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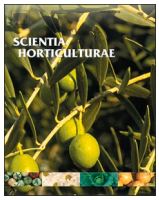

Review

# The tomato *long shelf-life* fruit phenotype: Knowledge, uncertainties and prospects

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ABSTRACT

The *long shelf-life* (LSL) phenotype is characteristic of diverse Mediterranean tomato landraces and is defined by the outstanding durability of the fruit, showing no symptoms of deterioration for up to ten months after harvest. The genetic basis behind this abnormal phenotype is yet to be fully characterized, though many ripening-related mutants have been identified and studied during the past decades. New technologies have allowed for a deeper understanding of the genetic background of this trait and have revealed that the LSL phenotype is dependent on more traits beyond the ethylene synthesis and response pathways. Nevertheless, full determination of ethylene synthesis levels in mutants of commercial interest, such as *rin, nor* and *alc*, warrants some further research as to describe to what extent these mutations affect ethylene response. Available data suggests that singular mutations are not enough to lead to the LSL phenotype. Particularly, the role of the *alc* allele is not entirely clear, as newer studies suggest this mutation alone is not responsible for this phenotype in landraces that naturally present it, summed to the fact that Italian LSL landraces do not harbor this particular allele. The molecular traits underlying the LSL phenotype have been increasingly studied for the past decades and it becomes clear that it is far more complex than originally believed, and that traits other than the *alc* mutation must be involved in this phenotype. This review aims to recapitulate the research performed in this area and highlight the questions that are still to be answered to fully explain the basis of this outstanding fruit phenotype.

## 1. Introduction: Interest of LSL landraces and ripening-related mutants

Extending fruit shelf-life after harvest has been widely explored in fleshy fruit crops to allow longer commercial periods, handling and exportation. In tomato (*Solanum lycopersicum* L.), this has been promoted through early harvest –before fully ripen– and storage atmosphere modification, mostly cold, low oxygen and high $CO_2$, which commonly requires ripening treatments with ethylene before commercialization (Majidi et al., 2014). Early harvest results in lower quality fruits, which is worsened by storage and ripening acceleration treatments during postharvest (Klee and Tieman, 2013, 2018). Nevertheless, shelf-life gain achieved in most cultivars after such processes does not surpass 90 days after harvest (Majidi et al., 2014). Despite many advances have been done in *Arabidopsis* (Giovannoni, 2004; Girin et al., 2009; Roeder and Yanofsky, 2006), tomato has served as a model for the study of fleshy climacteric fruits development and ripening partly because of its worldwide importance in human diet and also due to its small genome (c. 900 Mb), short life cycle, well characterized diploid genetics and availability of 'omics' data that has enabled the extensive study of this plant (Chen et al., 2020; The Tomato Genome Consortium, 2012).

The "long shelf-life" (LSL) or "long storage" fruit phenotype is defined by the long durability of the fruit, free of deterioration for a long period ranging between five to ten months after harvest, showing no signs of wrinkling (Bota et al., 2014; Casals et al., 2012; Ercolano et al., 2014). LSL tomatoes are picked fully ripen from the vine, and unlike most commercial tomato cultivars, they do not require temperature storage, leading to better fruit quality (Casals et al., 2015; Conesa et al., 2020; Renard et al., 2013). LSL landraces are commonly cultivated outdoors in summer, harvested between July and September, and stored in ventilated sheds with environmental conditions similar to the ambient ones occurring from harvest to early spring next year (Conesa et al., 2020).

The LSL phenotype is a defining trait in landraces from Southern Italy regions, mainly Campania and Sicily, and from Eastern Spain regions, especially the Balearic Islands, Catalonia and Valencian Country. Besides these geographic origins, it is not rare to find such landraces

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scattered in many other regions of the Mediterranean basin and across the Iberian Peninsula to Portugal (Conesa et al., 2020).

At least in regions of high prevalence of those landraces, the LSL trait has been selected during centuries to allow having fleshy fruits especially in the winter period, when its availability was lowest. Vine ripe fruits are disposed in bunches, either harvesting fruits attached to the inflorescence pedicels, or sewing individual tomato pedicels to a main rope to thread strings of variable length, and then are stored hung on ventilated sheds. Consequently, such landraces are an important part of the local heritage and bear local names referring to the storage systems and to the long-storage trait. Most common names are 'de Ramellet' (Balearic Islands), 'de Penjar' (Catalonia and Valencian Country), 'da serbo', 'del piennolo', 'da appendere', and 'd'inverno' (Italy). Other terms have been used to describe phenotypes that share many key factors with the LSL phenotype, such as the "delayed fruit deterioration" (DFD) tomato cultivar first described by Saladié et al. (2007), with behavior highly similar to 'de Ramellet' or 'de Penjar'. Most of those landraces have been selected under rainfed conditions in agronomic environments where water scarcity has been a prime, so many genotypes are also highly tolerant to abiotic stresses like drought and high temperature (Conesa et al., 2020). Notwithstanding, there is still no clear information in literature allowing to do a comprehensive classification of the existing LSL landraces based on genetic diversity and similarity among each region's populations. This has been partially done in some Italian regions and reflects a relatively low genetic difference among landraces bearing different local names (Tranchida-Lombardo et al., 2018b). Consequently, diversity in local names seems not to reflect genetic diversity within LSL landraces. A compilation of local names for LSL landraces in literature can be found in Conesa et al. (2020).

Diverse spontaneous mutants have been described bearing monogenic mutations leading to different degrees of ripening impairment, like *Never-ripe* (*Nr*) (Rick and Butler, 1956), *Colorless non-ripening* (*Cnr*) (Thompson et al., 1999), *Green ripe* (*Gr*) (Kerr, 1958), *ripening inhibitor* (*rin)* (Robinson and Tomes, 1968; Tigchelaar et al., 1978), *non-ripening* (*nor)* (Tigchelaar et al., 1978) and *alcobaça* (*alc*) (Almeida, 1961; Kopeliovitch et al., 1981; Leal and Tabim, 1974). Particularly, Western Mediterranean LSL landraces of 'de Penjar' and 'de Ramellet' are characterized by bearing the *alc* mutation (Bota et al., 2014; Casals et al., 2012), and their LSL phenotype has been attributed to this mutation, which has also been argued as an explanation for the LSL phenotype in Italian 'da serbo' landraces (Bota et al., 2014; Casals et al., 2012; Ercolano et al., 2014; Mercati et al., 2015). Nevertheless, the *alc* mutation is absent in LSL landraces from Campania like 'Corbarino' and 'Lucariello' (Tranchida-Lombardo et al., 2018a), and therefore cannot explain their LSL phenotype.

In the description of the *alc* mutation, from the 'Alcobaça' ripening mutant at the homonym Portuguese location, authors reported shelf-life longer than 300 days after harvest (Almeida, 1961; Leal and Tabim, 1974), which is similar to the reported for diverse 'de Ramellet' LSL accessions (Bota et al., 2014). However, any further literature report for shelf-life in *alc* mutants has always been, to the best of our knowledge, below 40 days (Dias et al., 2003; Kopeliovitch et al., 1981; Mutschler, 1984; Mutschler et al., 1988, 1992; Yu et al., 2017).

This review aims to address the uncertainties surrounding LSL landraces and ripening-related tomato mutants and offer new insights on the topic.

## 2. Ripening impairment and the LSL fruit phenotype

### 2.1. Tomato ripening-related mutants

Ripening-related mutations in tomato produce different degrees of ripening impairment and pleiotropic effects. In some cases, like *Gr* and *Nr*, the fruit fails to ripen, remaining of a green-yellow color with no signs of fruit softening, and measuring shelf-life and fruit quality is meaningless (Barry and Giovannoni, 2006; Lanahan et al., 1994; Monteiro et al., 2011). In other mutants, homozygosity produces severe pleiotropic effects, not only preventing normal softening and colouration, but also altering sugars, acids, volatiles and other quality traits defining nutritional value, flavor and aroma. However, these pleotropic effects are minimized in heterozygosis and, as compared to non-mutant counterparts, result in firmer fruits having slower fruit ripening and longer shelf-life (Fujisawa et al., 2013; Garg et al., 2008; Kitagawa et al., 2005). In this regard, *rin* and *nor* mutations are common in heterozygosis in breeding programs (Garg et al., 2008; Klee and Giovannoni, 2011; Kosma et al., 2010), achieving shelf-lives up to 54 days after harvest (Dhatt et al., 2001; Garg et al., 2008a). With respect to the *alc* mutation, also used in heterozygosis in breeding programs, the pleiotropic effects of the homozygous mutant are less pronounced than those for *rin* and *nor* (Dhatt et al., 2001; Kosma et al., 2010; Mutschler et al., 1992). Therefore, ripening-related mutants *alc, nor* and *rin* gained a considerable attention to prolong shelf-life of tomato. The fruits of these mutants, generally characterized by an absence of a ripening-associated ethylene burst, or lower levels of ethylene during the onset of ripening, are unable to ripen in the presence of exogenous ethylene. They are also useful in research and breeding of cultivated tomatoes for postharvest quality.

