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Opinion

Applying the Solanaceae Strategies to Strawberry Crop Improvement

Amelia Gaston,¹ Sonia Osorio,² Béatrice Denoyes,^{1,*} and Christophe Rothan^{1,*}

Strawberry is a fruit crop species of major horticultural importance, for which fruit quality and the control of flowering (for fruit yield), runnering (for vegetative propagation), and the trade-off between the two are main breeding targets. The octoploid cultivated strawberry has a limited genetic basis. This raises the question of how to identify important gene targets and successfully exploit them for strawberry improvement. In this Opinion article we propose to apply to woodland strawberry, a wild diploid species displaying wide diversity, the strategies successfully employed in recent years for the identification of genetic variations underlying fruit quality and fruit yield traits in solanaceous crops (tomato, potato). Next we propose to use gene editing technologies to translate the findings to cultivated strawberry.

How Lessons Learned from the Solanaceae Can Improve Strawberry

In the past years, fruit yield and quality have been studied in depth in the model fleshy-fruit species tomato. Recently, natural and artificial tomato **genetic diversity** (see [Glossary](#)) has been explored to an unprecedented level through deep sequencing and other omics technologies to discover the genetic architecture of fruit yield, fruit flavor, and fruit nutritional quality [1–6]. Remarkably, these findings were immediately translated to crop improvement through the creation of new tomato lines exhibiting enhanced yield and fruit quality or even for *de novo* domestication of wild related species through **clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated system 9 (Cas9)** gene editing technology [7–9].

Strawberry is a fleshy fruit of considerable commercial and nutritional importance. Like tomato, strawberry has sympodial shoot growth and bears fruit for which **volatiles** and primary metabolites (flavor) and specialized metabolites (color, nutrition) are of utmost importance. Like potato, which belongs to the Solanaceae family together with tomato, strawberry propagates by both sexual (fruits) and asexual (**stolons** or **runners**) reproduction [10,11]. However, strategies used in the Solanaceae for gene and **allele** discoveries can hardly be applied directly to cultivated strawberry because of its octoploid status ([Box 1](#)) and limited genetic diversity [12,13]. By contrast, the wild diploid woodland strawberry (*Fragaria vesca*) benefits from a wide distribution in the Northern Hemisphere and hence from very large genetic diversity. Several additional diploid *Fragaria* species with more limited distributions are found in the wild ([Figure 1](#)). Thanks to the considerable advances in phenotyping and genotyping technologies, these genetic resources can now be harnessed to identify genetic variations responsible for remarkable agronomic traits such as plant flowering and runnering aptitude or fruit flavor. In addition, the high-quality genome sequence of cultivated strawberry, recently released [14], showed the expression dominance of *F. vesca* in cultivated strawberry. Because the diploid and octoploid strawberry species can be efficiently engineered by CRISPR/Cas9 gene editing [15,16], the findings from *F. vesca* can now be transferred to cultivated strawberry via gene editing, which is well adapted for targeting multiple traits or multiple homoeoalleles ([Box 1](#)) in polyploid crop species.

Tomato and Strawberry Genetic Diversity

Tomato and its 12 closely related species belong to the *Solanum* genus in the large Solanaceae family [17], are diploid ([Box 1](#)) and originate from the Andean region of South America. Cultivated tomato *Solanum lycopersicum* derives from the wild *Solanum pimpinellifolium* via the domesticated cherry tomato. Domestication and selection triggered a genetic bottleneck resulting in a loss of genetic diversity [18], but later improvement and breeding contributed to the development of thousands of new cultivated varieties with large phenotypic diversity. Explorations of the tomato's center of

Highlights

Cultivated octoploid strawberry, whose production increases continuously, displays limited genetic variability, which impedes its improvement. Woodland strawberry, one of its wild diploid ancestors, exhibits wide phenotypic diversity. Tomato and potato are major crops of the Solanaceae family.

Fruit yield and quality are major breeding targets in strawberry and tomato. Stolon production is a trait important for both strawberry (vegetative propagation) and potato (tuber production) that has recently received much attention.

In tomato, the exploitation of genetic diversity combined with multiomics and/or gene editing strategies recently led to considerable improvement of fruit yield and quality.

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Box 1. Polyploidy

Polyploidy has long been recognized as a major force in speciation and evolution. The haploid number (n) represents the number of chromosomes present in haploid cells such as gametes and the number x represents the number of chromosomes present in the haploid cells of the species showing the lowest level of ploidy in the series. In diploids, one set of chromosomes is present ($2n = 2x$), while more than one set are present in polyploids resulting from whole-genome duplication [two in tetraploids ($2n = 4x$), three in hexaploids ($2n = 6x$), etc.]. As an example, in the strawberry (*Fragaria*) genus, for which the basic number of chromosomes is $x = 7$, species can be diploid such as *Fragaria vesca* ($2n = 2x = 14$), hexaploid such as *Fragaria moschata* ($2n = 6x = 42$), octoploid such as *Fragaria virginiana* ($2n = 8x = 56$), or decaploid such as *Fragaria iturupensis* ($2n = 10x = 70$).

An allopolyploid octoploid species results from the merger of four diploid genomes from related species, considered as four subgenomes. The cultivated strawberry *Fragaria* × *ananassa*, which is a hybrid between the two octoploid species *F. virginiana* and *Fragaria chiloensis*, comprises the four *F. vesca*, *Fragaria viridis*, *Fragaria iinumae*, and *Fragaria nipponica* subgenomes [14]. The consequences of polyploidy on crop breeding are essential. In diploid species such as tomato, only two copies or alleles of the same gene at the same loci on homologous chromosomes will contribute to the expression of a given gene and thus to the value of the corresponding trait. Therefore, the identification and selection of plants carrying the beneficial allelic variant and hence the trait of interest can be relatively straightforward even for multigene control. In polyploids, transcripts from a given gene can be represented by a combination of transcripts from various homoeoalleles. Allelic dosage or more complex interactions between homoeoalleles can therefore strongly affect phenotypes. In an octoploid species such as cultivated strawberry, determining which allele or which combination of up to eight homoeoalleles is responsible for the trait of interest can prove extremely difficult. As a consequence, breeding superior varieties by selecting the beneficial homoeoallelic variants or by modifying simultaneously several homoeoalleles via gene editing can be challenging. However, a recent study showed that *F. vesca* is the dominant subgenome in cultivated strawberry and controls several metabolic pathways including flavor, which makes this species the ideal target for the discovery of beneficial allelic variants and for their subsequent transfer to cultivated strawberry.

origin further enabled the collection and characterization of vast natural genetic diversity. In addition, artificially induced mutant collections provide new genetic diversity [17].

