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Reducing crop losses by gene-editing control of organ developmental physiology

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Some physiological processes in reproductive organs, if not controlled, can lead to crop loss even in the absence of environmental stress. These processes may occur pre- or post-harvest, and in diverse species and include abscission processes in cereal grain, e.g., shattering and in immature fruit, e.g., preharvest drop, preharvest sprouting of cereals, and postharvest senescence in fruit. Some of the molecular mechanisms and genetic determinants underlying these processes are now better detailed, making it possible to refine them by gene editing. Here, we discuss using advanced genomics to identify genetic determinants underlying crop physiological traits. Examples of improved phenotypes developed for preharvest problems are provided, and suggestions for reducing postharvest fruit losses by gene and promoter editing were made.

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Introduction

The factors underlying crop loss are variable. The extent of these losses depends on how efficiently a given crop's physiological traits can withstand the combined effects of biotic and abiotic stress, which vary in frequency and intensity throughout the lifecycle [1–3]. However, independent of environmental stressors, crop loss can result from failed physiological processes or those not compatible with agricultural production (Box 1). If these processes can be inhibited or delayed, more product can be harvested, marketed, and consumed. The goal of this review is to examine instances where gene editing can reduce crop loss in cereals and horticultural crops in the absence of environmental stress, by 'correcting' physiological processes or by refining their timing. For preharvest processes that occur in the field, causal genes have only just been identified, and the challenge is to modify these processes with no ill effects on crop yield and productivity [4]. This refinement is possible in part because genome editing is facilitating the genetic dissection of complex developmental traits. In contrast, many genes implicated in the postharvest fruit ripening–senescence pathway have been discovered, therefore, we focus on how a deeper understanding of their effect and their interconnectedness would be promising for precision breeding aimed at reducing crop loss. What becomes clear is the juxtaposition of physiological processes that evolved for adaptation in natural ecosystem versus how those processes have to be further modified for modern agriculture.

Processes implicated with losses in the field and at harvest

Preharvest sprouting

In cereals, preharvest sprouting (PHS) describes when germination occurs in mature seeds (Box 1). This leads to significant losses due to lower harvestable yield, lower grain quality, storability, and germination capacity, and the associated higher moisture content increases mycotoxin contamination of the grain [5]. PHS results from an imbalance between *dormancy* and *germination* (Box 1) [5]. Dormancy evolved so that seed germination in wild species would occur only when conditions are favorable, however, selection during domestication was for weak dormancy that permits uniform germination of crops almost independent of the prevailing conditions [6]. If dormancy is too weak, however, the spatiotemporal signals that trigger germination malfunction and PHS occur.

The relative balance of abscisic (ABA) and gibberellic acid has been implicated in the regulation of dormancy control and hence in the occurrence of PHS [5]. When edited, several genes, many ABA-related, have modified dormancy and germination traits and as a result, improved PHS. Rice overexpressing *OsMFT2*, a positive regulator of ABA response genes, has delayed germination and a lower rate of PHS [7]. A reduction of PHS in rice was also obtained by controlling glutaredoxin-

Box 1 Definitions of physiological processes.

PREHARVEST SPROUTING: Seeds germinate while still in the maternal tissue, and not after dispersal.

DORMANCY: Viable seed remains in suspension even if conditions permit germination.

ABSCISSION: The separation of organs from the mother plant before harvest.
- *Shedding*: the dispersal of leaves and seeds from plants.
- *Preharvest drop*: fruit falls to the ground before harvest.
- *Seed shattering*: loss of grain and seed from plants.

RIPENING: The coordinated physiological processes and compositional changes that make fruit attractive to frugivores.

SENESCENCE: The final stage of organ development during which spoilage occurs.

mediated ABA and reactive oxygen species signaling [8]. CRISPR-Cas9 (Clustered Regularly Interspaced Palindromic Repeats-Cas9) mutations of *OsABA8ox* and especially *OsABA8ox1* increased ABA levels and seed dormancy (SD6) in japonica rice [8], and PHS resistance also improved, with no significant effects on the main agronomic traits [9]. Additional genes that regulate ABA-mediated dormancy have been discovered. Two bHLH (basic Helix Loop Helix) transcription factors (TFs), that is, *SD6* and *Inducer of C-repeat binding factors expression 2*, function antagonistically to control SD6 by directly regulating ABA metabolism. Novel *SD6* alleles created by CRISPR–Cas9 editing reduced PHS in rice and wheat [6]. Genes involved in diverse pathways not related to ABA, also influence PHS [5]. CRISPR–Cas9-targeted mutagenesis of wheat *TaQsd1*, which encodes an alanine amino transferase, prolonged the dormancy and germination period and prevented PHS [10]. Given the many pathways that intertwine to regulate the dormancy–germination nexus, it seems likely that additional genes will be discovered [5].

