pericarp tissue serves to distinguish different categories or types of fruits (Roth, 1977). The presence of phenolic compounds in tomato and many other fruits presents a signal that the fruit is now ready for consumption. Phenolic substances are critical in phenylpropanoid metabolism which works by increasing pigmentation in fruits and aids in the formation of lignin polymers' building blocks. Biosynthesis of lignin is thought to play a role in determining cell wall properties and phenolic compounds form

cross-linkages between cell wall components (Abdel-massih, Baydoun, Waldron, & Brett, 2007). SHP genes are involved in tomato fruit lignification and are subsequent dehiscence. Their activities are restricted to the carpels and their activities are mainly on ovules and carpel margins (Liljegren, et al., 2000). Lignification mainly occurs in the pericarps. FUL-SHP networks are thought to promote lignification in tomato and many other fleshy fruits and are thought to determine fruit morphology and evolution of their functions (Cristina & Chloe, 2014). FUL-SHP orthologues could be involved in modulation of lignified endocarp properties, this has been suggested by temporal expression studies (Tani, Polidoros, & Tsaftaris, 2007).

Ethylene and Tomato ripening

Ethylene is a phyto-hormone that plays important roles in the tomato and other fruits in terms of developmental regulation and control of responses due to environmental pressures. In the tomato and other climacteric fruits, respiration increase is accompanied by a similar increase in ethylene levels which coordinate and synchronize the ripening process. Non-climacteric fruits on the other hand do not experience elevated levels of respiration during ripening. However, same biochemical changes take place i.e. change of fruit color, texture, taste and aroma which suggests that genes involved are the same which are differentially expressed due to evolution of and their regulators could have been conserved via evolution processes. Fruit softening is an important textural change in climacteric fruits due to cell wall hemicellulose and pectin solubilization and depolymerization by a variety of hydrolases (Rose, Saladie, & Catala, 2004). The role played by ethylene in fruit ripening has been extensively studied in the tomato plant (Adams-Phillips, et al., 2004, Giovannoni, 2007). Presence of ethylene initiates ripening and completes it in various stages. They have been designated as *System-1* and *System-2*. *System-1* is found in unripe fruits and other vegetative tissues, its regulated via autoinhibition, while *System-2* is characterized by mass increase in ethylene presence and leads to senescence and is regulated via autocatalysis (Lelievre, Latche, Jones, Bouzayen, & Pech, 1998 and Inaba, 2007). Exogeneous application of ethylene in climacteric fruits promotes ethylene biosynthesis and ripening via system-2 (Nakatsuka, et al., 1998) Synthesis of ethylene biochemically starts with the enzyme S-Adenosyl-Methionine Synthase (SAM) that catalyzes adonsylation of methionine's sulphur atom. SAM is then metabolized to 5'-methylthioadenosine (MTA) which is incorporated into the methionine cycle to enable recovery of the sulphur atom and 1-aminocyclopropane-1-carboxylic acid, ACC, which is the first compound in ethylene biosynthesis. ACC synthase(ACS) is the enzyme that catalyzes this reaction, it is occurs in the presence pyridoxine-phosphate. ACC is then finally converted to ethylene in the presence of oxygen by ACC Oxidase enzyme (ACO) (Bleecker & Kende, 2000 and Beatriz & James, 2008).

Many genes are involved in ethylene biosynthesis but the ACO and ACS genes are the most characterized. Barry, Llop-Tous, & Grierson (2000) reported that nine genes encode ACS i.e. LeACS1A-B and LeACS2-8. Four of them are expressed during fruit ripening; LeACS1A, LeACS2, LeACS4 and LeACS6 . LeACS6 is the main gene for ACS involved in ethylene synthesis in green fruits though LeACS1 was also expressed in the same tissues. The expression of LeACS1 and LeACS4 get induced during the transition to ripening which is dependent on the RIN-MADS transcription factor (Vrebalov, et al., 2002). ACO enzyme is encoded by five genes in tomato where three of them, LeACO1,3 and 4 are expressed differentially (Van-der-Hoeven, Ronning, Giovannoni, Martin, & Tanksley, 2002). LeACO 1 and 4 levels increase massively at the immature green stage of a fruit and are highly expressed during climacteric ripening. Their levels get sustained throughout the ripening period. ACO and ACS genes have also been characterized in the peach (Tatsuki, Haji, & Yamaguchi, 2006), melon (Yamamoto, et al., 1995) and many other fruits.

Application of 1-methylcyclopropene (1-MCP) inhibits ethylene activity and thus interferes with ripening in climacteric fruits where it is an absolute requirement for initiation and progression of ripening till completion (Sisler, 2006). 1-MCP is best applied after ripening has been initiated. Preclimacteric application results in severe inhibition of ripening which can be problematic to recover. It was determined that activity of 1-MCP is influenced by internal levels of ethylene (Zhengke, Donald, Brandon, & JingPing, 2009). This is an important discovery which can be exploited for industrial or commercial scale horticultural farming.