The position of these mutations in the genome is very diverse (Fig. 1), although most affect pathways related to ethylene, causing either insensitivity to ethylene and low endogen production of hormone (Barry and Giovannoni, 2006; Giovannoni, 2007). At least *rin* and *nor* mutants are considered non-climacteric, while *alc* partially climacteric (Garg et al., 2008a; Kosma et al., 2010).

Genetic screening of diversity across two Italian LSL landrances, 'Corbarino' and 'Lucariello', led to the discovery of great variation in genes associated to ethylene response, relating it to the LSL phenotype (Tranchida-Lombardo et al., 2018a). Nevertheless, the genetic determinants of the LSL phenotype in those landraces remains unclear since no polymorphism for either *nor* or *alc* mutations was found.

### 2.2. The maturation process in tomato

In addition to the role of ethylene in fruit ripening, maintenance of

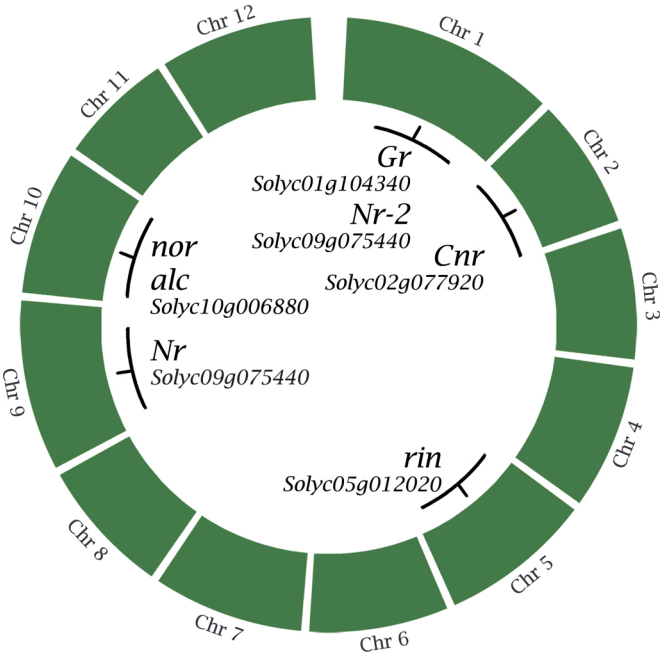


**Fig 1.** Chromosome localization of ripening-related mutations: *Green Ripe* (*Gr*), *Never Ripe* (*Nr*), *Never Ripe-2* (*Nr-2*), *Colorless Non-Ripening* (*Cnr*), *Ripening Inhibitor* (*rin*), *Non-Ripening* (*nor*) and *alcobaça* (*alc*), on a radial representation of the *S. lycopersicum* genome. Codes below each mutation correspond to the Sol Genomics Network (https://solgenomics.net/) for each loci.

the fruit integrity for months after harvesting without temperature storage implies a decrease in respiratory metabolism and transpirational water loss. Fruit ripening is mediated by both intrinsic developmental signals and extrinsic environmental factors, and results in significant changes on the color, aroma, taste, texture, and other physiochemical properties of the fruit, ultimately determining its quality.

In tomato, the stages of maturation can be defined as mature green (MG), breaker stage (BR), and red ripe (RR), (Giovannoni, 2004). The time between stages can vary widely among cultivars. The transition from MG to BR is triggered by concurrent peaks in respiration and in ethylene production in the climacteric rise, a point in the maturation process at which several ripening-related mutants fail to further develop mature fruits due to ethylene insensitivity and/or lack of ethylene production, such as *rin, Cnr* and *Nr*. In non-mutant tomatoes, ethylene burst results in unavoidable ripening leading to the RR stage and, in general, RR fruits continue to overripen (OR), causing higher degrees of water loss that favor pathogen attacks (Fig. 2). At this point in the maturation process, fruit respiration is lowered, though levels of gas exchange may vary from cultivar to cultivar (Giovannoni, 2004).

Excessive softening is the main factor limiting fruit shelf-life and post-harvest storage. Studies on transgenic plants with modified cell wall alteration show that the processes regulating fruit softening are vastly complex and involve the activity of a large group of cell wall-modifying enzymes (Brummell and Harpster, 2001; Rose et al., 2003; Saladié et al., 2007). Reverse genetic studies on the expression of cell wall modifying proteins have revealed that polygalacturonase (PG) activity is largely responsible for pectin depolymerization on the cell wall, although suppression of PG activity only slightly reduces fruit softening while extending fruit shelf-life (Brummell and Harpster, 2001). Expansins, another group of cell wall proteins, have been extensively characterized in tomato, being determined as the responsible for the stretching and loosening of isolated cell walls, and the disruption of hydrogen bonds between polymers of the cell wall matrix (Bennett and Labavitch, 2008).

Two systems are responsible for ethylene biosynthesis in fruits (McMurchie et al., 1972). System 1 determines the production of basal ethylene levels in all tissues, including those of non-climacteric fruits. On the other hand, System 2 operates exclusively during the onset of ripening in climacteric fleshy fruits, when autocatalytic synthesis begins. It is worth noticing that in climacteric fruit ripening, System 1 is autoinhibited with ethylene, and its synthesis stops at the onset of ripening (Barry et al., 2000; Liu et al., 2015). Interestingly, mutants with impaired ethylene production and sensitivity such as *rin, Cnr* and *Nr* continue to produce basal levels of ethylene throughout the entire maturation process (Giovannoni et al., 1989; Lincoln and Fischer, 1988), suggesting that System 2 failure is responsible for these mutants' phenotype (Fig. 2). Altogether, any alteration on both the ethylene synthesis and the ethylene signaling pathways may result in aberrant ripening of the fruit.

Ethylene biosynthesis reactions are catalyzed in three distinct steps by S-adenosyl-L-methionine synthetases (SAMS), ACC synthases (ACS), and ACC oxidases (ACO), respectively. The mechanisms underlying autocatalytic ethylene biosynthesis were first identified by investigating the functions of ACOs and ACSs due to their higher levels when compared to SAMS (Barry et al., 2000; Satoh and Yang, 1988). Among the known *ACO* genes in tomato, only two display ethylene-dependent expression patterns and are strongly up-regulated concurrently with the ethylene peak (Liu et al., 2015).

Ethylene signaling plays an equally important role in the process of fruit ripening. Studies on *Arabidopsis* complementing the data available on *S. lycopersicum* have revealed that the diverse responses to ethylene are mainly transduced by *ETHYLENE RESPONSE* (*ETR*s) receptors, which start the ethylene signaling cascade (Chen et al., 2018). *CONSTITUTIVE TRIPLE RESPONSE* (*CTR*) regulators play an important role as mediators between ETRs and *Ethylene-Insensitive 2* (*EIN2*), a mediating factor in Arabidopsis. *LeCTR1*, a *CTR1* homologue, activates the TFs *EIN3/EIN3*-like (*EIL*) and triggers the expression of ethylene response factors (*ERF*s) in tomato (Chen et al., 2020; Liu et al., 2015).

### 2.3. Maturation in LSL landraces

Contrary to ripening-related mutants, LSL tomato cultivars show a normal climacteric ripening process with apparently no signs of impairment in the ethylene synthesis and signaling pathways, although can show some coloration defects (Conesa et al., 2020). It is interesting to note that LSL tomato cultivars from Catalonia, Valencian Country and Balearic Islands are homozygous for the *alc* allele (Bota et al., 2014; Casals et al., 2012), and this does not lead to an impaired phenotype. There are apparently no differences in the ripening process from MG to RR between LSL and non-LSL tomatoes, especially in timing, indicating that respiration might be similar. However, there is a notorious difference from RR to OR, so that LSL landraces' fruits may somehow dramatically reduce respiration to ensure the fruit integrity and organoleptic traits are maintained for up to ten months after harvest. How RR fruits' respiration is minimized in LSL tomatoes remains a question, allowing discussion on possible altered processes and metabolic pathways that might explain the LSL phenotype. Some evidence has been described in 'de Penjar' tomatoes, showing downregulation of citrate and upregulation of malate (Kumar et al., 2018), which has been related to respiration attenuation (Centeno et al., 2011).