Abscission

The process of leaf, grain, or fruit separation from the mother plant, is described as abscission, and it has important functions in plant development (Box 1). For example, fruit drop (Box 1) during early development occurs in several species and evolved as a physiological self-regulatory mechanism to establish an optimal reproductive–vegetative balance. In some fruit species, for example, olive, this process is pronounced contributing to reduced yield at harvest. In other crops, for example, peach and apple, the intensity of fruitlet drop is not optimal, so fruit thinning using manual, mechanical, or chemical methods is unavoidable and must be performed to guarantee an adequate fruit size at harvest. The shedding of mature reproductive organs, that is, seeds and fruits of cultivated crops, inevitably causes yield losses and reduced harvesting efficiency.

Seed-shattering

Seed-shattering (Box 1) is essential for seed dispersal in natural ecosystems, but is undesirable in cultivation, so there was heavy selective pressure for nonshattering mutants during domestication [11]. In some species, this trait is tightly linked to others that are commercially valuable and so have been introgressed from wild species into cultivars [11]. In rice, because seed shattering improves threshability, this trait has been retained in several cultivars, especially in *indica* types [12]. Several genes that play a role in abscission zone (AZ) differentiation in rice have been identified [13,14]. The YABBY transcription factor *Sh1* is associated with loss of seed shattering in cereals [13–15]. Editing the rice homolog *OsSh1* (Rice Shattering 1) in a Chinese high-yielding *indica* rice cultivar which has an easy-shattering phenotype, led to remarkable resistance to shattering compared with the wild type [16]. In green millet (*Setaria viridis*), a mutation of the MYB (Myeloblastosis) transcription factor gene *Less Shattering1 (SvLes1)* was associated with reduced shattering [16]. Furthermore, CRISPR–Cas9 editing of *SvLes1* produced novel alleles that led to high tensile strength of the AZ as fewer seeds were released from the inflorescence in a wind tunnel experiment, thereby confirming a reduced shattering habit [16].

Fruit preharvest drop and abscission zone formation

Preharvest drop is an abscission-related event leading to quantitative losses and/or reduced harvesting efficiency. Preharvest drop of mature fruits is characteristic of specific crops such as citrus and apple, with the severity of drop varying among cultivars. The phenomenon is the result of early activation of the AZ, leading to a reduction of cell-to-cell adhesion and, hence, to the drop of the fruit. This causes a significant reduction of harvested produce; only breeding resistant varieties, and, under certain environmental conditions and for specific genotypes, the use of plant growth regulators such as ethylene antagonists, have so far limited the incidence of this problem in the field.

õPhysical damage of fruit during harvest can lead to crop loss. Machine-harvested tomato fruit with the floral stem attached causes wounding of other fruit during transportation. Such tomatoes have an AZ, called a ‘joint’ in their pedicel, causing the calyx and stem to remain attached to the fruit when they separate from the inflorescence axis [17].

Some tomato species lack this joint, facilitating stem-free fruit removal [17]. The first locus associated with a failure to develop an AZ in the pedicel was the MADS-box *jointless* (*j*) locus [18], then, the *jointless2 (J2)* locus was identified as a key regulator of AZ development in tomato [19]. *J2* loss-of-function mutants are 'j', that is, do not have an AZ, and this reduces the frequency of fruit drop and enables better mechanical harvesting [20]. However, introgression of this mutation into fresh market lines led to undesirable inflorescence branching, abnormally large number of flowers, and a higher incidence of fruit cracking and off-shapes [21,22]. This was overcome in Florida tomato breeding lines, where the *j2* alleles were specifically edited using CRISPR–Cas9 to avoid the linkage drag associated with sexual hybridization. The edited lines have both the 'j' phenotype and normal inflorescence architecture [23], and in a field trial, there was no difference in fruit yield [17].

