

Review

Domestication of Crop Metabolomes: Desired and Unintended Consequences

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The majority of the crops and vegetables of today were domesticated from their wild progenitors within the past 12 000 years. Considerable research effort has been expended on characterizing the genes undergoing positive and negative selection during the processes of crop domestication and improvement. Many studies have also documented how the contents of a handful of metabolites have been altered during human selection, but we are only beginning to unravel the true extent of the metabolic consequences of breeding. We highlight how crop metabolomes have been wittingly or unwittingly shaped by the processes of domestication, and highlight how we can identify new targets for metabolite engineering for the purpose of *de novo* domestication of crop wild relatives.

Domestication and Crop Improvement

Human-guided domestication began ~12 000 years ago in the Middle East and Fertile Crescent, and subsequently occurred across the world including events in China, Mesoamerica and the Andes, Near Oceania, Sub-Saharan Africa, and eastern North America [1–3]. Despite our simplified title, we distinguish here between domestication, diversification, and crop improvement events, wherever possible, given that in both evolutionary and phenotypic terms they are clearly distinct processes [4]. Large surveys have revealed that domesticated plant species span some 160 taxonomic families with >2500 species having undergone some extent of the process and ~300 being fully domesticated [2,3,5]. Current models integrating archaeological, genetic, and genomic evidence suggest that domestication is a multistage process consisting of (i) the onset of cultivation, (ii) an increased frequency of desirable alleles, (iii) the formation of domesticated populations, and finally (iv) deliberate breeding. That said, delineating the history of domestication is highly complex in many species because of the presence of multiple domestication events and frequent post-domestication exchanges with the progenitor species [6–8]. Moreover, it is important to note that some species such as *Oryza nivara* and Brazil nut are cultivated without domestication, and there has been insightful analysis regarding the genetic bottlenecks associated with the initial selection [9]. Taken together, these studies have greatly enhanced our understanding of trait evolution and have provided considerable insight into both convergent and parallel evolution during domestication [10]. For example, the stay green gene, *SGR*, underpins seed dormancy in a range of species [11], a subset of fruit weight quantitative trait loci map to the same genomic region in tomato (*Solanum lycopersicum*) and pepper (*Capsicum annum*) [12], and the glutinous grain improvement traits of rice (*Oryza sativa*), sorghum (*Sorghum bicolor*), barley (*Hordeum vulgare*), and millet (*Pennisetum glaucum*) are all defined by different mutations in orthologs of the *Waxy* gene [2]. By contrast, although initial views on the appearance of the classical traits of the domestication syndromes, such as the loss of seed shattering in cereals, were considered to be cases of parallel evolution [13], genetic mapping studies have recently revealed that multiple traits are often associated with non-homologous genes [14]. For example, the canonical domestication gene, *TEOSINTE BRANCHED 1 (tb1)* of maize (*Zea mays*) [15] has minor effects on branching in foxtail millet [16], and even within separate barley lineages different

Highlights

Next-generation sequencing has dramatically boosted our ability to study selection during domestication and crop improvement.

Domestication of our crop species is characterized by a reduction in allelic diversity and massive changes in both their gene expression and visible phenotype.

An increasing number of studies suggest that metabolism is also considerably affected.

However, in contrast to gene expression changes which are largely conserved, changes at the level of the metabolome appear to be species-specific.

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genes are causal for shattering [17]. These and many other examples prove that both common and distinct mechanisms underlie the phenotypic convergence of traits in the domestication syndrome. An excellent review on the role of the domestication of vegetatively propagated crops is provided by Denham *et al.* [18]. The majority of crop domestication genes reported to date are involved in diverse developmental processes that largely produced changes in morphological phenotypes [10,19,20]. Prominent amongst these genes are transcription factors, and many studies have shown that domestication has led to major transcriptional reprogramming (Table 1).