## 3. Genetic insights on tomato ripening impairment and the LSL phenotype

Classifying ripening-related mutants based on their phenotype would not reflect the reasons underlying ripening impairment. Besides the phenotype, mutants could be characterized according to the metabolic route affected. The ripening process in tomato is regulated by a highly complex network of genes and transcription factors (Fig. 2). Different impairments in the same metabolic pathway result in similar

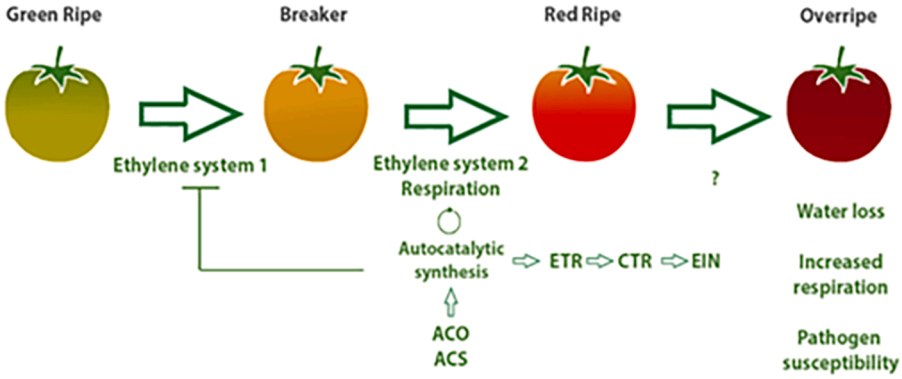


**Fig. 2.** Model for the maturation process in *S. lycopersicum*, where ACO stands for ACC (1-aminocyclypropane-carboxylate) oxidases, ACS for ACC synthases, ETR for *ETHYLENE RESPONSE*, CTR for *CONSTITUTIVE TRIPLE RESPONSE* and EIN for *Ethylene-Insensitive*. System 1 ethylene production is detected at basal levels throughout plant development, while System 2 ethylene production starts at the onset of ripening along with increased respiration rates. The molecular processes that lead to the overripe state of the fruit are not yet fully characterized.

phenotypes, although the severity of the impairment may differ depending on how upstream or downstream it is affected. In addition to the position at which the pathway is affected, the severity of the impairment may depend on how it is produced (Barry and Giovannoni, 2006; Lanahan et al., 1994). Nevertheless, the genetic basis underlying ripening-related phenotypes is variable among mutants, and mutations responsible are spread across the genome (Fig. 1). As exceptions, mutations among the most studied mutants are considered allelic, such as *nor* and *alc*, and also *Gr* and *Nr-2* (Barry et al., 2005; Barry and Giovannoni, 2006; Garg and Cheema, 2014; Kumar et al., 2018).

An important characteristic of these mutations is their dominant or recessive nature. While dominant mutations, such as *Gr, Nr, Nr-2* and *Cnr*, result in an irreparable ripening impairment that leads to inedible fruits, recessive mutations, like *rin, nor* and *alc*, have potential to be used commercially in their heterozygous state with relatively minor alterations in the ripening process (Garg et al., 2008b; Wang et al., 2020). Promising results for increased shelf-life in non-LSL tomatoes have been found via hybrid, backcrossed and recombinant inbred lines (RILs) obtained from crossing wild and cultivated tomato accessions, since shelf-life is usually longer in the formers. Genotypes resulting from the use of wild *S. lycopersicum* var. *cerasiforme* and *S. pimpinellifolium* parents showed fruits with increased shelf-life after harvest as compared to their wild parent, with also an impact on increased fruit quality (Rodríguez et al., 2006, 2010). In this regard, authors included also the mutant *nor* in some of the crosses and found increased shelf-life when this mutant was crossed with the wild *cerasiforme*, highlighting the potential of the wild germplasm to breed for increased shelf-life (Rodríguez et al., 2005, 2010). Nevertheless, maximum shelf-life obtained among such genotypes is notoriously shorter than the commonly reported in LSL landraces (Conesa et al., 2020).

### 3.1. Mutations with commercial potential

Among the tomato ripening-related mutants that have aided a better understanding of the complex process of fleshy climacteric fruit, three of them have been identified to affect the initiation of ripening: *Cnr, rin* and *nor*. Because of the global effects of these mutations on ripening, the proteins encoded by these loci have been determined to be key in the control of ripening prior to the production of ethylene in tomato. Of the three, *rin* has been the most extensively used for commercial purposes and largely investigated in many studies (Ito et al., 2017; Kopeliovitch et al., 1979; Lincoln and Fischer, 1988).

The transcription factor *MADS-RIN* acts as an essential regulator of the ripening process in tomato, as it affects the accumulation of many gene transcripts, proteins and their potential post-translational modifications (Giovannoni, 2004). The MADS-box family transcription factors often act as multimers (Honma and Goto, 2001), and the MADS-box proteins *TOMATO AGAMOUS-LIKE 1* (*TAGL1*) and two homologues of *Arabidopsis FRUITFULL* in tomato (*FUL1, FUL2*) act as coregulators of ripening with *RIN* (Bemer et al., 2012; Fujisawa et al., 2012; Itkin et al., 2009; Martel et al., 2011; Shima et al., 2014; S. Wang et al., 2014). Initially described by Robinson and Tomes (1968), the *rin* mutation was firstly thought to correspond to a loss-of-function event (Vrebalov et al., 2009). More recent studies have demonstrated that *rin* generates an active hybrid transcription factor (*RIN-MC*) with a repressor function, although the factor that takes on the role of ripening initiator in the absence of *RIN* remains unknown (Ito et al., 2017; Li et al., 2019).

Sequencing studies have successfully analyzed the RIN-binding sites across the genome, showing *RIN* binds to demethylated sites in the promoter regions of ripening genes (Zhong et al., 2013), proving that *MADS-RIN* is central in the regulation of ripening. Additionally, involvement of homologues in other plants have been found to play equally essential roles, even in non-climacteric species (Elitzur et al., 2016; Seymour et al., 2011).

A review by Ito et al. (2008) denoted that *RIN* regulates other transcription factors involved in fruit ripening, among them the *NOR* transcription factor. Ethylene mediates the induction of ripening-related genes under the control of mediating transcription factors, and the ethylene signaling pathway enhances the expression of *RIN, FUL1* and *NOR* in a positive feedback loop (Fujisawa et al., 2013). Studies on the function of *NAC-NOR* and the *nor* mutant have lagged behind the study of *MADS-RIN* and *rin*. In the case of the *nor* mutant, the synthesis of ethylene is considerably reduced, and fruits do not manage to fully ripen. The *nor* mutation was caused by the deletion of two adenines in the third exon of the *NAC-NOR* (Fig. 3), part of the NAC gene family. This shift in the reading frame causes the *NAC-NOR* gene to encode a truncated NOR protein much shorter than its wild-type counterpart (186 amino acids respect to WT's 345 amino acids). This fact results in a modified transcriptional activation region, but the DNA-binding region is fully conserved in *nor* mutants (Giovannoni et al., 2004), suggesting that the *nor* mutant phenotype is a consequence of a loss-of-function of the *NAC-NOR* gene (Gao et al., 2020). Interestingly, the mutation that occurs naturally in the *NAC-NOR* region in 'de Penjar' tomato inhibits several metabolic processes and yields in prolonged shelf-life of the fruit (Kumar et al., 2018), and overexpression of *NAC-NOR* accelerates the senescence of the tomato plant (Ma et al., 2019).

Another mutant of high interest, *alc*, presents an allelic mutation at the *NAC-NOR* gene (Fig. 3). The fruits of the homozygous genotype are characterized by reduced carotenoid content, increased firmness and increased shelf-life (Faria et al., 2003). The effects are additive and use of the allele in the heterozygous condition can extend shelf-life and minimize fruit loss. At the molecular level, thymine is replaced by adenine at position 317 in the wild type *NAC-NOR* gene (Casals et al., 2011).