Strawberry belongs to the genus *Fragaria* in the Rosaceae family. *Fragaria* comprises 22 wild species [19] ranging from diploids ($2n = 2x = 14$) to decaploids ($2n = 10x = 70$; Box 1). The main cultivated strawberry *Fragaria* × *ananassa* (8x) originated in the 18th century in Europe from a hybridization by chance between two octoploid species, the wild *Fragaria virginiana* (North America) and the domesticated *Fragaria chiloensis* (South America) [14]. Early breeding practices reduced its genetic diversity, which was later enlarged by crosses with both wild parental species aimed at transferring desirable traits such as remontancy for *F. virginiana* or adaptation to mild coastal climates for *F. chiloensis* [12]. In addition to the octoploid *F. chiloensis*, cultivated in Chile for ~1000 years, other cultivated species are the hexaploid *Fragaria moschata*, or musk strawberry, cultivated in Europe since the 14th century, and the diploid *F. vesca* or woodland strawberry, which was a common garden strawberry in Europe from Roman times until it was superseded by the hexaploid and then by the octoploid scarlet imported in Europe in 16th century and finally by *F.* × *ananassa* in the 18th century [19].

Wild *Fragaria* diploid species occur naturally almost throughout the Holarctic. Among the wild diploid species, *F. vesca* and its subspecies [19] show the most widespread distribution [20] (Figure 1). After *F. vesca*, the largest distribution is that of the Eurasian *Fragaria viridis*. Other *Fragaria* diploid species display a much more restricted distribution, examples of which are the *Fragaria iinumae* and *Fragaria nipponica* species circumscribed mainly to islands of Japan and East Russia. Interestingly, *F. vesca* is one of the four diploid progenitors of the octoploid cultivated strawberry and displays a dominant expression bias over the other three subgenomes from *F. viridis*, *F. iinumae*, and *F. nipponica* [14]. As a consequence of its extensive distribution, *F. vesca* diversity is high, as shown for fruit size, shape, and color traits in a subset of *F. vesca* accessions (Figure 1). It therefore constitutes a major genetic resource, still underexploited, for the discovery of natural allelic variants

Glossary

Allele: one of a number of alternative forms of the same gene occupying a given position on a chromosome.

Carpel: female part of a flower that comprises the ovary, stigma, and style. The fruit pericarp tissue that develops from the ovary wall of the flower and surrounds the seeds is fleshy in tomato fruit (fused carpels) and dry and hardened in the strawberry achene (unique carpel).

CENTRORADIALIS/TERMINAL FLOWER 1/SELF-PRUNING

(CETS) family: gene family with a prominent role in the regulation of flowering or tuberization, with members acting as florigen, tuberigen, and floral repressor.

Clustered regularly interspaced short palindromic repeats

(CRISPR)/CRISPR-associated system 9 (Cas9): CRISPR and its associated nuclease Cas are 'molecular scissors' designed to target and edit a given sequence. This can introduce mutations and thus create a new allelic variant or replace a wild allele with an engineered allele.

Florigen: long-distance signal promoting flowering.

Genetic diversity: variation in a DNA sequence between distinct individuals of a given species (or population). Genetic diversity is reflected in phenotypic diversity; for example, variations in fruit shape, color, or flavor between individuals and populations.

Genome-wide association study (GWAS): a powerful approach that examines the genetic variations (e.g., SNPs) over the genome of many individuals and correlates them with variations in phenotypic traits. To precisely pinpoint the genetic variations associated with a given trait requires access to cost-effective and high-resolution genome-wide genotyping technologies, such as whole-genome sequencing, and to highly diverse genetic resources representing a wide genetic diversity. The structure and genetic relatedness of genetic diversity has to be taken into account when constituting GWAS panels.

Meristem: in plants, groups of cells that achieve stem-cell renewal and coordinate organ

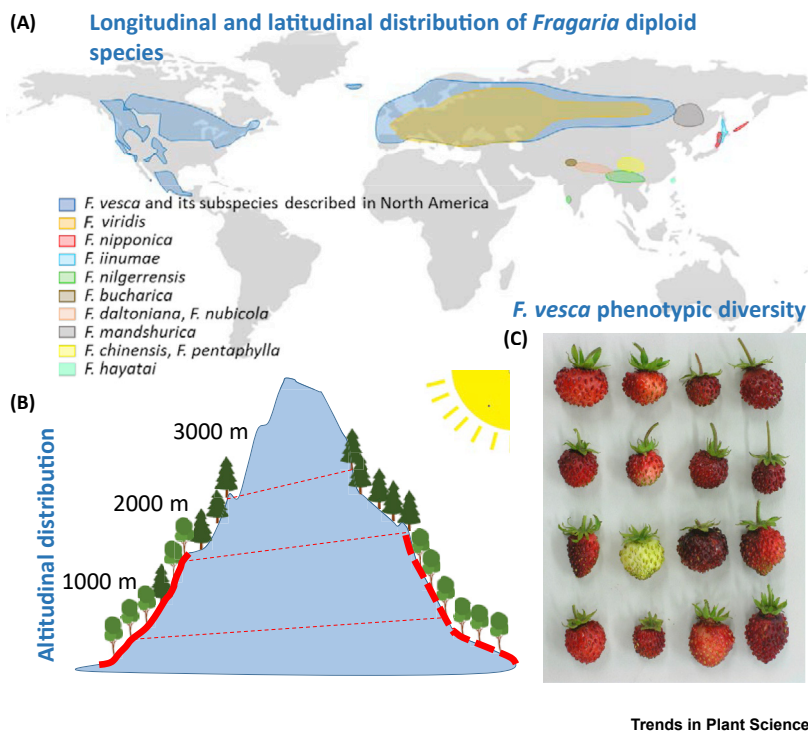


Figure 1. Distribution of Diploid Strawberry Species and Fruit Diversity in Subspecies of Woodland Strawberry.

(A) Geographic distribution of the twelve *Fragaria* diploid species. The woodland strawberry *Fragaria vesca* is the most widely distributed in the Northern Hemisphere. (B) Altitudinal distribution of woodland strawberry. *F. vesca* is present at a wide range of altitudes (e.g., from 150 m to 1700 m above sea level, depending on south or north exposure, in the Pyrénées mountains). (C) Example of fruit phenotypic diversity in *F. vesca* (different subspecies and formae). Photograph from Klaus Olbricht. Map available at https://www.sccpre.cat/show/imhibo_world-map-png-high-resolution-world-map-vector/.

responsible for environmental tolerance and for plant and fruit agronomic traits. In addition, new artificially induced genetic diversity has been generated in *F. vesca* by mutagenesis [11].

