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IMPROVING FRUIT SHELF LIFE: LESSONS FROM TOMATO

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ABSTRACT: Post-harvest spoilage of food crops is one of the major roadblocks to achieving food security. In addition to improving the storage facilities, gaining insights into the natural processes of post-harvest degradation of these crops hold promise for mitigating future food crisis to a great deal. The review article summarizes some of the key advancements in the molecular understanding and biotechnological interventions to improve the shelf life of tomato as a suitable model of a fleshy climacteric fruit.

KEYWORDS: Fruit Shelf Life; Tomato; Molecular Mechanisms; Hormones; Transgenic.

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1.INTRODUCTION

We are faced with the daunting task of having to feed millions of mouths every day, especially with the global population skyrocketing and rapid urbanization eating daily into the arable land. For ensuring global food security our focus has, therefore, been on increasing crop yield through various breeding as well as biotechnological approaches. While the former is time-consuming, labour-intensive and often infeasible, the latter is constrained by frequent development of resistance and on many occasions by perceived health risks associated with the genetically modified crops. However, hunger and malnourishment stem as much from population explosion as well as the ever-dwindling cultivable land as they do from our under-preparedness in saving the harvested crops. Post-harvest crop loss costs the global economy an astonishing US$750 billion annually and the reduction in such losses or damages by only 1 percent could save around US$40 million every year enabling us to take care of the unfed millions. The paucity of proper storage and other infrastructural facilities

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may be the root cause of this problem. Even though the majority of the existing storage facilities can reduce the risk of post-harvest diseases, they are rather inefficient at preventing the natural process of crop loss through an inevitable biological phenomenon called senescence. As a result, there have been considerable efforts around the world to tame the process in crops, especially the fleshy fruits and vegetables, in order to increase their shelf lives. Research aimed at shelf life prolongation basically centres around two broad themes. One is to delay the ripening and softening process of the harvested fruits and vegetables and the other is to prevent microbial infection. Sustained efforts over decades have lead to the recent transcriptome profiling of fruit development and ripening process in tomato, an appropriate model to study fruit ripening and shelf life related phenomena in fleshy fruits [1]. This review discusses the current molecular understanding of the shelf life improvement strategies in tomato, which not only serves as a model system in this field, but also is commercially the fourth most important crop in the world today.

Ethylene Intervention

Plant hormones play crucial roles in regulating fruit ripening and related events [2]. Ethylene as a gaseous hormone particularly orchestrates diverse functions in plants. It plays a vital positive role in senescence and fruit ripening, especially in climacteric fruits, such as tomato, which typically experience an ethylene induced respiratory burst during the ripening process [3]. All ethylene based interventions to shelf life improvement in tomato seek to reduce the levels of ethylene. Pharmacological approaches with compounds that interfere with one or more components of ethylene biosynthesis, perception, signal transduction and response have been critical in attempting to achieve this goal [4]. However, the effects of such molecules could be transient and they might pose serious health hazards to humans even in trace amounts, limiting the scope of their use. But molecular genetics based technologies directed at targeted silencing or overexpression of certain genes involved in ethylene biosynthesis and/or signal transduction pathways have proven useful and they probably offer suitable alternatives. In the very early days research, transgenic interventions sought to bring down ethylene amounts. Several early interventions relied on tinkering with ethylene biosynthesis pathway enzymes. In one such strategy truncated versions of the ACC synthase gene (ACS), which catalyzes the formation of the ethylene precursor 1-Aminocyclopropane-1-carboxylic acid (ACC), was introduced in tomato resulting in the silencing of the gene. Metabolizing or shunting away ACC should, in principle, have a similar outcome. Indeed expressing the bacterially derived gene encoding ACC deaminase, an enzyme that breaks down ACC into ammonia and 2-ketobutyrate, in tomato, showed similar results. In recent years RNA interference has proven to be a useful tool to achieve the same end. Self life extension up to 45 days was accomplished by employing RNAi based silencing of three homologs of the ACC synthase gene [5].Disrupting ethylene perception could be a better choice than biosynthesis inhibition to delay fruit ripening, since ethylene governs a multitude of physiological events other than fruit ripening in plants. There