Contrary to *Nr, Gr, Nr-2* and *Cnr*, tomatoes heterozygous for the *rin* mutation have long been successfully commercialized. Several varieties have been breed from ripening-related mutants, such as the 'Red Centre' (HRAS 87–70 x rin-HRAS 81–85) and 'Juliette' (79T-I x rin-795,054–1) from *rin*, which have approximately 40 days shelf-life at 20 °C. The *nor* gene has been exploited in other varieties, such as 'Vasilisa' or 'Changline'. Polish hybrid cultivar 'Rafal' has an exceptional storage life of about 100 days and provided a maximum of four weeks of harvesting delay (Seroczynska et al., 1998).

Available data confirms that LSL landraces do not harbor dominant mutations and, from the recessive mutations that have been liked to longer shelf-life, *alc* alone is unable to explain the LSL phenotype, and the variation existing among different LSL landraces.

## 4. LSL tomato landraces as an untapped resource for genetic studies

Modern techniques have allowed for a deeper understanding of the genetic, molecular and physiological background that leads to the LSL phenotype. Although, there are still many open questions to fully understand its genetic basis and underlying determinants. The incorporation of Genome Wide Association Studies (GWAS), RNA-seq and CRISPR/Cas9 technology in tomato research has brought new insights to the discussion.

Advances in DNA sequencing technology have been used for in depth characterization of diverse tomato accessions, and particularly to obtain a reference genome for tomato (Causse et al., 2013; The Tomato Genome Consortium, 2012). This first de novo sequencing project notoriously increased chances for sequencing further tomato genomes and served as a platform to ease the design of chips allowing fast genotyping of large arrays of genetic markers based on this genome as reference (Tranchida-Lombardo et al., 2018a).

The role of *rin* and *nor* has therefore been re-examined as to assess to which extent the ripening process is altered in those mutants. Contradicting the previous hypothesis that the *rin* mutation functions as a ripening inducer, research has shown that CRISPR/Cas-9 mediated RIN-knockout mutants do not fail to initiate ripening (Elitzur et al., 2016; Seymour et al., 2011; Vrebalov et al., 2002), suggesting that *rin* is not a

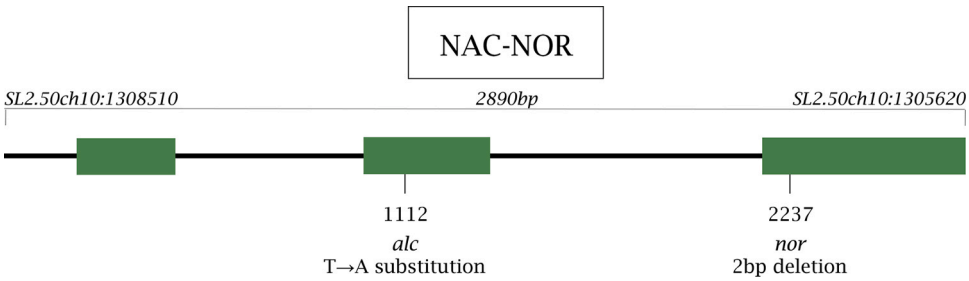


**Fig. 3.** Localization of the ripening-related mutations *Non-Ripening* (*nor*) and *Alcobaça* (*alc*) within the genomic sequence of the NAC-NOR region (Solyc10g006880). The *alc* mutation occurs due to a thymine to adenine replacement in position 1112. In turn, *nor* occurs due to a two base pair deletion at position 2237. Boxes indicate exonic regions; lines indicate intronic regions.

null mutation but rather a gain-of-function mutation that produces a ripening-inhibitor protein (Ito et al., 2017; Li et al., 2018, 2019). Very similarly, over-expression of the *NOR* gene in *nor* mutants did not recover the full ripening phenotype. CRISPR/Cas-9-edited *NOR* tomatoes showed a decrease of ethylene synthesis and fruit softening when compared to wild-type tomatoes, though not as significant as in *nor* mutants (Gao et al., 2020). Moreover, overexpressed *NOR* fruit presented increased levels of ethylene synthesis and fruit softening. In addition, to suggest the *NOR* gene is indeed key to tomato fruit ripening, the results from this study also point that the *nor* mutation is a gain-of-function mutant that leads to ethylene synthesis repression, very similarly to how the *rin* mutation generates a ripening-inhibitor protein (Gao et al., 2019, 2020; Kumar et al., 2014; Wang et al., 2019). Although it is clear that correct ethylene synthesis is essential for a full ripening, the exact levels at which ripening transcription factors and NAC-NOR alleles affect ethylene production have not been widely reported.

To the best of our knowledge, the first whole genome re-sequencing for a tomato LSL landrace was that for the Italian 'Vesuviano' (Ercolano et al., 2014). Later on, two more Campania LSL landraces, 'Lucariello' (a 'Vesuviano' type landrace) and 'Corbarino', were re-sequenced (Tranchida-Lombardo et al., 2018a). Knowledge on the determinants of fruit quality and extended shelf-life were among the prime aims behind it. All these new studies have paved the way for new questions and re-examination of the old ones. It is not yet entirely clear whether the *NOR* gene and more specifically the *alc* allele are key to the LSL phenotype in local landraces of the Mediterranean basin, as it was assumed so far (Bota et al., 2014; Casals et al., 2012, 2015; Figàs et al., 2018; Mercati et al., 2015). Interestingly, 'Corbarino' and 'Lucariello' landraces do not present any mutation in the *NOR* gene, so they do not bear the *alc* mutation, in contrast to the 'de Penjar' and 'de Ramellet' accessions (Bota et al., 2014; Casals et al., 2012). This, added to the high amount of SNPs related to ripening that these three Italian landraces harbor, suggests that the genetic background behind the LSL phenotype is much broader than a single point mutation in the *NOR* gene.

In this regard, introduction of the *alc* mutation in 'M82' via CRISPR/Cas9 displayed an increase in shelf-life of up to 40 days (Yu et al., 2017), which is similar to the reported for *rin* and *nor* heterozygous mutants used for commercial purposes (Garg et al., 2008b; Wang et al., 2020), and to most reports for the *alc* mutant (Dias et al., 2003; Kopeliovitch et al., 1980; Mutschler, 1984; Mutschler et al., 1988, 1992), but far shorter than the five to ten months shelf-life commonly reported for LSL landraces bearing the *alc* mutation (Bota et al., 2014; Casals et al., 2012). Altogether, it seems unfeasible that the LSL phenotype is explained solely by the *alc* mutation, and the relationship between *alc* and the LSL phenotype could be a matter of "linked evolution" occurring in 'de Penjar' and 'de Ramellet' landraces (Bota et al., 2014; Casals et al., 2015; Kumar et al., 2018; Seymour et al., 2013), but not in Italian LSL landraces, at which the *alc* mutation has been suggested as responsible, but not confirmed to our knowledge (Mercati et al., 2015). These doubts become increased after two observations: first, large landrace collections homozygous for *alc* display very different shelf-lifes (Bota et al., 2014), and second, the LSL phenotype can be severely impaired by cultivation conditions in some landraces but not others (Conesa et al., 2014).

To this end, modern sequencing techniques have allowed to identify novel genes and loci that can aid in the explanation of the LSL phenotype. In Italian landraces 'Corbarino' and 'Lucariello', ethylene-responsive transcription factor *ERF13* harbors a splicing-site mutation, affecting the onset of ripening. The *TAGL1* TF, involved in cuticle development and fruit softening, and several other genes likely associated in texture and firmness were also identified as potential key factors to explain the LSL phenotype in some Italian landraces, including 'Vesuviano' and 'San Marzano' (Ercolano et al., 2014; Tranchida-Lombardo et al., 2018a). Analysis at transcript levels revealed down-regulation of *fatty acyl-ACP thioesterase (FATB)*, involved in cuticle formation, could play a significant role in the differences in cuticle composition in 'de Penjar' and 'de Ramellet' landraces in contrast to WT. Cell wall modifying genes, such as β-GALACTOSIDASE (β-GAL), α-GALACTOSIDASE (α -GAL), α-MANNOSIDASE (α -MAN), β-MANNOSIDASE (β-MAN), POLYGALACTURONASE-β (PG- β), PECTIN METHYLESTERASEs (PME), EXPANSINs (EXP), and DEOXYHYPUSINE SYNTHASE (DHS), are downregulated in 'de Penjar' accessions, and System 2 ethylene synthesis genes ACS2, ACS4 and ACO1 show consistently lower expression in LSL landraces (Barry and Giovannoni, 2007; Kumar et al., 2018; Pech and Latché, 2013).