Transcriptional control and tomato fruit ripening

Promoter regions of genes involved in control of tomato fruit ripening have been isolated and studied to help in identifying their functional regulatory elements in order to understand fully the mechanisms through which expression these genes is regulated. One such gene promoter region that has been well characterized is of the LeACO1 gene. The -1855 to -396 region of the LeACO1 gene confers expression that depends on the presence of ethylene. It has two repeat regions with homology to 2A11 and E4, which are ripening specific genes. Several other ethylene responsive and stress related motifs are located in this region. The -396 region confers expression which

is independent of ethylene influence (Blume & Grierson, 1997). ACS2 and ACS4's regulatory elements that control their expression have been reported. They share a wound responsive element. LeACS4 promoter has a sequence resembling the Anaerobiosis responsive element found in maize's alcohol dehydrogenase genes (Lincoln, et al., 1993). Putative cis-elements in the promoter region of LeACS6 that negatively regulate ethylene responses have been localized between -347 and -266 in the upstream region the starting point of translation (Lin, Fan, Wan, Charnig, & Wang, 2007). There is evidence showing that ACC synthase enzyme activity is affected by protein phosphorylation which strengthens the idea that synthesis of ethylene is regulated at many intervals from transcription to its activity (Argueso, Hansen, & Kieber, 2007) E4 and E8 profiles contrast which makes them interesting for analysis. E4 gene transcription is stimulated by both *system-1* and *system-2* ethylene. E8 gene transcription is only induced in mature fruits, i.e. by *system-2*, a strong indicator that they are both developmentally regulated and are tissue specific. (Lincoln, Cordes, Read, & Fischer, 1987 and James & Robert, 1988). A minimum of two cis-elements cooperate to make E4 promoter region responsive to ethylene. One element is upstream between -150 and -121bp and the other is downstream between -40 and +65 (Xu, Goldman, Coupe, & Deikman, 1996) E8 gene promoter has a sequence that spans from -409 to -263 which regulates its expression during ripening. Other enhancer elements whose activity is specific to anthers, leaves and pollen are found in the E8 promoter region (Deikman, et al., 1998). Polygalacturonase enzyme is only expressed in mature fruit tissues, and its transcription gets activated only during ripening. Different regions in the PG promoter region positively and negatively interact with regulatory elements that mediate gene expression. The promoter region sequence from -4822 to -1412 together with a 1.8kb 3' end flanking region determine ripening specific expression while -1412 to -150 promoter region has elements which direct spatial expression of genes of the inner and outer pericarp (Montgomery, Pollard, Deikman, & Fischer, 1993 and Nicholass, Smith, Schuch, Bird, & Grierson, 1995) Tomato ripening pleiotropic mutations have greatly contributed to the understanding of mechanisms involved in fleshy fruit ripening. They include colorless non-ripening (*Cnr*), Ripening inhibitor (*Rin*), Never Ripe (*Nr*), Green ripe (*Gr*) and high pigment-1 and 2 ripe. They have been genetically mapped and cloned. *Cnr* is recessive and *Rin* mutation is dominant and thus inhibit ripening (Manning, et al., 2006). The *Rin* mutation encodes for a MADS-box protein, which is partially deleted, belonging to the SEPALATTA clade. *Cnr* encodes for an epigenetic change that changes promoter methylation of the SQAMOSA promoter binding protein. *Nr* encodes for an ethylene receptor gene and *Gr* encodes for a major ethylene signalling component which was done via positional cloning of the underlying gene that regulates dominant ripening mutation (Barry & Giovannoni, 2006). TAGL1, a member of the AGAMOUS clade of the MADS-box genes gets induced early during carpel development and late during the onset of ripening. Its repression via RNAi interference prevented carpel thickening and inhibited ripening (Itkin, et al., 2009 and Pan, McQuinn, Giovannoni, & Irish, 2010). Most positive transcriptional regulators like AGAMOUS, SHATTERPROOF, MADS-box, FRUITFULL e.t.c., that effect ripening pathways have been described. A negative effector SIAP2a, belonging to the APETALA2 gene family, has been described. Its induced during ripening but its repression led to acceleration of ripening, elevated levels of ethylene production and increased carotenoid accumulation (Chung, et al., 2010 and Karlova, et al., 2011).