are seven ethylene receptors in tomato, some of which have been implicated in fruit ripening while the rest play diverse other physiological roles [6].The occurrence of fruit ripening related ethylene receptors- *ETR3/NR*, *ETR4*- is the result of two genome triplication events followed by neofunctionalization in the *Solanum* lineage and together with certain other genes originating from such evolutionary events, they regulate ethylene biosynthesis, aroma, colouration, fruit tenderness and succulence, and contribute greatly to fleshy fruit development in tomato [7]. In Arabidopsis ethylene signal transduction cascade, in the absence of ethylene, the receptors (ETR1, ETR2, ERS1, ERS2 and EIN4), which are localized in the endoplasmic reticulum (ER), are activated and trigger dimerization as well as activation of the associated CTR1, a mitogen activated triple kinase (MAPKKK) type Ser/Thr protein kinase. CTR1, in turn, phosphorylates and inhibits EIN2, an essential signal transducer in activating all kinds of ethylene response [8]; [9]. EIN2 is located in the ER membrane and comprises an N-terminal hydrophobic membrane spanning domain having similarity with NRAMP family of metal ion transporters and a C-terminal hydrophilic domain housing a highly conserved nuclear localization sequence (NLS) [10]; [11]. Ethylene inhibits as well as triggers the degradation of its receptors, thereby turning on ethylene signal transduction through the inactivation of the kinase activity of CTR1 leading to dephosphorylation by an as yet unidentified phosphatase and the cleavage of the C-terminal domain of EIN2 by an unknown protease [11]; [12]. The C-terminal domain of EIN2 becomes translocated into the nucleus where it allows the transcription factors EIN3 and EIL1, as dimers, to turn on early ethylene responsive genes [13]; [14]. At the post-translational level, the C-terminal domain of EIN2 inhibits EBF1/2(F-box proteins) mediated degradation of EIN3 via the ubiquitin-proteasome pathway, thereby stabilizes EIN3/EIL1 by destabilizing EBF1/2 [15]. At the translational level, the C-terminal domain of EIN2 also binds to the polyuridine (poly-U) motifs in the 3'-UTR of *EBF1/2* mRNA and in association with UPF1, a critical regulator of the Nonsense Mediated Decay (NMD) pathway, and other factors, it inhibits *EBF1/2* mRNA translation causes its sequestration in cytoplasmic P-bodies and possibly degradation of *EBF1/2* mRNAs [16]; [17]. Both the kinase CTR1 and the receptor ETR1 form ER membrane bound complex with EIN2.EIN2 and ETR1 establish physical interaction through their C-terminal NLS motif and the kinase domains, respectively, and this contact appears necessary for ethylene response to be activated, despite the receptors negatively regulating ethylene signalling [8]. Expressing synthetic peptide NOP-1 (LKRYKRRL-NH2) that mimic the NLS motif (LKRYKRRL) of C-terminal EIN2 from Arabidopsis, it has been possible to disrupt ETR1-EIN2 physical interaction in tomato, thereby turning off ethylene response and extending shelf life [18]; [19].Although this model posits that the interaction between the ethylene receptor ETR1 with EIN2 is a prerequisite for the initiation of ethylene signalling, one can hardly rule out the possibility of NOP-1 interfering with the signalling by competitively binding to EIN2 C-terminal domain interacting partners acting downstream.

Cell Wall Targeting

The firmness in tomato fruit is attributed to the presence of mainly three kinds of polysaccharides- cellulose, hemicelluloses and pectins- in the cell wall. In particular, the pectic substances, which are the cementing materials, serve to maintain the integrity of the fruit and are subject to a number of hydrolytic enzymes [20].As the fruit matures and senesces, various hydrolytic enzymes act on these materials causing it to soften or rot [3]; [21]. Consequently, a great deal of efforts has been made to inactivate such enzymes or silence the genes coding for them. In fact, the first ever genetically engineered food approved for human consumption by U. S. Food and Drug Administration (FDA) was a transgenic tomato called FlavrSavr. In FlavrSavr tomato the polygalacturonase (PG) gene, which encodes an enzyme that degrades the cell wall pectin, was silenced through anti-sense technology, leading to an enhanced shelf-life [22]; [23]; [24]. Still, molecular interventions targeting polygalacturonase (PG) and pectin methyl esterase (PME) have found limited success [25]; [26]. For this reason, another pectolytic enzyme pectate lyase (PL) has received much attention from the scientific attention [27]; [28]. Of the five PL genes identified to be expressed using RT-qPCR in the tomato cv Alisa Craig, one allele (Solyc03g111690) was found to be particularly highly induced in the ripening fruit [26]. RNAi lines, where this particular gene suppressed, not only had firmer fruits with longer shelf life (14 days at 20 °C), but these fruits were also better in terms of palatability, texture, aroma and taste, resulting from little changes in the fruit metabolites and the expression alteration in only a few genes (fewer than 120 out of 15,500 genes) compared to the control tomato plants [26]. Furthermore, calcium in the form of calcium pectate maintains cell wall integrity and transgenic expression of a $H+/Ca^{2+}$ exchanger from Arabidopsis in tomato have also lead to the development of firmer fruits through increases levels of Ca^{2+} in the tissue [29].Free N-glycan amounts see a rise as the fruit begins to ripen [30]. N-glycan processing enzymes involved in the cleavage mediated degradation of these polysaccharides have been recently implicated in the rotting of tomatoes. Indeed, RNAi mediated suppression of two such enzymes, α-mannosidase (α-Man) and β-D-N-acetylhexosaminidase (β-Hex) had a tremendous positive effect on the tomato shelf life. Fruits from these RNAi lines remained fresh for as long as 30 days, holding great promise for long-term storage of tomato the ambient temperature [31].Interestingly, *beta-glucuronidase (GUS)* reporter assay combined with Electrophoretic Mobility Shift Assay (EMSA) revealed that the MADS box transcription factor RIPENING INHIBITOR (RIN), which had traditionally been extensively used in plant breeding programs for shelf life prolongation, has been shown to bind to *α-Man* promoter as a positive regulator, establishing a much stronger molecular connectivity in tomato shelf life regulatory processes [32]; [33]; [34]; [35]. Moreover, tinkering with auxin response by suppressing *Sl-ARF4* has lead to improved shelf-life in line with its role in governing cell wall architecture [36].