It is also worth mentioning that some 'de Penjar' and especially 'de Ramellet' accessions have some fruit color heterogeneity during ripening, mostly orange spots in the closest part to the fruit pedicel (Fig. 4; Conesa et al., 2020), which could be related to slight pleiotropic effects resulting from the *alc* mutation. This is not common in Italian LSL landraces, to our knowledge.

A study by Kumar et al. (2018) showed that the prolonged shelf-life of some 'de Penjar' accessions is connected to an attenuation of different metabolic processes, such as cell wall degradation, although the genetic background that leads to this phenotype can vary from accession to accession. Both alleles known to affect the NOR gene, *nor* and *alc*, appear to have a quite similar effect on fruit duration. However, a novel allele was found to present a stop codon in the NOR protein after six amino acids. This genotype, however, still leads to a LSL fruit with the capability of full ripening, lasting for similar periods after harvest than those having the *alc* mutation (Kumar et al., 2018). This particular development raises the question of whether the *NOR* gene is relevant at all during the ripening process of tomato fruits and suggests that neither the *nor* nor the *alc* mutations alone can lead to the LSL phenotype. Thus, the explanation to the LSL phenotype lies in the genetic background of these Mediterranean landraces.

In addition to the key role of ethylene in fleshy climacteric fruits ripening, other factors must contribute in order to fully explain the LSL phenotype. Fruit cuticle is reportedly an important factor contributing to longer shelf-life after harvest in non-LSL tomatoes (Matas et al., 2009), and differences in cuticle size result in diversity in resistance to desiccation and pathogens. A study by Kosma et al. (2010) showed that *rin, nor* and *alc* mutants' cuticle diverged vastly from wild-type

**Fig. 4.** Tomatoes from 'de Ramellet' accessions present a visible yellow-orange gradient near the pedicel of the fruit.

tomatoes, suggesting that the LSL phenotype involves more than an ethylene synthesis impairment. This cuticle development is more akin to other non-climacteric fruits, such as cherries, which deposit maximum levels of wax at early stages of development (Knoche and Peschel, 2007). Still, cuticle properties ensuring a minimum water loss by transpiration might notoriously contribute to the fruit integrity maintenance for such a long period. In turn, a limited permeability of the cuticle to $CO_2$ and $O_2$ could also explain higher reduction in respiration than in non-LSL tomatoes (Heuvelink, 1996; Kader and Saltveit, 2003; Mojevic and Tesanovic, 2011). Cuticle development in ripening-related mutants is not impaired, as opposed to other ripening related processes such as cell wall disassembly (Giovannoni, 2004; Kosma et al., 2010). However, how larger cuticle may affect respiration has not been extensively characterized yet, and this may present a very interesting window for fleshy climacteric fruit ripening and longer shelf-life phenotypes research.

## 5. Conclusion

Altogether, besides of the *alc* mutation, current evidence suggests that the LSL phenotype may be related to further genetic determinants, among which fruit cuticle properties seem to be a cornerstone. This could also explain the impairment in the LSL phenotype occurring in some 'de Ramellet' accessions when cultivated under high water availability. Therefore, future research on the determinants of extended fruit shelf-life and the LSL phenotype might focus on genes related to cuticle formation, of which many have been already highlighted in several studies.

## Declaration of Competing Interest

The authors declare no interest influencing this paper.

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## References

Almeida, J.L.F., 1961. Um novo aspecto de melhoramento do tomato. Agricultura 10, 43–44.

Barry, C.S., Giovannoni, J.J., 2006. Ripening in the tomato green-ripe mutant is inhibited by ectopic expression of a protein that disrupts ethylene signaling. In: Proceedings of the National Academy of Sciences of the United States of America, 103, pp. 7923–7928. https://doi.org/10.1073/pnas.0602319103.

Barry, C.S., Giovannoni, J.J., 2007. Ethylene and fruit ripening. J. Plant Growth Regul. 26 (2), 143–159. https://doi.org/10.1007/s00344-007-9002-y.

Barry, C.S., Llop-Tous, M.I., Grierson, D., 2000a. The regulation of 1-aminocyclopropane-1-carboxylic acid synthase gene expression during the transition from system-1 to system-2 ethylene synthesis in tomato. Plant Physiol. 123 (3), 979–986. https://doi.org/10.1104/pp.123.3.979.

Barry, C.S., McQuinn, R.P., Thompson, A.J., Seymour, G.B., Grierson, D., Giovannoni, J. J., 2005. Ethylene insensitivity conferred by the Green-ripe and Never-ripe 2 ripening mutants of tomato. Plant Physiol. 138 (1), 267–275. https://doi.org/10.1104/pp.104.057745.

Bemer, M., Karlova, R., Ballester, A.R., Tikunov, Y.M., Bovy, A.G., Wolters-Arts, M., de Barros Rossetto, P., Angenent, G.C., de Maagd, R.A., 2012. The tomato fruitfull homologs tdr4/ful1 and mbp7/ful2 regulate ethylene-independent aspects of fruit