Major Traits to Improve in Strawberry

Plant and Fruit Yield

Fruit yield, one of the most important and complex crop traits, is determined by fruit number and weight. Fruit number depends on plant and inflorescence architecture (Box 2), which determines the number of inflorescences and the number of flowers/fruits in a truss, and on the extent of the fruit production period. Fruit weight mostly depends on fruit size and shape [21]. In strawberry, there is a trade-off between fruit yield and the production of aerial stolons (or runners), an important trait in nurseries for vegetative plant propagation via daughter plants [10] (Figure 2).

Considerable advances have been made in recent years regarding fruit yield control in tomato via plant and inflorescence architecture, thanks to the dissection of the molecular mechanisms for meristem maturation [22–24] and to the study of the available genetic diversity from Solanaceae species and tomato mutant collections [1]. Several key players in tomato yield have been unraveled including the florigen SFT and anti-florigen SP [from the CENTRORADIALIS/TERMINAL FLOWER 1/SELF-PRUNING (CETS) family], the balance of which modulates shoot termination and branching and therefore plant architecture [1,25], and the TMF transcription factor, S locus (WOX9 homolog), and MADS-box J2 and EJ2, which determine inflorescence architecture [2,7,23] (Figure 2). Moreover,

formation from the periphery of the meristem.

Metabolome: the total complement of metabolites present in a cell, tissue, organ, or organism.

Plant architecture: spatial arrangement of vegetative and reproductive structures along the stem that produces the complexity of plant organization. It results from the complex regulation of hierarchically organized meristems.

Primary metabolism: reactions and pathways absolutely vital for plant survival.

Quantitative trait locus (QTL): locus (chromosomal region) associated with the quantitative variation of a trait.

Receptacle: enlarged flower stem.

Runners: aerial stolons producing daughter plants in strawberry.

Single-nucleotide polymorphism (SNP): substitution of a single nucleotide at a specific position in the genome.

Specialized metabolism: reactions and pathways that fulfil a multitude of important functions for growth and development, including the interaction of the plant with the environment.

Stolon: specialized and highly elongated axes developed from an axillary meristem (AM). In potato, underground stolons produce tubers in the tuberization process; in strawberry, stolons are called runners.

Transcriptome: the total complement of transcripts present in a cell, tissue, organ, or organism.

Tuberigen: long-distance signal promoting tuberization.

Volatiles: volatile organic compounds that comprise a wide diversity of low-molecular-weight secondary metabolites with high vapor pressure under ambient conditions.

Box 2. Plant and Inflorescence Architecture

Tomato and strawberry are sympodial species in which the main axis, issued from the shoot apical meristem (SAM), terminates in an inflorescence under inducible conditions. The vegetative growth of the plant is maintained by the uppermost AM leading to a sympodial axis, which can in turn terminate in an inflorescence; this process will be reiterated.

In addition, in potato and strawberry, the AM along the axis can produce lateral axes terminated by an inflorescence or can develop specialized and highly elongated axes, named stolons. In strawberry, stolons are aerial and produce daughter plants, whereas in potato stolons are underground and produce tubers, both processes allowing asexual reproduction. AM fate thus regulates the trade-off between flowering (fruit yield) and runnering (daughter-plant yield) in strawberry.

The inflorescence meristem is indeterminate and may continuously initiate determinate floral meristems, which produce flowers. As an example, depending on meristem activity, the number of flowers in Solanaceae may range from one (pepper) to hundreds in some species and in several tomato mutants [71].

customized combination of allelic variants of architecture-associated genes resulted in spectacular improvement in crop productivity not only in cultivated tomato but also in its wild ancestor *S. pimpinellifolium* and in the orphan species *Physalis peruviana* [1,3,7,9,26]. To date, the large variability in plant and inflorescence complexity has not been studied in depth in *Fragaria* species [27].

The fruit production period can be extended in cultivated strawberry, which is a short-day species, thanks to the natural perpetual flowering homoeoallelic variant introduced from *F. virginiana* [28]. In woodland strawberry, mutation in the *TFL1* floral repressor considerably prolongs fruit production [29,30], whereas in tomato mutation in its *SP* homolog results in determinate growth and thus shortens the flowering period [25]. In tomato, domestication-driven loss of day-length-sensitive flowering is due to allelic variations in the florigen repressor *SP5G* and in the circadian clock gene *EID1* [2,31], while in potato an allelic variant of the *CDF1* mediator between light regulation and the **tuberigen** pathway is responsible for the loss of photoperiodic sensitivity in tuberization [32]. In both Solanaceae species, these mutations permitted the adaptation to new geographic regions. Together these findings highlight how improvement of strawberry productivity can benefit from Solanaceae studies on plant architecture and on the florigen/tuberigen pathway and its regulation by light.

Fruit weight has long received considerable attention in tomato because of its association with domestication and crop productivity [21]. Various combinations of natural, mutated and, more recently, gene edited variants of genes regulating the floral meristem were shown to have a huge effect on **carpel** number and fruit size in tomato [7,21,33]. However, because the strawberry fruit is a swollen **receptacle** and is not formed from carpels, the most interesting target genes are those active in the ovary and in growing fruit, such as *FW2.2*, *SUN*, or *OVATE* [21,33].

Regarding daughter-plant yield, there is a clear convergence between strawberry and potato because tubers are also produced by stolons, which are underground in this species (Box 2). In strawberry, the study of natural and induced mutants led to the identification of GA pathway components (GA20ox and DELLA proteins) for the control of runnering [10,11], while the same hormonal pathway and the tuberigen *SP6A* were shown to regulate stolon formation and tuberization in potato [34–36] (Figure 2).