Many studies have also shown how the contents of a handful of metabolites have been altered during the initial unintended and the protracted artificial selection phases during domestication. Indeed, a seemingly somewhat overlooked study on the domestication traits of 203 global food crops highlighted that changes in secondary metabolites are highly common domestication traits [21]. The emergence of a sweet, fleshy fruit, for example, can be considered to be an evolutionary adaptation to promote seed dispersal by animals; deliberate human selection subsequently transformed many fruit crops into obligate domesticates [22]. Most of the fruits traits shaped initially by zoochory

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Table 1. Genes Underlying Domestication That Are Directly Related to Metabolic Changes

Crop species	Gene	Function	Phenotypes	Refs
Apple	<i>Ma</i>	Transporter	Fruit acidity	[120,121]
Cucumber	<i>CmBr</i> , <i>CmBt</i>	Cytochrome P450s (CYPs)	Triterpenes/bitter taste	[79]
Melon	<i>CmPH</i>	Transporter	Acidity, sweetness	[80]
Watermelon	<i>QBRX2-1</i> , <i>CITST2</i> , <i>LCYB</i> , <i>CIAGA2</i>	Transporters, lycopene α -cyclase, α -galactosidase	Sweetness, sugars, carotenoids	[82,101,122]
Citrus sinensis	<i>Ruby</i> (MYB)	Transcription factor	Anthocyanin	[117]
Almond (<i>Prunus dulcis</i> Miller)	<i>bHLH1</i> to <i>bHLH5</i>	Transcription factor	Bitter and toxic cyanogenic diglucoside amygdalin	[123]
Grape	<i>VvmybA1</i> (MYBA1)	Transcription factor	Anthocyanin biosynthesis/color	[124]
Grape	<i>VvMYBA2</i>	Transcription factor	Anthocyanin biosynthesis/color	[125]
Potato	<i>GLYCOALKALOID METABOLISM 4</i> (GAME4)	Cytochrome P450	Steroidal alkaloids	[106]
Tomato	<i>Solyc10 g085230</i>	UDP-glycosyltransferase	Steroidal alkaloids	[54]
Tomato	<i>Lin5</i>		Sugars	[126]
Tomato	<i>E8</i> (Solyc09g089580)	1-Aminocyclopropane-1-carboxylate oxidase	Volatiles	[126]
Tomato	<i>TomloxC</i>	Lipoxygenases	Lipids and volatiles	[39]
Tomato	<i>LIP1</i>	Lipase	Lipids and volatiles	[127]
<i>Ziziphus jujuba</i> Mill.	Currently still not resolved		Fruit sweetness	[67]
<i>Glycine max</i>	<i>R2R3 MYB</i>	Transcription factor	Anthocyanin (seed color)	[128]
Rice	<i>Black hull 4</i> (<i>Bh4</i>)	Transporter	Color	[129]
Maize	<i>Teosinte branched 1</i> (<i>tb1</i>)	Transcription factor	Regulating phytohormones such as gibberellins, abscisic acid, and jasmonic acid	[76]
<i>Chenopodium quinoa</i>	<i>TSARL1</i> (<i>bHLH</i>)	Transcription factor	Saponins	[130]
Rice, potato	<i>Dihydroflavonol-4-reductase</i> (DFR)	Dihydroflavonol reductase	Anthocyanin and proanthocyanidin	[131,132]
Tomato, maize	<i>MYB12</i>	Transcription factor	Flavonoids	[54,133]
Barley, rice	<i>sd1</i>	Enzyme (oxidation)	Hormone biosynthesis	[133,134]

(the dispersal of plant spores or seeds by animals), and later by human cultivation, involved color and flavor-related compounds, with a trend towards an increased sugar content and a reduction in bitterness and acidity [23]. Over the past few years such studies have, however, expanded to the metabolome level, considerably broadening the type of questions that can be addressed. These studies initially characterized broad patterns of change during domestication, for example revealing that wheat domestication was first characterized by a reduction in unsaturated fatty acids during the primary domestication event, and altered amino acid content then accompanied the secondary domestication event [24]. As we describe in the following text, in recent years genomics and transcriptomics have been used to study the effect of domestication on a wide range of species, including the major cereals and a range of other crops. We discuss aspects of these studies that seem most likely to impact on metabolism. We focus mainly on the application of metabolomics to unravel the effect of domestication on the metabolome in multiple important crop species, distinguishing, where possible, metabolic changes that are suggestive of being direct targets of selection from those where metabolic variation between wild and domesticated forms appears instead to be derived from processes other than selection (e.g., hitch-hiking, pleiotropy, or drift). In doing so, we use published data to highlight recent advances and insights into how crop metabolomes have been wittingly or unwittingly shaped by the processes of domestication and crop improvement. We suggest that metabolic change is a major feature of both processes and we discuss evidence supporting this conclusion derived from genomics and transcriptomics. Finally, we provide a roadmap to (i) gain a better understanding of the metabolic consequences of domestication and breeding, (ii) better characterize existing plant genetic resources, and (iii) identify new targets for metabolite engineering and gene editing approaches for the purpose of *de novo* domestication of crop wild relatives.