ripeningW. Plant Cell 24 (11), 4437–4451. https://doi.org/10.1105/tpc.112.103283.
Bennett, A.B., Labavitch, J.M., 2008. Ethylene and ripening-regulated expression and function of fruit cell wall modifying proteins. Plant Science 175 (1–2), 130–136. https://doi.org/10.1016/j.plantsci.2008.03.004.
Bota, J., Conesa, M.À., Ochogavia, J.M., Medrano, H., Francis, D.M., Cifre, J., 2014. Characterization of a landrace collection for Tomàtiga de Ramellet (Solanum lycopersicum L.) from the Balearic Islands. Genet. Resour. Crop Evol. 61 (6), 1131–1146. https://doi.org/10.1007/s10722-014-0096-3.
Brummell, D.A., Harpster, M.H., 2001. Cell wall metabolism in fruit softening and quality and its manipulation in transgenic plants. Plant Mol. Biol. 47 (1–2), 311–339. https://doi.org/10.1023/A:1010656104304.
Casals, J., Cebolla-Cornejo, J., Roselló, S., Beltrán, J., Casañas, F., Nuez, F., 2011. Long-term postharvest aroma evolution of tomatoes with the alcobaça (alc) mutation. Eur. Food Res. Technol. 233 (2), 331–342. https://doi.org/10.1007/s00217-011-1517-6.
Casals, J., Marti, R., Casañas, F., Cebolla, J., 2015. Sugar-and-acid profile of penjar tomatoes and its evolution during storage. Sci. Agric. 72 (4), 314–321. https://doi.org/10.1590/0103-9016-2014-0311.
Casals, J., Pascual, L., Cañizares, J., Cebolla-Cornejo, J., Casañas, F., Nuez, F., 2012. Genetic basis of long shelf life and variability into Penjar tomato. Genet. Resour. Crop Evol. 59 (2), 219–229. https://doi.org/10.1007/s10722-011-9677-6.
Causse, M., Desplat, N., Pascual, L., Le Paslier, M.C., Sauvage, C., Bauchet, G., Bérard, A., Bounon, R., Tchoumakov, M., Brunel, D., Bouchet, J.P., 2013. Whole genome resequencing in tomato reveals variation associated with introgression and breeding events. BMC Genom. (1), 14. https://doi.org/10.1186/1471-2164-14-791.
Centeno, D.C., Osorio, S., Nunes-Nesi, A., Bertolo, A.L.F., Carneiro, R.T., Araújo, W.L., Steinhauser, M.C., Michalska, J., Rohrmann, J., Geigenberger, P., Oliver, S.N., Stitt, M., Carrari, F., Rose, J.K.C., Fernie, A.R., 2011. Malate plays a crucial role in starch metabolism, ripening, and soluble solid content of tomato fruit and affects postharvest softening. Plant Cell 23 (1), 162–184. https://doi.org/10.1105/tpc.109.072231.
Chen, T., Qin, G., Tian, S., 2020. Regulatory network of fruit ripening: current understanding and future challenges. New Phytol. 228 (4), 1219–1226. https://doi.org/10.1111/nph.16822.
Chen, Y., Grimplet, J., David, K., Castellarin, S.D., Terol, J., Wong, D.C.J., Luo, Z., Schaffer, R., Celton, J.M., Talon, M., Gambetta, G.A., Chervin, C., 2018. Ethylene receptors and related proteins in climacteric and non-climacteric fruits. Plant Sci. 276, 63–72. https://doi.org/10.1016/j.plantsci.2018.07.012. July.
Conesa, M.A., Fullana-Pericàs, M., Granell, A., Galmés, J., 2020. Mediterranean long shelf-life landraces: an untapped genetic resource for tomato improvement. Front. Plant Sci 10, 1–21. https://doi.org/10.3389/fpls.2019.01651. January.
Conesa, M.A., Galmés, J., Ochogavía, J.M., March, J., Jaume, J., Martorell, A., Francis, D. M., Medrano, H., Rose, J.K.C., Cifre, J., 2014. The postharvest tomato fruit quality of long shelf-life Mediterranean landraces is substantially influenced by irrigation regimes. Postharvest Biol. Technol. 93, 114–121. https://doi.org/10.1016/j.postharvbio.2014.02.014.
Dhatt, A.S., Singh, S., Cheema, D.S., Dhaliwak, M.S., 2001. Heterosis for quality traits nor, rin and alc alleles in tomato. Crop Improv.t 28, 177–186.
Dias, T.J.M., Maluf, W.R., Faria, M.V., Freitas, J.A., de, Gomes, L.A.A., Resende, J.T.V., Azevedo, S.M.de., 2003. Alcobaça allele and genotypic backgrounds affect yield and fruit shelf life of tomato hybrids. Sci. Agric. 60 (2), 269–275. https://doi.org/10.1590/s0103-90162003000200010.
Elitzur, T., Yakir, E., Quansah, L., Zhangjun, F., Vrebalov, J., Khayat, E., Giovannoni, J.J., Friedman, H., 2016. Banana MaMADS transcription factors are necessary for fruit ripening and molecular tools to promote shelf-life and food security. Plant Physiol. 171 (1), 380–391. https://doi.org/10.1104/pp.15.01866.
Ercolano, M.R., Sacco, A., Ferriello, F., D'Alessandro, R., Tononi, P., Traini, A., Barone, A., Zago, E., Chiusano, M.L., Buson, G., Delledonne, M., Frusciante, L., 2014. Patchwork sequencing of tomato San Marzano and Vesuviano varieties highlights genome-wide variations. BMC Genom. (1), 15. https://doi.org/10.1186/1471-2164-15-138.
Faria, M.V., Maluf, W.R., Azevedo, Márcio De, S., M., Andrade-Júnior, Carvalho, V., Augusto Gomes, L., A., Moretto, P., Licursi, V, 2003. Yield and post-harvest quality of tomato hybrids heterozygous at the loci alcobaça, old gold-crimson or high pigment. Genet. Mol. Res. 2 (3), 317–327.
Figàs, M.R., Prohens, J., Raigón, M.D., Pereira-Dias, L., Casanova, C., García-Martínez, M.D., Rosa, E., Soler, E., Plazas, M., Soler, S., 2018. Insights into the adaptation to greenhouse cultivation of the traditional mediterranean long shelf-life tomato carrying the alc mutation: a multi-trait comparison of landraces, selections, and hybrids in open field and greenhouse. Front. Plant Sci. 871, 1–16. https://doi.org/10.3389/fpls.2018.01774. December.
Fujisawa, M., Nakano, T., Shima, Y., Ito, Y., 2013. A large-scale identification of direct targets of the tomato MADS box transcription factor RIPENING INHIBITOR reveals the regulation of fruit ripening. Plant Cell 25 (2), 371–386. https://doi.org/10.1105/tpc.112.108118.
Fujisawa, M., Shima, Y., Higuchi, N., Nakano, T., Koyama, Y., Kasumi, T., Ito, Y., 2012. Direct targets of the tomato-ripening regulator RIN identified by transcriptome and chromatin immunoprecipitation analyses. Planta 235 (6), 1107–1122. https://doi.org/10.1007/s00425-011-1561-2.
Gao, Y., Wei, W., Fan, Z., Zhao, X., Zhang, Y., Jing, Y., Zhu, B., Zhu, H., Shan, W., Chen, J., Grierson, D., Luo, Y., Jemrić, T., Jiang, C.Z., Fu, D.Q., 2020. *Re*-evaluation of the nor mutation and the role of the NAC-NOR transcription factor in tomato fruit ripening. J. Exp. Bot. 71 (12), 3560–3574. https://doi.org/10.1093/jxb/eraa131.