Fruit Quality*Flavor Is a Major Fruit Quality Trait*

Fleshy-fruit quality is also complex (Figure 3A) and is difficult to improve by selection. One of the most important traits is fruit flavor (taste and smell), which is determined in both tomato and strawberry by complex interactions between sugars (mainly glucose, fructose, and sucrose), organic acids (mainly citrate, malate, and glutamate), and a set of tens to hundreds of volatile compounds. Major compounds produced by **primary** and **specialized metabolism** are summarized in Figure 3B. The

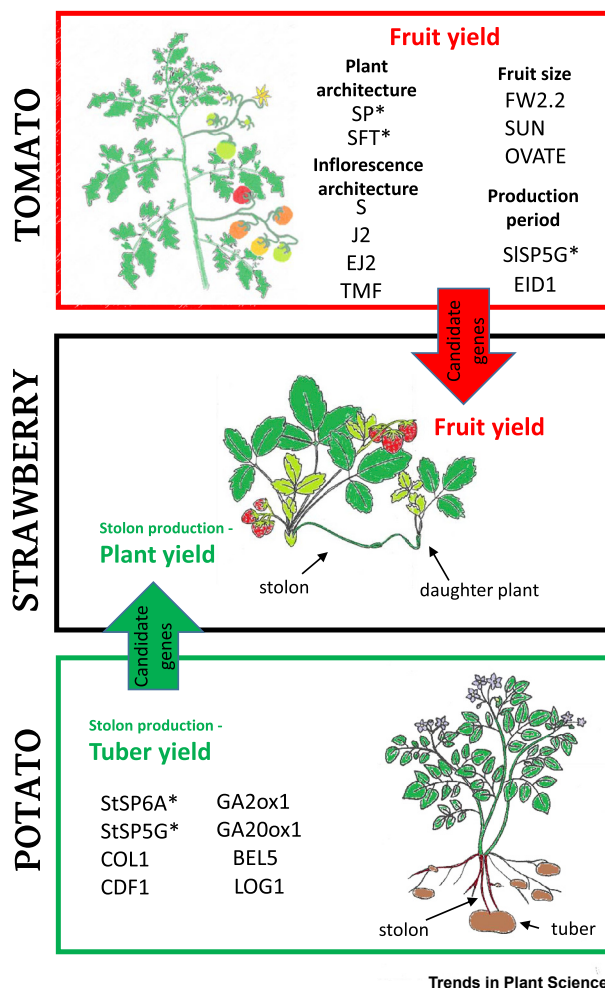
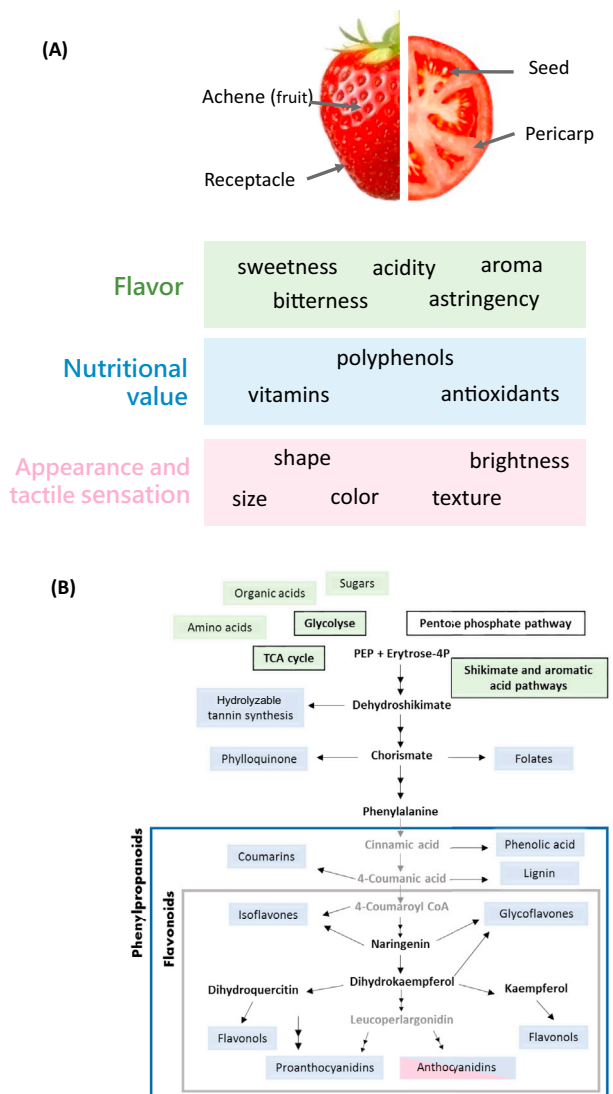


Figure 2. Fruit and Plant Yield-Associated Traits and Candidate Genes.

Nonexhaustive list of major genes from tomato and potato that may contribute to the improvement of yield-associated traits in strawberry. The fruit yield trait can benefit from insights on plant and inflorescence architecture, fruit size determination, and day-length control of flowering in tomato. The daughter-plant yield trait can benefit from insights on stolon production and tuberization in potato. Tomato (*Sl*, *Solanum lycopersicum*): SP, SELF-PRUNING; SFT, SINGLE FLOWER TRUSS; S, COMPOUND INFLORESCENCE [homolog of WUSCHEL-RELATED HOMEODOMAIN 9 (WOX9)]; J2, JOINTLESS 2; EJ2, ENHANCER-OF-JOINTLESS 2; TMF, TERMINATING FLOWER; FW2.2, FRUIT WEIGHT 2.2; SUN; OVATE; SIS5G, SELF-PRUNING 5G; SIEID1, EMPFINDLICHER IM DUNKELROTEN LICHT 1. Potato (*St*, *Solanum tuberosum*): StSP6A, SELF-PRUNING 6A; StSP5G, SELF-PRUNING 5G; COL1, CONSTANS-LIKE 1; CDF1, CYCLIN DOF FACTOR 1; GA2ox1, GIBBERELIN 2 OXIDASE 1; GA20ox1, GIBBERELIN 20 OXIDASE 1; BEL5, arabidopsis BEL1-like; LOG1, LONELY GUY 1. *, belongs to the CETS family.

sugar:acid ratio is widely adopted as a breeding target [37], but specific volatiles may also enhance the perception of sweetness independent of sugar content [38]. In strawberry fruit, for example, positive correlations between sweet aroma intensity and a subset of lactones and terpenoids have been reported [39].

Until recently, modern breeding has been mainly focused on plant production, yield (Figure 2), biotic resistance, and postharvest handling rather than on fruit quality. Consequently, modern varieties frequently lack the pleasant flavor characteristics required by consumers because they contain



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Figure 3. Fruit Quality.