Genome-Level Assessment of the Effects of Domestication

Essentially, the investigation of the genetic basis of phenotypic changes related to domestication has followed two main routes. In the first, classical quantitative genetic studies have been applied to the progeny of a controlled cross between a wild and a domesticated parent to identify associations between genetic polymorphisms and the phenotypic trait under investigation. This approach led to identification of genes controlling the large morphological and physiological changes between wild and domesticated forms: for example, genes related to the increase of fruit size [25] and seed mass [26] in tomato, or to the degree of shoot branching [27] as well as the regulation of flowering time [28] in maize.

Quantitative trait locus (QTL) mapping, however, has limitations. The power of any study is limited by the genotyping depth (although this is of lesser concern today, with the development of next-generation sequencing) and by the extent of the genetic variation captured in the population of wild and the domesticated individuals that may not be entirely representative of the domestication history of the species. Given that the QTL approach is also often restricted to the detection of polymorphisms associated with large phenotypic effects, these studies may give the impression that domestication was a relatively simple genetic process, controlled by few major loci. To overcome the limitations of QTL mapping, a second approach, that of linkage disequilibrium (LD) mapping, was introduced. Essentially, instead of studying the progeny of a controlled cross, LD mapping takes advantage of historical recombination which uses large panels of accessions, thus increasing the mapping resolution by checking for associations between phenotypes and genetic polymorphisms at the population level. Genome-wide associations of large sets of accessions have generally uncovered a more complex architecture of domestication, with the identification of numerous large and small effect loci underlying the differences between domesticated forms and their wild progenitors [29].

The second class of approaches for studying the genetic basis of domestication involve population-level analysis of genetic diversity. This approach is not biased towards a specific

phenotype; instead, the objective is to detect signatures of selection from DNA polymorphism data in the form of selective sweeps or population subdivision [30,31]. Genomic scans for selection in populations of wild and domesticated accessions have revealed a large number of putative regions under selection, thus uncovering many putative genes, not only those related to morphological changes, which could have been targeted by domestication [32]. However, it is important to note that, given the correct choice of parents in the development of the biparental crosses that underlie QTL mapping populations, insight into domestication could obviously be achieved. As such, the approaches should probably be regarded as complementary.

Although the studies mentioned so far have provided a large catalog of mutations – mostly SNPs – that are causal for traits affected by domestication and diversification, the impact of larger structural variants as well as genomic rearrangements and their relationships to the evolutionary processes occurring during domestication has received considerably less attention. The majority of causal polymorphisms identified so far, for example, have been assigned to SNPs, but more recent studies have started to uncover the contribution of larger genetic polymorphisms (e.g., copy-number variations and differential insertions of transposable elements, TEs) that affect domestication traits [19,33,34].

TEs, in particular, are ubiquitous genetic elements which possess the ability to integrate into different locations in the genome. TEs represent the largest majority of structural variants (SVs) and are present in nearly all genomes sequenced to date. In the case of *Arabidopsis thaliana* (arabidopsis), as well as other plants with small genome size, TEs may constitute up to 30% of the genome, but can reach proportions >80% in case of large genomes such as those of hexaploid wheat and maize [35]. Considering their large abundance, widespread distribution, and especially the mutagenic and regulatory roles TEs might have in plant genomes, it was long questioned whether these mobile elements could have contributed to the emergence of adaptive traits during plant evolution [36]. TE mobilization, in fact, in addition to mutations generated by the transposition itself (e.g., upon integration of the mobile element into a new genomic location), may also determine a wide range of effects on the function, processing, and expression of the genes in the vicinity of the integration site [37]. The largest majority of TE integration events were probably deleterious, such that these variants were promptly eliminated by natural selection. Although it is thus difficult to estimate the consequences of these TE-induced loss-of-function mutations over long evolutionary timescales, the process of plant domestication, which started at around 12 000 years ago [38], could offer instead the possibility to see the recent consequences of TE activity on the evolution of plant metabolic phenotypes under the influence of human selection. Indeed, some novel metabolic traits, apparently selected during domestication, have been documented to originate from TE insertions and subsequent rearrangements (Box 1), as well as larger insertions and deletions (e.g., [39]).