Gao, Y., Zhu, N., Zhu, X., Wu, M., Cai-Zhong, J., Grierson, D., Luo, Y., Shen, W., Zhong, S., Fu, D.Q., Qu, G., 2019. Diversity and redundancy of the ripening regulatory networks revealed by the fruitENCODE and the new CRISPR/Cas9 CNR and NOR mutants. Hortic. Res. 6 (1) https://doi.org/10.1038/s41438-019-0122-x.
Garg, N., & Cheema, D. (2014). *Genetic improvement of tomatoes involving rin, nor and alc alleles*.
Garg, N., Cheema, D.S., Dhatt, A.S., 2008a. Utilization of rin, nor, and alc alleles to extend tomato fruit availability. Int. J. Veg. Sci. 14 (1), 41–54. https://doi.org/10.1080/19315260801890526.
Garg, N., Cheema, D.S., Pathak, D., 2008b. Heterosis breeding in tomato involving rin, nor and alc alleles: a review of literature. Adv Hortic Sci 22 (1), 54–62. https://doi.org/10.1400/91111.
Giovannoni, J.J., 2004. Genetic regulation of fruit development and ripening. Plant Cell 16, 170–180. https://doi.org/10.1105/tpc.019158. SUPPL.
Giovannoni, J.J., 2007. Fruit ripening mutants yield insights into ripening control. Curr. Opin. Plant Biol. 10 (3), 283–289. https://doi.org/10.1016/j.pbi.2007.04.008.
Giovannoni, J.J., DellaPenna, D., Bennett, A.B., Fischer, R.L., 1989. Expression of a chimeric polygalacturonase gene in transgenic rin (ripening inhibitor) tomato fruit results in polyuronide degradation but not fruit softening. Plant Cell 1 (1), 53–63. https://doi.org/10.1105/tpc.1.1.53.
Giovannoni, J.J., Tanskley, S., Vrebalov, J., Noensie, F., 2004. NOR gene compositions and methods for use thereof. Patent US 6. *762,347 B1*.
Girin, T., Sorefan, K., Østergaard, L., 2009. Meristematic sculpting in fruit development. J. Exp. Bot. 60 (5), 1493–1502. https://doi.org/10.1093/jxb/erp031.
Heuvelink, E., 1996. Tomato Growth and yield : Quantitative Analysis and Synthesis. Wageningen University.
Honma, T., Goto, K., 2001. Complexes of MADS-box proteins are sufficient to convert leaves into floral organs. Nature 409 (6819), 525–529. https://doi.org/10.1038/35054083.
Itkin, M., Seybold, H., Breitel, D., Rogachev, I., Meir, S., Aharoni, A., 2009. TOMATO AGAMOUS-LIKE 1 is a component of the fruit ripening regulatory network. Plant J. 60 (6), 1081–1095. https://doi.org/10.1111/j.1365-313X.2009.04064.x.
Ito, Y., Kitagawa, M., Ihashi, N., Yabe, K., Kimbara, J., Yasuda, J., Ito, H., Inakuma, T., Hiroi, S., Kasumi, T., 2008. DNA-binding specificity, transcriptional activation potential, and the rin mutation effect for the tomato fruit-ripening regulator RIN. Plant J. 55 (2), 212–223. https://doi.org/10.1111/j.1365-313X.2008.03491.x.
Ito, Y., Nishizawa-Yokoi, A., Endo, M., Mikami, M., Shima, Y., Nakamura, N., Kotake-Nara, E., Kawasaki, S., Toki, S., 2017. *Re*-evaluation of the rin mutation and the role of RIN in the induction of tomato ripening. Nat Plants 3 (11), 866–874. https://doi.org/10.1038/s41477-017-0041-5.
Kader, A., Saltveit, M., 2003. Atmosphere Modification. Potharvest Physiology and Pathology of Vegetables, pp. 229–246.
Kerr, E.A., 1958. Mutations of chlorophyll retention in ripe fruit. Rep. Tomato Genet. Coop 8 (22).
Kitagawa, M., Ito, H., Shiina, T., Nakamura, N., Inakuma, T., Kasumi, T., Ishiguro, Y., Yabe, K., Ito, Y., 2005. Characterization of tomato fruit ripening and analysis of gene expression in F1 hybrids of the ripening inhibitor (rin) mutant. Physiol. Plant 123 (3), 331–338. https://doi.org/10.1111/j.1399-3054.2005.00460.x.
Klee, H.J., Giovannoni, J.J., 2011. Genetics and control of tomato fruit ripening and quality attributes. Annu. Rev. Genet. 45, 41–59. https://doi.org/10.1146/annurev-genet-110410-132507.
Klee, H.J., Tieman, D.M., 2013. Genetic challenges of flavor improvement in tomato. Trends Genet. 29 (4), 257–262. https://doi.org/10.1016/j.tig.2012.12.003.
Klee, H.J., Tieman, D.M., 2018. The genetics of fruit flavour preferences. Nat. Rev. Genet. 19 (6), 347–356. https://doi.org/10.1038/s41576-018-0002-5.
Knoche, M., Peschel, S., 2007. Deposition and strain of the cuticle of developing European plum fruit. J. Am. Soc.r Hort.l Sci. 132 (5), 597–602. https://doi.org/10.21273/jashs.132.5.597.
Kopeliovitch, E., Mizrahi, Y., Rabinowitch, H.D., Kedar, N., 1980. Physiology of the tomato mutant alcobaca. Physiol. Plant 48 (2), 307–311. https://doi.org/10.1111/j.1399-3054.1980.tb03260.x.
Kopeliovitch, E., Rabinowitch, H.D., Mizrahi, Y., Kedar, N., 1979. The potential of ripening mutants for extending the storage life of the tomato fruit. Euphytica 28 (1), 99–104. https://doi.org/10.1007/BF00029179.
Kopeliovitch, E., Rabinowitch, H.D., Mizrahi, Y., Kedar, N., 1981. Mode of inheritance of Alcobaca, a tomato fruit-ripening mutant. Euphytica 30 (1), 223–225. https://doi.org/10.1007/BF00033685.
Kosma, D.K., Parsons, E.P., Isaacson, T., Lü, S., Rose, J.K.C., Jenks, M.A., 2010. Fruit cuticle lipid composition during development in tomato ripening mutants. Physiol. Plant 139 (1), 107–117. https://doi.org/10.1111/j.1399-3054.2009.01342.x.
Kumar, Rahul, Khurana, A., Sharma, A.K., 2014. Role of plant hormones and their interplay in development and ripening of fleshy fruits. J. Exp. Bot. 65 (16), 4561–4575. https://doi.org/10.1093/jxb/eru277.
Kumar, Rakesh, Tamboli, V., Sharma, R., Sreelakshmi, Y, 2018. NAC-NOR mutations in tomato Penjar accessions attenuate multiple metabolic processes and prolong the fruit shelf life. Food Chem. 259, 234–244. https://doi.org/10.1016/j.foodchem.2018.03.135. March.
Lanahan, M.B., Ching, Yen Hsiao, Giovannoni, J.J., Klee, H.J, 1994. The Never ripe mutation blocks ethylene perception in tomato. Plant Cell 6 (4), 521–530. https://doi.org/10.1105/tpc.6.4.521.
Leal, N.R., Tabim, M.E., 1974. Testes de conservaçao natural pos colheita, alem dos 300 dias, de frutos de alguns cultivares de tomateiro (Lycopersicon esculentum Mill.) e hibridos destes com "alcobaca.". Rev. Ceres 21, 310–328.
Li, S., Chen, K., Grierson, D., 2019. A critical evaluation of the role of ethylene and MADS transcription factors in the network controlling fleshy fruit ripening. New Phytol. 221 (4), 1724–1741. https://doi.org/10.1111/nph.15545.
Li, S., Xu, H., Ju, Z., Cao, D., Zhu, H., Fu, D., Grierson, D., Qin, G., Luo, Y., Zhu, B., 2018. The RIN-MC fusion of MADs-box transcription factors has transcriptional activity