(A) Tomato is a berry where seeds are enclosed in fleshy pericarp. Strawberry is botanically not a fruit but a swollen receptacle that bears multiple fruits (achenes) on its surface. The achene is an indehiscent dry fruit in which a unique seed is enclosed in a sclerified pericarp. Strawberry and tomato fruits share common sensorial and nutritional quality traits including fruit appearance and tactile sensation (size, shape, color, brightness, or texture), flavor (sugars, organic acids, volatiles, bitter and astringent compounds), and nutritional value (vitamins, polyphenols). (B) Specialized metabolism is important in both tomato and strawberry. Compounds derived from specialized metabolism are, for example, essential for fruit color (e.g., the red anthocyanins in strawberry) and health benefits (vitamins, phenolics, and other antioxidants). An outline of the most important shikimate- and phenylalanine-derived secondary compounds pathways in tomato and strawberry fruit is shown. Single and double arrows indicate single and multiple reactions, respectively.

only a small variation of the flavor chemicals present in wild genetic resources. This is due to extreme genetic selection and loss of allelic diversity during domestication [4–6,40,41]. Additionally, breeding for increased sugars content may be detrimental to fruit size, which is a major trait [6].

A Wild Reservoir for Flavor Improvement in Genetic Resources

Interestingly, strawberry and tomato wild relatives are significantly different from cultivated varieties, not only for fruit size but also for flavor attributes [5,6,42–44]. In tomato, this diversity has been instrumental for the discovery by **quantitative trait locus (QTL)** mapping of allelic variants responsible for desirable flavor-related traits (e.g., starch and sugar content [45], fructose-to-glucose ratio [46], flavor volatiles [47]). In parallel, **genome-wide association studies (GWASs)** of hundreds of tomato modern varieties, landraces, and wild relatives led to the identification of causal **SNPs** associated with variations in fruit size [6], steroidal alkaloids [6], malate [48], fruit color [6,18], and flavor components [4,5]. Building a correlation network with tens of millions of SNPs, tens of thousands of transcripts (**transcriptome**), and hundreds of metabolites (**metabolome**) revealed new metabolic genes and pathways underlying major fruit quality traits [6]. Recently, GWAS data sets representing a very large tomato diversity (775 accessions) were further combined and analyzed to pinpoint gene polymorphisms likely to be responsible for sugars, acids, and flavor-related volatiles [49].

In strawberry, pathways and candidate genes for key flavor volatiles were uncovered through genetic, genomic, and chemical analyses [42,43,50,51]. A striking example is that of the very attractive and fruity note of *F. vesca*, which is found only in rare cultivated varieties such as ‘Mara des Bois’ and is due to the highly aromatic methyl anthranilate (MA) volatile [44]. Combined genetic, transcriptome, and volatile analyses of MA- and non-MA-producing plants from a *F.* × *ananassa* population succeeded in the identification of a methyltransferase enzyme responsible for the last step of MA synthesis [44]. These findings indicate that breeding desirable strawberry varieties with substantial flavor improvement is achievable by restoring or introducing specific volatiles from wild species to modern varieties. Fortunately, many more attractive flavor components can be found by screening natural diversity from *F. vesca* and other wild species [39,52,53].

Applying the Solanaceae Strategies to Strawberry Crop Improvement

Woodland Strawberry *F. vesca* as a Model for Cultivated Strawberry

Major breeding targets are common to tomato and strawberry (Figures 2 and 3). In cultivated strawberry, as in tomato, wild alleles from parental species (e.g., the perpetual flowering locus [28]) can be introduced by crossing. Its genome size of 813 Mb (900 Mb in tomato) [14] makes it suitable for GWASs [12] and it can be genetically transformed [16]. However, direct transfer of the strategies and findings from tomato to cultivated strawberry is not trivial because of the complexity of its octoploid genome (Box 1). Woodland strawberry recently emerged as a model for cultivated strawberry and for the Rosaceae family [54]. It is diploid, has a short generation time and can be genetically transformed [15]. It has a high-quality genome sequence of only 219 Mb [54,55] and a gene expression atlas is available [56]. Moreover, its genome is dominant in the octoploid for certain biological pathways including fruit flavor [14]. Wide natural and induced genetic diversity (Figure 1) [11] and populations of introgression lines [57] are available, which have already allowed the discovery of causal mutations of the perpetual flowering [29,30] and runnerless [10,11] traits.

Which Strategies to Exploit *F. vesca* Diversity and Transfer the Findings to Cultivated Strawberry?

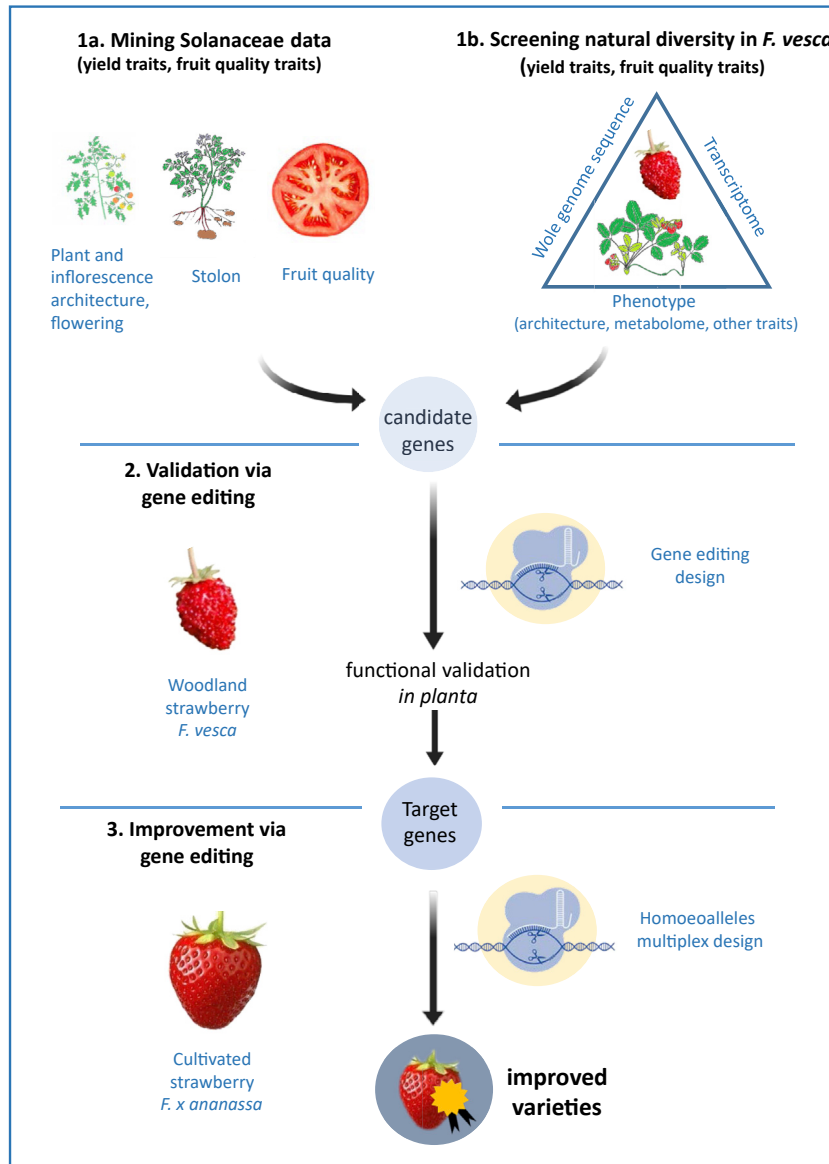
*Exploiting *F. vesca* Genetic Diversity*