Greater understanding of the 'j' trait was developed with the discovery that there is a negative epistatic interaction between *j2* and *ENHANCER OF JOINTLESS2 (EJ2)*. The latter is a cryptic variant of *J2*, that is, a mutation that would only influence the 'j' phenotype under certain genetic and environmental conditions [23]. The negative relationship between *j2* and *EJ2* was neutralized by CRISPR–Cas9 editing of other genes associated with 'j' locus — *suppressor of branching 1* and *3*, which led to a 'j' phenotype and normal inflorescence [23]. Discovering these interactions expands the breeding toolkit, and provides more flexibility and precision in crop improvement programs. While the physiology and regulation of abscission by AZ activation are well described, the molecular mechanisms underlying fruit AZ differentiation are less known.

If the function of 'j' and other MADS-box genes is conserved across plant species, fundamental studies discovering additional AZ-associated genes might be translated for managing immature and mature fruit drop in different species using gene editing. This is the case of climacteric melon in which the CRISPR–Cas9-mediated knockout of *CmNAC-NOR* (Melon NAC-NON RIPENING) (a homolog of the tomato *NOR* gene) altered the formation of the fruit AZ [24].

Processes implicated with postharvest losses

A third of all crops are never consumed because their natural physiological processes after-harvest are not optimal for modern agriculture [3,25]. Horticultural crops are especially perishable; however, postharvest losses of the relatively metabolically inert grain and legumes are of such a scale that they should not be ignored [3,25]. Losses from mycotoxin contamination of cereals are costly, but approaches for reducing its occurrence are beyond the scope of this review and the reader is referred to others [26,27]. Likewise, overviews of postharvest losses of horticultural crops due to biotic and abiotic stress are also available [28–33]. Studies where fruit have edited alleles of cell wall-modifying enzymes [34] and other ripening TFs [35] that ripen without excessive softening offer exciting developments for commercialization, but are not discussed here. In this entry, we focus on how the knowledge derived from editing regulatory genes in tomato, especially those that control ripening and senescence, could reduce postharvest losses.

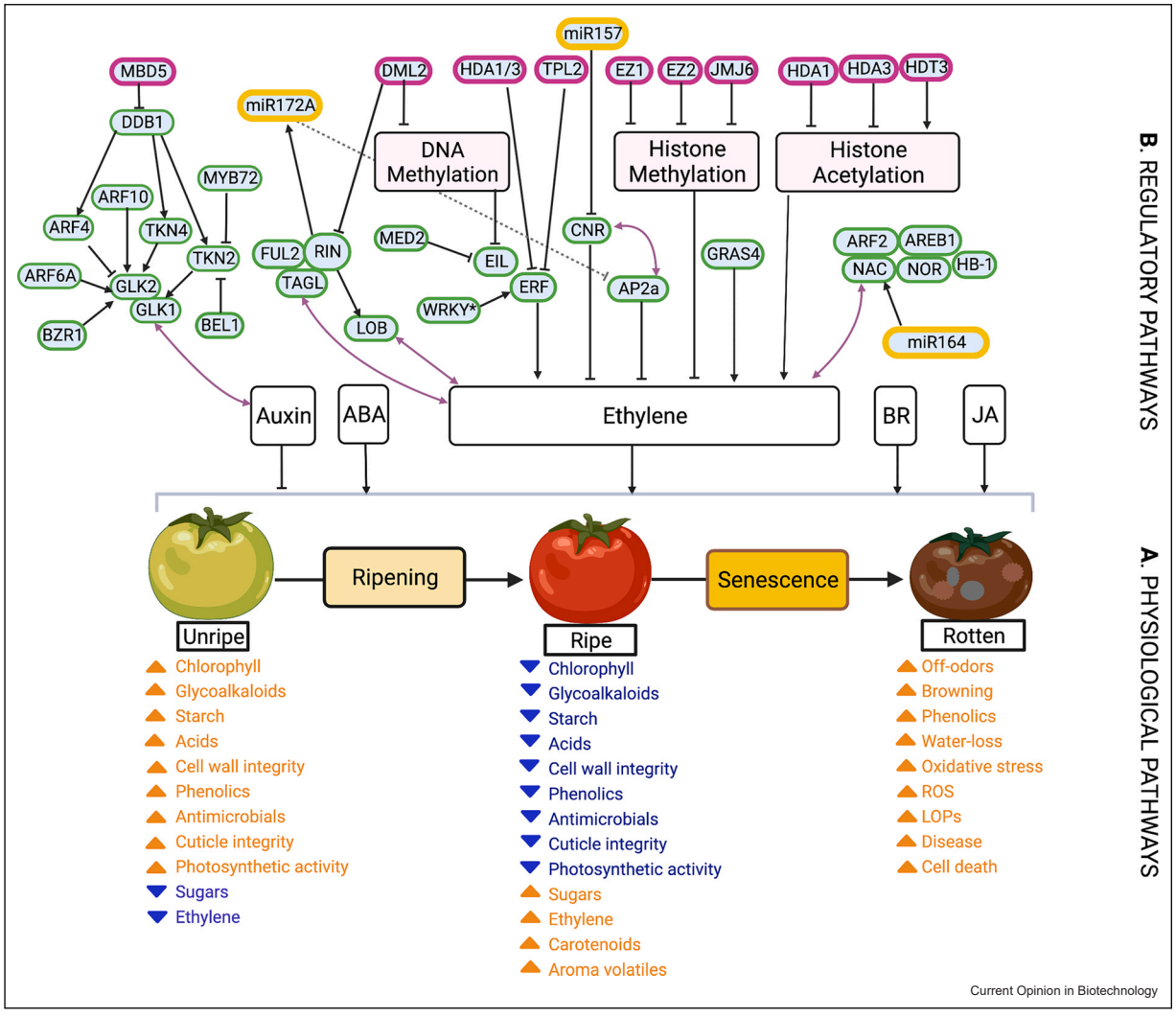
Controlling fruit progression to senescence