MiRNAs and Fruit development

They are approximately 21nt endogenous RNAs that play major roles in gene expression in a species-specific manner in plants (Fan, et al., 2014). MiRNAs represent a new and very important form of regulating genes (Jones-Rhoades, Bartel, & Bartel, 2006). They have been identified in developing tomato fruits (Pilcher, et al., 2007). They function as fine tuning agents of gene regulation which if misexpressed can produce pleiotropic effects that can alter development (Palatnik, et al., 2003). Various methods have been used to identify miRNAs. Homology search using bioinformatics has been used to identify them. The disadvantage of this method is that it is not novel and species-specific. 13 MiRNAs candidates distributed in 9 MiRNA gene families have been identified using homology search from 578000 tomato sequences. This was done using known miRNAs from plant species against tomato nucleotide sequences. Second, cloning has enabled detection of MiRNAs but quantities detected by this method are not enough. Third, high throughput sequencing has identified many tomato miRNAs whose presence have been confirmed using Northern blot analysis (Zhuo, et al., 2011).

In order to obtain insights into miRNA targets, researchers need to investigate them under three categories. i.e. the transcription factors they encode that can be involved in plant growth and development, proteins they encode which may be involved in many metabolic processes, and lastly their hormonal, signalling and stress responses (Yin, Li, Han et al., 2008). Functional analysis of tomato sequences have revealed viral (Voinnet, 2001 and Finnegan & Matzke, 2003) and phosphate stress related miRNAs (Gu, Xu, Chen, et al., 2010). The most

important step in determining miRNA functions is first to search for gene targets, identify them and find out their functions through transgenics.

The Chemistry of flavor and ripening

Among the most critical aspects in tomato ripening and flavor chemistry is the conversion of chloroplasts to chromoplasts. It leads to loss of chloroplast's photosynthetic ability when thylakoid structures get disassembled. Chromoplasts are characterized by high number of plastoglobules which are principal sites where carotenoids accumulate (Egea, et al., 2010). Ripening is a multipurpose issue in fruit development since it also serves to attract consumers to assist in fruit dispersal. Lycopene and beta carotene accumulation provide visual cues that serve to attract consumers that the fruits are ripe who help in dispersal (Iijima, et al., 2009) Volatiles are the principal compounds that determine unique tomato and other fruit flavors. Almost 400 volatile compounds have been identified in the tomato fruit. Sugars and amino acids also play major roles (Baldwin, Scott, Shewmaker, & Schuch, 2000). They are major constituents of the tomato fruit and contribute over 60% of dry matter. They contribute to soluble solids i.e. °Brix, and have a major role to play in the overall intensity of flavor (Carbonell-Barrachina, Ana, & Ruiz, 2005). During ripening, enzymes associated with amino acids and sugar metabolism accumulate. Some of these enzymes are; Phosphoenol pyruvate carboxykinase, phosphoenol pyruvate carboxylase, aldolase, glutamine synthetase, cytosolic aspartate aminotransferase among others (Franco & Robert, 2009). Of the more than 400 volatiles detected in ripe tomato fruits, only 16 have been predicted to contribute to tomato flavor. This was based on their concentrations in the fruit (Buttery, Teranishi, Hath, & Ling, 1987). Flavor intensity based on tasting of over 60 tomato varieties was determined by contributions of citric acid, fructose and six other volatiles i.e. 2-butylacetate, 3-methyl-1-butanol, 2-methylbutanol, 1-octen-3-one, cis-3-hexen-1-ol, and trans, trans-2,4-decadienal (Alan, 2012). There were much variations in the types of volatiles that contributed to flavor in the tomato varieties that were examined. This is surprising because tomato DNA sequence have low sequence diversity (Jose & Julin, 2009). Biosynthesis of volatiles is a highly regulated process from the onset till the completion of ripening. Volatile content in ripening tomato and other fruits can be increased by identifying QTLs that control their expression and exploiting them in breeding programmes (Graham, Mervin, James, & Gregory, 2013). Transgenic studies have proven that accumulation of lycopene (Gao, et al., 2008) and polyamine at the transcriptional level (Neily, et al., 2011) among many others lead to slowed biosynthesis of volatiles in ripening fruits.

CONCLUSIONS

Tomato genome sequencing, which was completed three years ago, will play major role in understanding fruit development and ripening genetics. Its genome was compared to its closest relative, the *Solanum pimpinellifolium*. The two species showed nucleotide divergence of over 0.5%, however when compared to Potato, *Solanum tuberosum*, they had divergence of over 8% which showed how the three species may have evolved (The Tomato Genome Consortium, 2012). Tomato ripening has been greatly and widely studied and thus serves as a model to investigate genetics involved in ripening of other fleshy fruits. Transgenics and R-DNA technology will greatly help in understanding pathways and genes involved in biosynthesis of fatty acids, volatiles and sugars, which have not been greatly researched on, and compounds secreted to enable the tomato to respond to biotic stress from pathogens (Li & Steffens, 2002) and from insect pests (Abdeen, et al., 2005) by evolving active & passive defensive mechanisms; and abiotic stresses (Patade, et al., 2013 and Wang, Wisniewski, Meilan, Cui, & Fuchigami, 2006).

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