The exploitation of artificially (Figure 4, Key Figure) induced diversity by mapping-by-sequencing to discover causal mutation is relatively straightforward in both tomato and woodland strawberry [11,58]. Exploitation of natural genetic diversity from woodland strawberry (Figure 1; e.g., for vernalization requirement [59], for fruit shape and flavor [53,57]) can be done by generating various types of populations [10,57,60] and by GWASs, as done in tomato [4–6,18,48,49]. For GWASs, the first step is to constitute representative *F. vesca* collections to control the effect of population structure [17,49]. Because of its small genome size and the plummeting costs of sequencing, large GWAS panels of tens or hundreds of available accessions of *F. vesca* [61] can now be sequenced. Phenotyping may be the limiting step. For fruit quality traits, analytical methods are essentially identical in the two species and the same strategy of integration of genetic, genomic, transcriptomic, and metabolomic data

Key Figure

Strategies for Strawberry Improvement

Key Figure How to improve strawberry by applying Solanaceae strategies ?



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Figure 4. Mining the tomato and potato literature and databases produces candidate genes for improvement of yield-associated traits (plant and inflorescence architecture, stolon formation, timing and duration of flowering period, fruit size) and of fruit quality traits in strawberry (1a). New candidate genes can be produced by genome-wide association studies (GWASs) of natural diversity from woodland strawberry; whole-genome sequence data can be further integrated with large-scale metabolome and transcriptome data (1b). After a validation step in the diploid woodland strawberry via gene editing (2), beneficial alleles can be engineered in octoploid cultivated strawberry to obtain strawberry varieties with the desirable traits (3).

[6] can be applied (Figure 4). For other complex traits, such as yield-associated traits, the development of more precise and higher phenotyping methods may be required.

Validation of Candidate Genes and Transfer of Beneficial Alleles to Cultivated Strawberry

Most findings from tomato and potato on yield and fruit quality traits (Figures 2 and 3) can be readily used to identify orthologous target genes in *F. vesca* (Figure 4). However, we must stress that the conservation of gene function and involvement in the same processes in strawberries is not guaranteed. For example, although modulation of flowering and plant architecture by FT/TFL1 floral regulators is well conserved in plants, FT/TFL1 duplication in different species often leads to conservation of the function of one paralog and neofunctionalization of the others [62]. Such functional diversification of FT-like genes can be observed, for instance, in potato, where *StSP3D* and *StSP6A* (Figure 2) are involved in two independent systemic signals: florigen and tuberigen [34]. Likewise, although there is a remarkable convergence between fleshy fruit species, regardless of the ontogeny of the fruit, the processes involved can be very different. To cite just a few examples: tomato fruit imports sucrose while strawberry fruit imports both sucrose and sorbitol, sugars can be transiently stored as starch in tomato but not in strawberry, and profiles of volatile organic compounds differ markedly between the two species. To cope with these discrepancies, new computational analyses, such as STATIS, which combines principal component analysis and pathway over-enrichment analysis [63], have been successfully used to identify the metabolic processes whose behavior is similarly affected during fruit development and ripening in tomato and strawberry.

Screening natural and induced diversity will additionally produce a wealth of candidate genes and allelic variants. Once identified, their effect on the trait of interest can be validated in diploid or cultivated strawberry through, for example, virus-induced gene silencing [64] or stable transformation [15,16]. Gene editing technologies using transcription activator-like effector nucleases (TALENs) or the CRISPR/Cas9 system are particularly attractive for engineering crops with desirable traits, as shown in many crop species including tomato, potato, and strawberry [65,66]. The CRISPR/Cas9 system has been successfully established in both *Fragaria* species [15,16]. It is a powerful gene editing technology allowing the introduction of mutations in target genes or even the replacement of a wild allele by an engineered allele, as recently demonstrated in tomato [67]. In this species, the creation of new allelic variants for up to six genes could redirect metabolic pathways controlling fruit nutritional value [68] and, in various studies on wild relatives, recapitulate the domestication process [26]. Furthermore, the creation of new expressional alleles by targeting the *cis*-regulatory regions of two genes controlling floral meristem size led to the production of fruits with increased size, a trait that could be further fixed in progeny [7].

However, because the octoploid genome is complex (Box 1), it is preferable to first validate the candidates in diploid species and then, for selected target genes, reproduce the beneficial allelic variations in cultivated strawberry [69]. Editing multiple homoeoalleles at a same locus is possible. It has been done with the CRISPR/Cas9 system in octoploid strawberry [16] and has already demonstrated its potential for trait engineering in polyploid crop species such as hexaploid wheat [70]. Examples from the Solanaceae clearly show how strawberry improvement for plant and yield traits and for fruit quality can benefit from these technologies.

Concluding Remarks and Future Perspectives

One of the current challenges in strawberry breeding is to together improve daughter-plant yield (for nurseries), fruit yield (for producers), and fruit quality (for consumers) and the balance between these traits. Cultivated strawberry is octoploid and, due to its history, has a low genetic diversity, which impedes the pace at which new desirable traits can be introduced. Recently, the deep sequencing and CRISPR/Cas9 gene editing revolutions modified the scale, precision, and speed at which genetic variations underlying fruit yield and fruit quality could be discovered and translated to tomato crop improvement. The application of the strategies successfully used in tomato to strawberry crop improvement is timely. The wide genetic diversity from woodland strawberry is still underexploited and the scientific context is now favorable to the rapid transfer of discoveries from wild to cultivated

Outstanding Questions

What proportion of woodland strawberry natural diversity is currently available in *ex situ* collections of genetic resources and how can it be increased?

Given that the evaluation of plant architecture is complex in strawberry but is essential for yield-associated traits, is it possible to increase its throughput?