In addition, several typical phenotypes of domesticated plants, such as for example the gigantism of leaf and other organs, are reminiscent of the effects of polyploidy on organ size [40,41]. A recent study dated the occurrence and the frequency of polyploidization events in a large group of wild and domesticated plants, analyzing the data in a probabilistic framework across a large phylogenetic scale. In contrast to wild plants, polyploidization is a frequent feature in the genomes of domesticates, and its occurrence pre-dated, in most cases, the onset of domestication [42]. The reason for this higher preponderance of polyploids among domesticated forms is probably related to the higher adaptive value conferred by the presence of multiple genomes. Polyploidization increases allelic diversity, fueling evolutionary novelties upon which natural (and artificial) selection can act, and provides genetic buffering during the reduction of genetic diversity caused by domestication bottlenecks [42]. Thus, for most plant species analyzed, it seems probable that incipient domesticates were already polyploids [5].

Box 1. Examples of TE-Induced Metabolic Phenotypes of Domestication

Arguably the best known example of a domestication phenotype induced by TE transposition into a metabolic gene is that in the gene encoding granule bound starch synthase (*GBSS1*) in foxtail millet (*Setaria italica*). Wild accessions of this species have a non-waxy endosperm, containing little or no amylose. The waxy endosperm in domesticated *S. italica* emerged from multiple integrations of various TEs into *GBSS1*, which is responsible for the synthesis of amylose. Although all wild accessions were non-waxy, and expressed a fully functional *GBSS1* gene, all Asian landraces instead carried alleles in which retrotransposons (RTs) and other class II TEs were inserted, causing loss-of-function mutations and resulting in the endosperm waxy phenotype [112,113]. Other cereals, such as barley and rice, also show convergence for the waxy phenotype upon domestication; in these cases, however, different mutations – not involving insertion of TEs – led to the loss of function of *GBSS1* and to the appearance of the waxy phenotype [114,115].

In *Vitis* spp., white grape varieties were found to be homozygous for the insertion of *Gret1* [a long terminal repeat (LTR) RT of the Ty3/gypsy family] immediately upstream of the coding sequence of *MybA1*, a transcription factor involved in the positive regulation of anthocyanin biosynthesis. The insertion of *Gret1* abolished or strongly reduced the expression of the *MybA1* gene; today, almost all white grape varieties are homozygous for this insertion. Because the two LTRs of *Gret1* have an almost identical DNA sequence, the insertion of this RT probably occurred recently in an ancestral black grape and was then brought to a homozygous state through crossing [116].

Plants of the *Citrus* genus (which includes sweet orange, mandarin, citron, and pummelo) offer another example of the impact of TEs in the diversification of a metabolic phenotype. Within the genus, the accumulation of anthocyanins in fruits, flowers, and leaves varies significantly among the various species and hybrids: the presence of these pigments has been linked to deletions, RT insertions, and nonfunctional alleles of the *Ruby* locus. The 'blood orange' phenotype, for example, was due to the insertion of a LTR-RT into the promoter of *Ruby*, causing its transcriptional activation [117]. Similarly, inactivating insertions of LTR-RTs have also been detected in *Noemi* [118,119]. Thus, during the domestication and subsequent diversification of *Citrus*, various selective pressures, both conscious and unconscious, led to the fixation of various combinations of functional and nonfunctional alleles of *Ruby* and *Noemi*. This allelic diversity probably explain the varying patterns of anthocyanin pigmentation observed in *Citrus* species today.