At peak ripeness, fruit are at a high risk for loss by physical damage, and beyond that time frame, senescence occurs, which renders the fruit unpalatable (Figure 1**a**) [36].

Both ripening and senescence have evolved to distribute and release seeds, either by frugivore dissemination, or by disintegration and rot by necrotic fungi. The complicated distribution logistics of the produce supply chain makes it necessary to control fruit ripening and senescence after-harvest to stymy losses and improve quality [25,36]. A desired outcome would be to widen the window between ripening and senescence, either by incrementally slowing the rate of progression toward aging, or by pausing and then restarting ripening, with minimal loss of quality (Figure 1**a**) [37]. Unlike the genes that regulate preharvest physiological processes, there has been an explosion in the discovery of the molecular components of ripening (Figure 1**b**; Table S1) [28–30]. The next step will be to understand how these factors interact with each other, and in turn, regulate their downstream targets. Fruit-ripening TFs (Box 2) regulate organ physiology by controlling gene(s), some of which are part of the fruit developmental pathways described in Figure 1**a** and which are part of hormonal signal transduction pathways [38]. These TFs may require coactivators or physical interaction with other proteins to function [39–41], and may be regulated by epigenetic modifications (Box 2) [32]. Collectively, these regulators drive fruit development to the senescent phase [42–45].

The goal of Figure 1**b** is to show our growing knowledge of the complexity of the global ripening network (Figure 1; Table S2), and to see this complexity as an extraordinary opportunity for modulating fruit quality and storability. Mutations in the cis-regulatory elements (CREs) of individual ripening TFs, and histone and DNA modifiers (Box 2), should generate gain-, loss-of-function, and transgressive alleles, creating quantitative trait variation for fruit ripening, which is nuanced, progressive, and of a large dynamic range [46]. Even richer data and genotypes would be uncovered by simultaneously editing the CREs of multiple ripening regulatory genes — a massive number of edited lines, with unique epistatic interactions, some presumably, with

Figure 1



A snapshot of some of the regulatory components that coordinate the physiological changes occurring during the ripening-to-senescence transition in tomato fruit, these components may be edited singly or in combination to delay ripening and senescence and hence reduce postharvest losses. **(a).** Multiple biological pathways that make fruit desirable for consumption, also make them susceptible to damage and loss. Several processes are accelerated (orange) or suppressed (blue) as fruit undergoes the final stages of development. Abbreviations: lipoxygenase products (LOPs), reactive oxygen species (ROS). **(b).** Some TFs (**green boxes**) regulate ethylene biosynthesis but are also in turn, regulated by ethylene, or may develop **regulatory loops** with others (**purple lines**). **Hormones**, (**white boxes**) are key parts of the fruit developmental pathways: ethylene is the 'central' ripening hormone in tomato fruit, yet ABA, brassinosteroids (BR), and jasmonic acid (JA) act antagonistically or synergistically to effect fruit ripening. **Epigenetic marks (pink boxes)** and the genes that determine these marks **(pink ovals)** and **microRNAs (yellow ovals)** are supra-regulatory agents. Arrowed lines indicate positive regulation, bunt-ended lines indicate inhibition. Lines are dashed for clarity. The full gene list and references are found in the Supplementary materials. This figure was drawn using BioRender.