What are the existing barriers for the transfer of beneficial alleles from woodland strawberry to cultivated strawberry, either by crossing (available diversity in octoploid related species) or by genetic engineering (regulatory issues)?

strawberry. We argue that efforts should be devoted to large-scale and multilevel analyses of woodland strawberry genetic resources and, once beneficial alleles have been discovered and validated, to transferring the findings to cultivated strawberry via conventional marker-assisted breeding or gene editing (see [Outstanding Questions](#)).

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References

- Park, S.J. *et al.* (2014) Optimization of crop productivity in tomato using induced mutations in the florigen pathway. *Nat. Genet.* 46, 1337–1342
- Soyk, S. *et al.* (2017) Bypassing negative epistasis on yield in tomato imposed by a domestication gene. *Cell* 169, 1142–1155
- Soyk, S. *et al.* (2017) Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. *Nat. Genet.* 49, 162–168
- Bauchet, G. *et al.* (2017) Identification of major loci and genomic regions controlling acid and volatile content in tomato fruit: implications for flavor improvement. *New Phytol* 215, 624–641
- Tieman, D. *et al.* (2017) A chemical genetic roadmap to improved tomato flavor. *Science* 355, 391–394
- Zhu, G. *et al.* (2018) Rewiring of the fruit metabolome in tomato breeding. *Cell* 172, 249–261
- Rodríguez-Leal, D. *et al.* (2017) Engineering quantitative trait variation for crop improvement by genome editing. *Cell* 171, 470–480
- Li, T. *et al.* (2018) Domestication of wild tomato is accelerated by genome editing. *Nat. Biotechnol.* 36, 1160–1163
- Zsögön, A. *et al.* (2018) *De novo* domestication of wild tomato using genome editing. *Nat. Biotechnol.* 36, 1211–1216
- Tenreira, T. *et al.* (2017) Specific gibberellin 20-oxidase dictates the flowering-runner decision in diploid strawberry. *Plant Cell* 29, 2168–2182
- Caruana, J.C. *et al.* (2018) Suppressor of runnerless encodes a DELLA protein that controls runner formation for asexual reproduction in strawberry. *Mol. Plant* 11, 230–233
- Hardigan, M.A. *et al.* (2018) Domestication of temperate and coastal hybrids with distinct ancestral gene selection in octoploid strawberry. *Plant Genome* 11, 180049
- Horvath, A. *et al.* (2011) Structured diversity in octoploid strawberry cultivars: importance of the old European germplasm. *Ann. Appl. Biol.* 159, 358–371
- Edger, P.P. *et al.* (2019) Origin and evolution of the octoploid strawberry genome. *Nat Genet* 51, 541–547
- Zhou, J. *et al.* (2018) Efficient genome editing of wild strawberry genes, vector development and validation. *Plant Biotechnol. J.* 16, 1868–1877
- Martín-Pizarro, C. *et al.* (2019) Functional analysis of the TM6 MADS-box gene in the octoploid strawberry by CRISPR/Cas9-directed mutagenesis. *J. Exp. Bot.* 70, 885–895
- Rothan, C. *et al.* (2019) Trait discovery and editing in tomato. *Plant J.* 97, 73–90
- Lin, T. *et al.* (2014) Genomic analyses provide insights into the history of tomato breeding. *Nat. Genet.* 46, 1220–1226
- Liston, A. *et al.* (2014) *Fragaria*: a genus with deep historical roots and ripe for evolutionary and ecological insights. *Am. J. Bot.* 101, 1686–1699
- Staudt, G. (1989) The species of *Fragaria*, their taxonomy and geographical distribution. *Acta Hort.* 265, 23–34
- van der Knaap, E. *et al.* (2014) What lies beyond the eye: the molecular mechanisms regulating tomato fruit weight and shape. *Front. Plant Sci.* 5, 227
- Park, S.J. *et al.* (2012) Rate of meristem maturation determines inflorescence architecture in tomato. *Proc. Natl Acad. Sci. U. S. A.* 109, 639–644
- MacAlister, C.A. *et al.* (2012) Synchronization of the flowering transition by the tomato TERMINATING FLOWER gene. *Nat. Genet.* 44, 1393–1398
- Huang, X.Z. *et al.* (2018) Control of flowering and inflorescence architecture in tomato by synergistic interactions between ALOG transcription factors. *J. Genet. Genomics* 45, 557–560
- Lifschitz, E. *et al.* (2014) Florigen and anti-florigen – a systemic mechanism for coordinating growth and termination in flowering plants. *Front. Plant Sci.* 5, 465
- Lemmon, Z.H. *et al.* (2018) Rapid improvement of domestication traits in an orphan crop by genome editing. *Nat. Plants* 4, 766–770
- Labadie, M. *et al.* (2019) Identifying phenological phases in strawberry using multiple change-point models. *J. Exp. Bot.* 70, 5687–5701
- Perrotte, J. *et al.* (2016) Narrowing down the single homoeologous FaPFRU locus controlling flowering in cultivated octoploid strawberry using a selective mapping strategy. *Plant Biotechnol. J.* 14, 2176–2189
- Iwata, H. *et al.* (2012) The TFL1 homologue KSN is a regulator of continuous flowering in rose and strawberry. *Plant J* 69, 116–125
- Koskela, E.A. *et al.* (2012) Mutation in TERMINAL FLOWER1 reverses the photoperiodic requirement for flowering in the wild strawberry *Fragaria vesca*. *Plant Physiol.* 159, 1043–1054
- Müller, N.A. *et al.* (2016) Domestication selected for deceleration of the circadian clock in cultivated tomato. *Nat. Genet.* 48, 89–93
- Kloosterman, B. *et al.* (2013) Naturally occurring allele diversity allows potato cultivation in northern latitudes. *Nature* 495, 246–250
- Wu, S. *et al.* (2018) A common genetic mechanism underlies morphological diversity in fruits and other plant organs. *Nat. Commun.* 9, 4734
- Navarro, C. *et al.* (2011) Control of flowering and storage organ formation in potato by FLOWERING LOCUS T. *Nature* 478, 119–122
- Navarro, C. *et al.* (2015) Conserved function of FLOWERING LOCUS T (FT) homologues as signals