Other than polyploidization, few large-scale genomic surveys have addressed the impact of other chromosome-level mutations on domestication processes. It was long known, for example, that chromosome inversions may contribute to local adaptation, life history, and morphological traits, and thus to the spread of reproductive isolation barriers between populations [43,44]. Six large inversions detected in the genome of cucumber, *Cucumis sativus*, for example, seem to differentiate the wild (*C. sativus* var. *hardwickii*) from the cultivated forms (*C. sativus* var. *sativus*), suggesting a role for these inversions in the domestication of cucumber [45]. In general, however, assessing the impact of these chromosome rearrangements would require precise dating of when the inversion arose during species evolution. Most of the inversions characterized to date in the genomes of crops seem in fact to have been fixed well before the onset of domestication [46].

Transcriptomics-Based Assessment of the Effects of Domestication

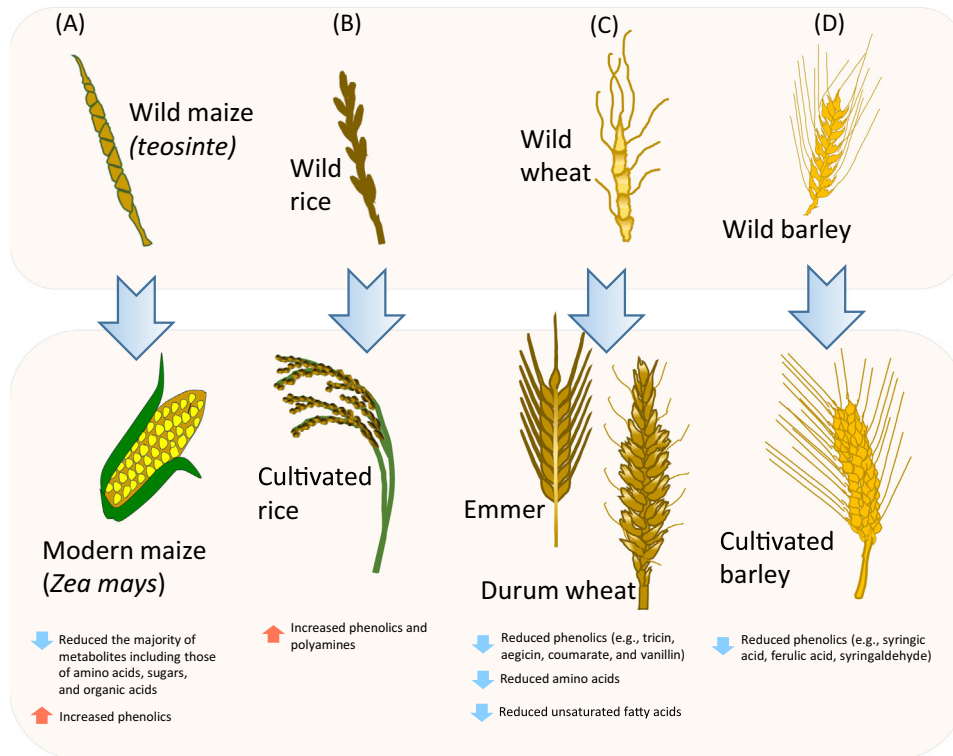
In recent years transcriptomics has been used to study the effect of domestication on a wide range of species including the major cereals rice [47,48], maize [49,50], wheat (*Triticum aestivum*) [51], and barley [52], as well as cotton (*Gossypium hirsutum*) [53], tomato [54,55], eggplant (*Solanum melongena*) [56,57], peppers [58], sunflower (*Helianthus annuus*) [59], common bean (*Phaseolus vulgaris*) [60], curcubita [61], and a range of more other crops including chickpea (*Cicer arietinum*) [62], lupin (*Lupinus albus*) [63], pistachio (*Pistacia vera*) [64], spinach (*Spinacia oleracea*) [65], lettuce (*Lactuca sativa*) [66], and jujube (*Ziziphus jujube*) [67]. A common theme that emerges from these studies is that domestication is associated with a large reduction in allelic diversity, with estimates suggesting that our current crops contain on average only 6% of the allelic diversity present in wild species gene pools [68]. Indeed, domestication results in massively decreased nucleotide diversity in common bean [60] and somewhat lower diversity in maize [50,69]. This reduced diversity is often associated with higher overall levels of expression, more uniform gene expression, and modified coexpression patterns. Because transcriptional changes that occurred during domestication and crop improvement have been recently reviewed elsewhere [70], we will not detail these studies extensively but instead focus on those changes that