incremental differences in fruit lifespan and quality. Some nonlinear relationships between gene dosage and trait intensity may be expected, which may create even more novel and diverse time-to-senescence phenotypes [46–48]. Further, promoter engineering might break the negative link between shelf life and quality [49], which is observed as a divergence in the fruit 'physiological state' and its 'chronological age' [50,51]. Dissecting these molecular switches would allow for precise breeding of traits that could reduce postharvest losses, by allowing flexible quality management along the supply chain and deliver high-quality fruit to consumers.

There are still many gaps in knowledge and unanswered questions that need to be addressed to control fruit senescence. (1) First, many of the genes specific to the onset of fruit senescence have not been functionally dissected. Determining accurate physiological markers

Box 2 Definitions of gene-regulatory factors and processes.

TFs: Proteins that bind to specific sequences in the promoter region of single or multiple genes to activate or repress their transcription to mRNA.

CREs: Specific sequence motifs in gene promoters to which TFs or other proteins bind to regulate gene transcription.

EPIGENETIC REGULATION: Modification of gene expression, by mechanisms that do not involve changes in DNA sequence. This includes DNA methylation, histone modification, and the action of noncoding RNAs such as microRNAs.

CHROMATIN: The complex formed with DNA and histone proteins. Chromatin structure determines the physical accessibility of TFs to gene promoters.

DNA METHYLATION: The reversible addition of methyl groups to DNA sequence. Methylation of DNA influences accessibility to DNA, and is determined by the balance between DNA methylase and demethylase activity.

HISTONE MODIFICATION: The process of acetylating, methylating, or phosphorylating histone proteins, which alters the accessibility of TFs to gene promoters. The degree of acetylation is regulated by the balance between the activities of acetylases and deacetylases, for example, HDA1 (Histone deacetlyation), HDA3, HDT3, and methylation are controlled by the relative activity of methylases and demethylases, for example, EZ1 (Enhancer of zeste - histone methyltransferases), EZ2, and JMJ6 (Jumonji - Histone demethylase).

MICRORNA: Noncoding RNAs that regulate gene expression, either by degrading mRNA, or by directly influencing translation**.** These include miR157, miR172A, and miR164.

for 'fruit senescence' and identifying genes that are initiated or negatively or positively co-expressed as senescence progressed might be helpful. (2) The mode of action of many of the 'characterized' genes that regulate ripening, although expanding, still remains unclear [49,52–55], and without more complete information, predicting their mode of action may not be as accurate as desired. (3) Genes that regulate the spatial development of ripening andsenescence are still being unearthed and they offer opportunities for controlling senescence [35,56,57].

Controlling vegetative organ progression to senescence

Differently from fruits, functional analyses of genes that would reduce losses of leafy greens and vegetables have only partially explored, but progress is being made [50]. In lettuce, modifying cell wall *xyloglucan endotransglucosylase/hydrolases* maintained turgor texture and postharvest shelf life [58]. Reducing browning in cut tissues by altering polyphenol oxidases has been successful in many species, improving the likelihood that they will be consumed and not discarded [25]. Leafy greens and green cruciferous vegetables could be marketable after extended storage if chlorophyll contents could be retained — generating favorable *stay-green* alleles by gene editing may be a viable approach [59]. Directing attention to these questions could expand the toolkit for preserving fruit and delaying the onset and progression of senescence.

Conclusion

Losses of yield in cereals and horticultural crops occur at almost every stage of the plant or organ lifecycle, as a result of a failure of, or poor timing of key physiological processes. We have provided examples of genes that have been edited, or are excellent candidates for editing, that could help to minimize these losses, both pre- and after-harvest. Delving into the signal transduction pathways of key physiological processes in plants would identify additional genes or gene networks that could be dissected to deliver precision phenotypes.

CRediT authorship contribution statement

Pietro Tonutti: Supervision; Conceptualization; Writing – original draft; Writing – review & editing. **Stefano Brizzolara**: Conceptualization; Writing – original draft; Writing – review & editing. **Diane M Beckles**: Conceptualization; Writing – original draft; Writing – review & editing; Visualization; Supervision.

Conflict of interest statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

No data were used for the research described in the article.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.copbio.2023.102925.

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