and modulates expression of many ripening genes. Plant Physiol. 176 (1), 891–909. https://doi.org/10.1104/pp.17.01449.
Lincoln, J.E., Fischer, R.L., 1988. Regulation of Gene Expression by Ethylene in Wild-Type and rin Tomato (Lycopersicon esculentum) Fruit. Plant Physiol. 88 (2), 370–374. https://doi.org/10.1104/pp.88.2.370.
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 Physiol. 169 (4), 2380–2390. https://doi.org/10.1104/pp.15.01361.
Ma, X., Balazadeh, S., Mueller-Roeber, B., 2019. Tomato fruit ripening factor NOR controls leaf senescence. J. Exp. Bot. 70 (10), 2727–2740. https://doi.org/10.1093/jxb/erz098.
Majidi, H., Minaei, S., Almassi, M., Mostofi, Y., 2014. Tomato quality in controlled atmosphere storage, modified atmosphere packaging and cold storage. J. Food Sci. Technol. 51 (9), 2155–2161. https://doi.org/10.1007/s13197-012-0721-0.
Martel, C., Vrebalov, J., Tafelmeyer, P., Giovannoni, J.J., 2011. The tomato MADS-box transcription factor RIPENING INHIBITOR interacts with promoters involved in numerous ripening processes in a COLORLESS NONRIPENING-dependent manner. Plant Physiol. 157 (3), 1568–1579. https://doi.org/10.1104/pp.111.181107.
Matas, A.J., Gapper, N.E., Chung, M.Y., Giovannoni, J.J., Rose, J.K., 2009. Biology and genetic engineering of fruit maturation for enhanced quality and shelf-life. Curr. Opin. Biotechnol. 20 (2), 197–203. https://doi.org/10.1016/j.copbio.2009.02.015.
McMurchie, E.J., McGlasson, W.B., Eaks, I.L., 1972. Treatment of fruit with propylene gives information about the biogenesis of ethylene. Nature 237 (5352), 235–236. B1.
Mercati, F., Longo, C., Poma, D., Araniti, F., Lupini, A., Mammano, M.M., Fiore, M.C., Abenavoli, M.R., Sunseri, F., 2015. Genetic variation of an Italian long shelf-life tomato (Solanum lycopersicon L.) collection by using SSR and morphological fruit traits. Genet. Resour. Crop Evol. 62 (5), 721–732. https://doi.org/10.1007/s10722-014-0191-5.
Mojevic, M., Tesanovic, D., 2011. Influence of short anoxia treatment and maturity on quality and storage life of tomatoes. In: J.Agric. Sci. Belgrade, 56, pp. 121–131. https://doi.org/10.2298/jas1102121m.
Monteiro, C.C., Carvalho, R.F., Gratão, P.L., Carvalho, G., Tezotto, T., Medici, L.O., Peres, L.E.P., Azevedo, R.A., 2011. Biochemical responses of the ethylene-insensitive Never ripe tomato mutant subjected to cadmium and sodium stresses. Environ. Exp. Bot. 71 (2), 306–320. https://doi.org/10.1016/j.envexpbot.2010.12.020.
Mutschler, M.A., 1984. Inheritance and linkage of the Alcobaca ripening mutant in tomato. J. Am. Soc. Hortic. Sci. 109 (4), 500–503.
Mutschler, M.A., Guttieri, M., Kinzer, S., Grierson, D., Tucker, G., 1988. Changes in ripening-related processes in tomato conditioned by the alc mutant. Theor. Appl. Genet. 76 (2), 285–292. https://doi.org/10.1007/BF00257857.
Mutschler, M.A., Wolfe, D.W., Cobb, E.D., & Yourstone, K.S. (1992). *Mutschler 1992*. 27 (April), 1985–1988.
Pech, J.C., Latché, A., 2013. Contribution of genomics to postharvest biology. Stewart Postharvest Rev. 9 (4), 1–6. https://doi.org/10.2212/spr.2013.4.1.
Renard, C.M.G.C., Ginies, C., Gouble, B., Bureau, S., Causse, M., 2013. Home conservation strategies for tomato (Solanum lycopersicum): storage temperature vs. duration-Is there a compromise for better aroma preservation? Food Chem. 139 (1–4), 825–836. https://doi.org/10.1016/j.foodchem.2013.01.038.
Rick, C.M., Butler, L., 1956. Cytogenetics of the Tomato. Adv. Genet. 8, 267–382. https://doi.org/10.1016/S0065-2660(08)60504-0.
Robinson, R.W., Tomes, M.L., 1968. Ripening inhibitor: a gene with multiple effect on ripening. Tomato Genet. Coop 18, 36–37.
Rodríguez, Gustavo, R., Pratta, G.R., Zorzoli, R., Picardi, L.A., 2005. Transgressive segregation for fruit quality traits in a cross between exotic and mutant genotypes of lycopersicon. N. Z. J. Crop Hortic. Sci. 33 (4), 373–379. https://doi.org/10.1080/01140671.2005.9514373.
Rodríguez, Gustavo, R., Pratta, G.R., Zorzoli, R., Picardi, L.A., 2006. Recombinant lines obtained from an interspecific cross between Lycopersicon species selected by fruit weight and fruit shelf life. J. Am. Soc. Hortic. Sci. 131 (5), 651–656. https://doi.org/10.21273/jashs.131.5.651.
Rodríguez, Gustavo Rubén, Pratta, G.R., Liberatti, D.R., Zorzoli, R., Picardi, L.A, 2010. Inheritance of shelf life and other quality traits of tomato fruit estimated from F1's, F2's and backcross generations derived from standard cultivar, nor homozygote and wild cherry tomato. Euphytica 176 (1), 137–147. https://doi.org/10.1007/s10681-010-0241-9.
Roeder, A.H.K., Yanofsky, M.F., 2006. Fruit Development in *Arabidopsis*. In: The Arabidopsis Book, Vol. 4. https://doi.org/10.1199/tab.0075.
Rose, J.K.C., Catalá, C., Gonzalez-Carranza, C.Z.H., Roberts, J.A., 2003. Plant cell wall disassembly. The Plant Cell Wall. Blackwell Publishing Ltd, pp. 264–324.
Saladié, M., Matas, A.J., Isaacson, T., Jenks, M.A., Goodwin, S.M., Niklas, K.J., Xiaolin, R., Labavitch, J.M., Shackel, K.A., Fernie, A.R., Lytovchenko, A., O'Neill, M. A., Watkins, C.B., Rose, J.K.C., 2007. A reevaluation of the key factors that influence tomato fruit softening and integrity. Plant Physiol. 144 (2), 1012–1028. https://doi.org/10.1104/pp.107.097477.
Satoh, S., Yang, S.F., 1988. S -Adenosylmethionine-dependent inactivation and radiolabeling of 1-Aminocyclopropane-1-Carboxylate Synthase isolated from tomato fruits. Plant Physiol. 88 (1), 109–114. https://doi.org/10.1104/pp.88.1.109.
Seroczynska, A., Niemirowicz, S.K., Korrenicwsk, L.A., 1998. Utilization of three non-ripening mutants In tomato breeding for prolonged shelf lite. Folia Hortic. 1 (1), 3–14.
Seymour, G.B., Chapman, N.H., Chew, B.L., Rose, J.K.C., 2013. Regulation of ripening and opportunities for control in tomato and other fruits. Plant Biotechnol. J. 11 (3), 269–278. https://doi.org/10.1111/j.1467-7652.2012.00738.x.
Seymour, G.B., Ryder, C.D., Cevik, V., Hammond, J.P., Popovich, A., King, G.J., Vrebalov, J., Giovannoni, J.J., Manning, K., 2011. A SEPALLATA gene is involved in the development and ripening of strawberry (Fragaria×ananassa Duch.) fruit, a non-climacteric tissue. J. Exp. Bot. 62 (3), 1179–1188. https://doi.org/10.1093/jxb/erq360.
Shima, Y., Fujisawa, M., Kitagawa, M., Nakano, T., Kimbara, J., Nakamura, N., Shiina, T., Sugiyama, J., Nakamura, T., Kasumi, T., Ito, Y., 2014. Tomato FRUITFULL homologs regulate fruit ripening via ethylene biosynthesis. Biosci. Biotechnol. Biochem. 78 (2), 231–237. https://doi.org/10.1080/09168451.2014.878221.
The Tomato Genome Consortium, 2012. The tomato genome sequence provides insights into fleshy fruit evolution. Nature 485 (7400), 635–641. https://doi.org/10.1038/nature11119.
Thompson, A.J., Tor, M., Barry, C.S., Vrebalov, J., Orfila, C., Jarvis, M.C., Giovannoni, J. J., Grierson, D., Seymour, G.B., 1999. Molecular and genetic characterization of a novel pleiotropic tomato-ripening mutant. Plant Physiol. 120 (2), 383–389. https://doi.org/10.1104/pp.120.2.383.
Tigchelaar, E., Mcglasson, W., Franklin, M., 1978. Natural and ethephon-stimulated ripening of F1 hybrids of the ripening inhibitor (rin) and non-ripening (nor) mutants of tomato (Lycopersicon esculentum Mill.). Funct. Plant Biol. 5 (4), 449. https://doi.org/10.1071/pp9780449.
Tranchida-Lombardo, V., Aiese Cigliano, R., Anzar, I., Landi, S., Palombieri, S., Colantuono, C., Bostan, H., Termolino, P., Aversano, R., Batelli, G., Cammareri, M., Carputo, D., Chiusano, M.L., Conicella, C., Consiglio, F., D'Agostino, N., De Palma, M., Di Matteo, A., Grandillo, S., …, Grillo, S., 2018a. Whole-genome re-sequencing of two Italian tomato landraces reveals sequence variations in genes associated with stress tolerance, fruit quality and long shelf-life traits. DNA Res. 25 (2), 149–160. https://doi.org/10.1093/dnares/dsx045.
Tranchida-Lombardo, V., Mercati, F., Avino, M., Punzo, P., Fiore, M.C., Poma, I., Patanè, C., Guarracino, M.R., Sunseri, F., Tucci, M., Grillo, S., 2018b. Genetic diversity in a collection of Italian long storage tomato landraces as revealed by SNP markers array. Plant Biosyst. 153 (2), 288–297. https://doi.org/10.1080/11263504.2018.1478900.
Vrebalov, J., Pan, I.L., Arroyo, A.J.M., McQuinn, R., Chung, M., Poole, M., Rose, J., Seymour, G., Grandillo, S., Giovannoni, J., Irish, V.F., 2009. Fleshy fruit expansion and ripening are regulated by the tomato SHATTERPROOF Gene TAGL1. Plant Cell 21 (10), 3041–3062. https://doi.org/10.1105/tpc.109.066936.
Vrebalov, J., Ruezinsky, D., Padmanabhan, V., White, R., Medrano, D., Drake, R., Schuch, W., Giovannoni, J., 2002. A MADS-Box Gene necessary for fruit ripening at the tomato ripening-inhibitor (Rin) locus. Science 296, 343–346.
Wang, R., Lammers, M., Tikunov, Y., Bovy, A.G., Angenent, G.C., de Maagd, R.A., 2020. The rin, nor and Cnr spontaneous mutations inhibit tomato fruit ripening in additive and epistatic manners. Plant Sci. 294, 110436 https://doi.org/10.1016/j.plantsci.2020.110436. October 2019.
Wang, R., Tavano, E.C., da, R., Lammers, M., Martinelli, A.P., Angenent, G.C., de Maagd, R.A., 2019. *Re*-evaluation of transcription factor function in tomato fruit development and ripening with CRISPR/Cas9-mutagenesis. Sci. Rep. 9 (1), 1–10. https://doi.org/10.1038/s41598-018-38170-6.
Wang, S., Lu, G., Hou, Z., Luo, Z., Wang, T., Li, H., Zhang, J., Ye, Z., 2014. Members of the tomato FRUITFULL MADS-box family regulate style abscission and fruit ripening. J. Exp. Bot. 65 (12), 3005–3014. https://doi.org/10.1093/jxb/eru137.
Yu, Q.H., Wang, B., Li, N., Tang, Y., Yang, S., Yang, T., Xu, J., Guo, C., Yan, P., Wang, Q., Asmutola, P., 2017. CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. Sci. Rep. 7 (1), 1–9. https://doi.org/10.1038/s41598-017-12262-1.
Zhong, S., Fei, Z., Chen, Y.R., Zheng, Y., Huang, M., Vrebalov, J., McQuinn, R., Gapper, N., Liu, B., Xiang, J., Shao, Y., Giovannoni, J.J., 2013. Single-base resolution methylomes of tomato fruit development reveal epigenome modifications associated with ripening. Nat. Biotechnol. 31 (2), 154–159. https://doi.org/10.1038/nbt.2462.