- for storage organ differentiation. *Curr. Opin. Plant Biol.* 23, 45–53
36. Abelenda, J.A. et al. (2016) Potato StCONSTANS-like1 suppresses storage organ formation by directly activating the FT-like StSP5G Repressor. *Curr. Biol.* 26, 872–881
 37. Zanor, M.I. et al. (2009) Metabolic characterization of loci affecting sensory attributes in tomato allows an assessment of the influence of the levels of primary metabolites and volatile organic contents. *J. Exp. Bot.* 60, 2139–2154
 38. Tieman, D. et al. (2012) The chemical interactions underlying tomato flavor preferences. *Curr. Biol.* 22, 1035–1039
 39. Ulrich, D. and Olbricht, K. (2016) A search for the ideal flavor of strawberry – comparison of consumer acceptance and metabolites patterns in *Fragaria × ananassa* Duch. *J. Appl. Bot. Food Qual.* 89, 223–234
 40. Schwieterman, M.L. et al. (2014) Strawberry flavor: diverse chemical compositions, a seasonal influence, and effects on sensory perception. *PLoS One* 9, e88446
 41. Vallarino, J.G. et al. (2018) Genetic diversity of strawberry germplasm using metabolomic biomarkers. *Sci. Rep.* 8, 14386
 42. Aharoni, A. et al. (2004) Gain and loss of fruit flavor compounds produced by wild and cultivated strawberry species. *Plant Cell* 16, 3110–3131
 43. Hoffmann, T. et al. (2011) Metabolic engineering in strawberry fruit uncovers a dormant biosynthetic pathway. *Metab. Eng.* 13, 527–531
 44. Pillet, J. et al. (2017) Identification of a methyltransferase catalyzing the final step of methylanthranilate synthesis in cultivated strawberry. *BMC Plant Biol.* 17, 147
 45. Petreikov, M. et al. (2006) Temporally extended gene expression of the ADP-Glc pyrophosphorylase large subunit (AglL1) leads to increased enzyme activity in developing tomato fruit. *Planta* 224, 1465–1479
 46. Shammai, A. et al. (2018) Natural genetic variation for expression of a SWEET transporter among wild species of *Solanum lycopersicum* (tomato) determines the hexose composition of ripening tomato fruit. *Plant J.* 96, 343–357
 47. Garbowicz, K. et al. (2018) Quantitative trait loci analysis identifies a prominent gene involved in the production of fatty acid-derived flavor volatiles in tomato. *Mol. Plant* 11, 1147–1165
 48. Ye, J. et al. (2017) An InDel in the promoter of AL-ACTIVATED MALATE TRANSPORTER9 selected during tomato domestication determines fruit malate contents and aluminum tolerance. *Plant Cell* 29, 2249–2268
 49. Zhao, J.T. et al. (2019) Meta-analysis of genome-wide association studies provides insights into genetic control of tomato flavor. *Nat. Commun.* 10, 1534
 50. Chambers, A.H. et al. (2014) Identification of a strawberry flavor gene candidate using an integrated genetic–genomic–analytical chemistry approach. *BMC Genomics* 15, 217
 51. Song, C. et al. (2016) Glucosylation of 4-hydroxy-2,5-dimethyl-3(2H)-furanone, the key strawberry flavor compound in strawberry fruit. *Plant Physiol.* 171, 139–151
 52. Gruner, P. et al. (2017) *Fragaria viridis* Weston: diversity and breeding potential of an underutilised strawberry species. *Acta Hort.* 1156, 203–208
 53. Ulrich, D. and Olbricht, K. (2013) Diversity of volatile patterns in sixteen *Fragaria vesca* L. accessions in comparison to cultivars of *Fragaria × ananassa*. *J. Appl. Bot. Food Qual.* 86, 37–46
 54. Shulaev, V. et al. (2011) The genome of woodland strawberry (*Fragaria vesca*). *Nat. Genet.* 43, 109–116
 55. Edger, P.P. et al. (2018) Single-molecule sequencing and optical mapping yields an improved genome of woodland strawberry (*Fragaria vesca*) with chromosome-scale contiguity. *Gigascience* 7, 1–7
 56. Hawkins, C. et al. (2017) An eFP browser for visualizing strawberry fruit and flower transcriptomes. *Hortic. Res.* 4, 17029
 57. Urrutia, M. et al. (2017) Genetic analysis of the wild strawberry (*Fragaria vesca*) volatile composition. *Plant Physiol. Biochem.* 121, 99–117
 58. Garcia, V. et al. (2016) Rapid identification of causal mutations in tomato EMS populations via mapping-by-sequencing. *Nat. Protoc.* 11, 2401–2418
 59. Koskela, E.A. et al. (2017) Altered regulation of TERMINAL FLOWER 1 causes the unique vernalisation response in an arctic woodland strawberry accession. *New Phytol* 216, 841–853
 60. Samad, S. et al. (2017) Additive QTLs on three chromosomes control flowering time in woodland strawberry (*Fragaria vesca* L.). *Hortic. Res.* 4, 17020
 61. Hilmarsson, H.S. et al. (2017) Population genetic analysis of a global collection of *Fragaria vesca* using microsatellite markers. *PLoS One* 12, e0183384
 62. Wickland, D.P. and Hanzawa, Y. (2015) The FLOWERING LOCUS T/TERMINAL FLOWER 1 gene family: functional evolution and molecular mechanisms. *Mol. Plant* 8, 983–997
 63. Klie, S. et al. (2014) Conserved changes in the dynamics of metabolic processes during fruit development and ripening across species. *Plant Physiol.* 164, 55–68
 64. Tian, J. et al. (2014) TRV-GFP: a modified tobacco rattle virus vector for efficient and visualizable analysis of gene function. *J. Exp. Bot.* 65, 311–322
 65. Ye, M.W. et al. (2018) Generation of self-compatible diploid potato by knockout of S-RNase. *Nat. Plants* 4, 651–654
 66. Nadakuduti, S.S. et al. (2018) Genome editing for crop improvement – applications in clonally propagated polyploids with a focus on potato (*Solanum tuberosum* L.). *Front. Plant Sci.* 9, 1607
 67. Dahan-Meir, T. et al. (2018) Efficient in planta gene targeting in tomato using geminiviral replicons and the CRISPR/Cas9 system. *Plant J.* 95, 5–16
 68. Li, R. et al. (2017) Multiplexed CRISPR/Cas9-mediated metabolic engineering of γ -aminobutyric acid levels in *Solanum lycopersicum*. *Plant Biotechnol. J.* 16, 415–427
 69. Koskela, E.A. et al. (2016) TERMINAL FLOWER1 is a breeding target for a novel everbearing trait and tailored flowering responses in cultivated strawberry (*Fragaria × ananassa* Duch.). *Plant Biotechnol. J.* 14, 1852–1861
 70. Wang, Y. et al. (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat. Biotechnol.* 32, 947–951
 71. Perilleux, C. et al. (2019) Turning meristems into fortresses. *Trends Plant Sci* 24, 431–442