Antioxidants

Antioxidants are secondary metabolites having huge health benefits. Anthocyanins act as antioxidant and anti-inflammatory compounds with hugely beneficial roles in human health [37]; [38]. Driven by the health promoting qualities, scientists have developed anthocyanin-rich tomato [39]; [40]. It is particularly noteworthy that antioxidants acts as efficient scavengers of post-harvest pathogen induced reactive oxygen species (ROS), especially helping to ward off necrotrophic pathogens which employ ROS mediated host cell death as a virulence strategy. In keeping with the role that the antioxidants play, it was found that hybrid tomato (Aft/Aft atv/atv) and those ectopically expressing Delila and Rosea1 transcription factors from Antirrhinum majus synthesize anthocyanins at high levels in response to high light are able to resist the necrotrophic post-harvest fungal pathogen Botrytis cinerea, thus extending shelf-life considerably [41]; [42]. Whereas total antioxidant content positively correlates with delay in over-ripening, prevention of microbial damage relies on specific types of antioxidant rather than total antioxidant content in tomato. Superoxide oxide scavenging potential determines susceptibility and different flavonoids vary in their abilities to detoxify superoxides, based on hydroxylation as well as methoxylation status of the B-ring of the flavonols. For instance, flavonoids with three –OH groups in the B-ring, which is introduced by the enzyme F3’5’H, confers resistance to *B. cinerea*. Introducing the gene encoding the enzymes in fruits lacking it holds promise in respect of a better management of post-harvest disease. Other covalent modifications of the flavonoids also differ in their ability to promote resistance [43]. Expressing yeast spermidine synthase (ySpdSyn) gene, which forms the polyamine spermidine, constitutively or under the control of the promoter of the fruit specific gene E8 have lead to increased shelf-life of tomato and is attributable to the accumulation of lycopene, an antioxidant, combined with the anti-senescence effects of polyamine [44].

Epigenetics and Ripening Associated Genes

Some naturally occurring rare mutations- ripening inhibitor (rin), nonripening (nor), and Colorless nonripening (Cnr) - are compromised in fruit ripening. RIN, NOR and CNR encode transcription factors in MADS box, NAC and SBP box families, respectively [21]; [45]. These mutants typically do not exhibit climacteric rise in respiration as well as ethylene production and stay green [46].RIN acts as a master transcriptional regulator of fruit ripening, regulating genes involved in almost all ripening associated developments and phenotypes starting from ethylene biosynthesis and perception (LeACS2, LeACS4, NR) through fruit colouration (PSY1) as well as aroma compound production (Tomlox C, ADH2, HPL) to cell wall integrity (PG, TBG4, LeEXP1) [21]. RIN also targets other ripening related genes including NOR, CNR, HB1, and TDR4 at the same time inducing its own expression [21]. Recent works have uncovered epigenetic mechanisms that regulate transcriptional as well as posttranscriptional events determining the transcript and protein levels of the master regulators (RIN, NOR and CNR) of fruit ripening in tomato [47].At the

transcriptional level, Ethylene Response Factors (ERFs) impinge on these natural mutants as three of these ERFs belonging to the subclass E, which are up-regulated during fruit ripening in the wild type plants, are drastically down-regulated [48]. Studies on epigenetics in Arabidopsis as well as in various other model organisms have lead to an improved understanding of the recurring themes operating in these organisms. Basically, epigenetics works at two different levels-the transcriptional and the post-trascriptional. Transcriptionally, genes are regulated by the accessibility of the promoters to the transcription machinery. Generallyhypermethylation of the promoter regions by a host of DNA methyltransferases is regarded as a repressive mark, while hypomethylation causes the genes to turn on. While DNA methyltransferases establishes and maintains the repressive marks, DNA demethylases act to remove these covalent modifications, maintaining a subtle balance in chromatin condensation and decondensation. Similarly, co-translational and post-translational modifications of the histones also fine-tune gene expression. Chromatin modifying enzymes -the ubiquitin ligases, deubiquitinases,histone acetyl transferases (HATs), histone deacetylases (HDACs), histone methyltransferses (HMTs) and histone demethylases- in conjunction with the chromatin remodelers regulate promoter accessibility and activity. Whereas acetylated histones are usually characteristic of actively transcribed genes, the removal of the acetylation marks by the HDACs shuts the genes off. These epigenetic changes are dynamic and spatio-temporally regulated. In Arabidopsis, various noncoding RNAs-more specifically the siRNAs and Long Noncoding RNAs (lncRNAs)-have also been implicated as active participants in DNA methylation. Post-transcriptionally, epigenetic regulation mainly involves translational inhibition and mRNA turnover under the control of several noncoding RNAs including miRNAs. In Arabidopsis and other model organisms, developmental repressor chromatin modifying enzymes form complexes with other proteins. Developmental repressive complexes form two distinct groups- Polycomb Repressive Complex 1 (PRC1) and Polycomb Repressive Complex 2 (PRC2). While PRC1 catalyzes histone H3 trimethylation at lysine 27 (H3K27me3) to establish gene repression, PRC2 stabilizes the repressive state via histone H2A monoubiquitination. Through transgenic tomato lines overexpressing SlMSI1, which encodes a component of PRC1, it has been shown that it represses transcription of RIN and its target genes including CNR and NOR, thereby negatively regulating fruit ripening [49]. A histone deacetylase SlHDA1 converges on the promoters of a number of fruit ripening related genes (RIN, E4, E8, Cnr, TAGL1, PG, Pti4 and LOXB) turning them off [50].Treatment with an inhibitor of DNA (Cytosine 5) methyltransferases (5-azacytidine) in combination with bisulfite sequencing have provided evidence for promoter hypermethylation and the inactivation of the CNR gene in the naturally occurring stay-green Cnr mutant tomatoes. Transcription factors also compete with chromatin modifying complexes for target promoter binding. Indeed, the promoters of RIN regulated genes including CNR are hypermethylated in rin loss-of-function mutants, indicating increased promoter occupancy and activity of the DNA

methylransferases in the absence of RIN transcription factor [51]. In the wild type tomatoes these promoters exhibit gradual hypomethylation as the green unripe fruits become ripe [51]; [52]. DNA demethylases strike a fine balance in the DNA methylation state. VIGS and RNAi mediated silencing of SlDML2, a DNA demethylase, unearthed a role for fruit ripening in wild type tomato and SlDML2 expression pattern in the fruit was found to coincide with fruit maturation. In keeping with its crucial role in fruit ripening, promoter hypermethylation in rin as well as Cnr mutants correlates with SlDML2 repression [53].Epigenetic reprogramming usually resets most of these epigenetic marks in the embryo so as to prevent their transgenerational inheritance. However, there are instances where the epigenetic state is passed on to the next generation. Indeed such a scenario exists in case of the non-ripening naturally occurring mutants, where promoter hypermethylation is apparently maintained generation after generation. At least in the natural Cnr mutants this is achieved through the CHG sequence specific maintenance DNA methyltransferase SlCMT3 which apparently acts via the RNA-directed DNA methylation (RdDM) pathway employing the RNA polymerase V (Pol V) generated long non-coding RNA (scaffold RNA) as well as the RNA polymerase IV transcribed and, Dicer and Argonaut dependent siRNA as in Arabidopsis [54]; [55]; [56]; [57]; [58]. MicroRNAs like SlymiR157 and SlymiR156 add to the complexity of the tomato fruit ripening process. Whereas SlymiR157 inhibits and/or destabilizes CNR mRNA and thereby negatively impacts fruit ripening, SlymiR156 hastens fruit softening probably in a CNR dependent fashion and it could target some of the key negative regulators of cell wall modifying enzymes [59]. Overexpression of SlymiR157 in transgenic lines may improve tomato storage life without any disagreeable effects on the various desirable fruit phenotypes, since miRNAs are normally gene-specific. Although the components of the RdDM are fairly well-established in Arabidopsis, the tomato RdDM pathway is rather speculative and poorly understood.

2. CONCLUSION

While raising transgenic plants, it is a common practice to emphasize only few chosen parameters to assess the probable impact on the overall health of the plants and the ecosystem, of the accompanying genetic modifications. In the development of transgenic tomatoes, it is imperative to investigate any adverse effects on the metabolome and proteome of these plants. Similarly safety of concerns about any ill-effects on human health should also be adequately addressed. Future research in tomato should aim to look for the translatability of the insights gained in tomato of extending shelf life to other major fleshy fruits and possibly vegetables.

CONFLICT OF INTEREST

There is no conflict of interest.

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