are known, or could be anticipated, to affect metabolism. Given that increased overall gene expression is a relatively highly conserved domestication trait, the fact that so many of the domestication genes cloned to date are transcription factors [3,10,19] is perhaps not surprising. Although many of these genes, such as *Rht1* in wheat [71], *VRN1* and 2 in barley and wheat [72,73], *Sh1* in sorghum, rice, and maize [74], and *GW8* in rice [75], control reproductive and morphological traits, we contend that transcription factor-mediated alterations in metabolism are also prominent manifestations of the domestication and improvement processes. Table 1 lists examples of domestication genes directly related to metabolism that have been identified. The majority of these transcription factors, including those underlying pigmentation, sweetness, or bitterness, correspond to traits such as color or taste that are sensed by humans. Moreover, almost half of the genes in Table 1, including some of the genes invoking the above-mentioned traits, are the result of transcription factor-mediated changes in metabolism. Interestingly, the classical domestication gene *Tb1* is on this list because studies in maize identified that it not only affects hormone-mediated branching [76,77] but also displays highly altered energy metabolism [76]. Given these facts, alongside the likelihood that mutation of many other of the domestication genes has consequences for metabolism, these combined data suggest that metabolic consequences of domestication and improvement are considerably broader than the morphological traits classically associated with the domestication syndrome.

Metabolomics-Based Assessment of the Effects of Domestication

Variation in crop species has been studied for decades for specific metabolic traits linked to agronomic traits; for example, the Illinois maize continuous selection experiment now running for well over 100 years has followed kernel protein and oil content [78]. Other examples of tracking metabolic variation include longstanding breeding for (i) reduced bitterness in potato (*S. tuberosum*), tomato, and cucumber [79], (ii) modified acidity in sweet melon (*Cucumis melo* var. *cantalupo*) [80], apple (*Malus domestica*) [81], (iii) modified sweetness in watermelon (*Citrullus lanatus*) [82], (iv) attractive color in citrus [83], melon [77], tomato [54], maize, rice, barley, soybean (*Glycine max*), grape (*Vitis vinifera*), apple, and common bean [84], (v) starchiness in rice [47,85], and (vi) aroma in a wide range of crops including tomato, pepper, cucumber, Brassicaceae, and onion (*Allium cepa*) [86]. Although the previously-mentioned studies were invaluable in identifying the genomic loci or even the genes underlying metabolic changes occurring on domestication, the combination of next-generation sequencing and metabolomics has greatly accelerated advances in our understanding of the metabolic changes accompanying domestication.

Early studies focusing on the levels of phenolics across an eggplant panel set up to establish the effects of domestication revealed that agronomic features aside from nutrition were prominently selected for [87], an observation that now appears to be a feature that is conserved throughout crop breeding [88]. Many of the early studies that adopted metabolomics to evaluate the domestication and improvement processes focused on cereals [24,89–93]. Indeed, the first such study was that of Beleggia *et al.* [24] who performed a relatively simple, but ground-breaking, evaluation of metabolic changes occurring during wheat domestication. They investigated the levels of 51 primary metabolites in the kernels of three *Triticum turgidum* L. subspecies (wild emmer, emmer, and durum wheat), finding that primary domestication was associated with a reduction in unsaturated fatty acids whereas a decrease in amino acid levels characterized secondary domestication (Figure 1). Importantly, these effects were partially independent of the associations of any of these metabolites with other domestication-related kernel traits. Moreover, the changes in metabolite content were coupled to alterations in metabolite correlation networks, suggesting that deep metabolic restructuring may have taken place during domestication and that this often resulted in a loss of nutritional quality [94]. In particular, the massive metabolic changes occurring specifically during the diversification phase of crops have been mostly driven by the strong



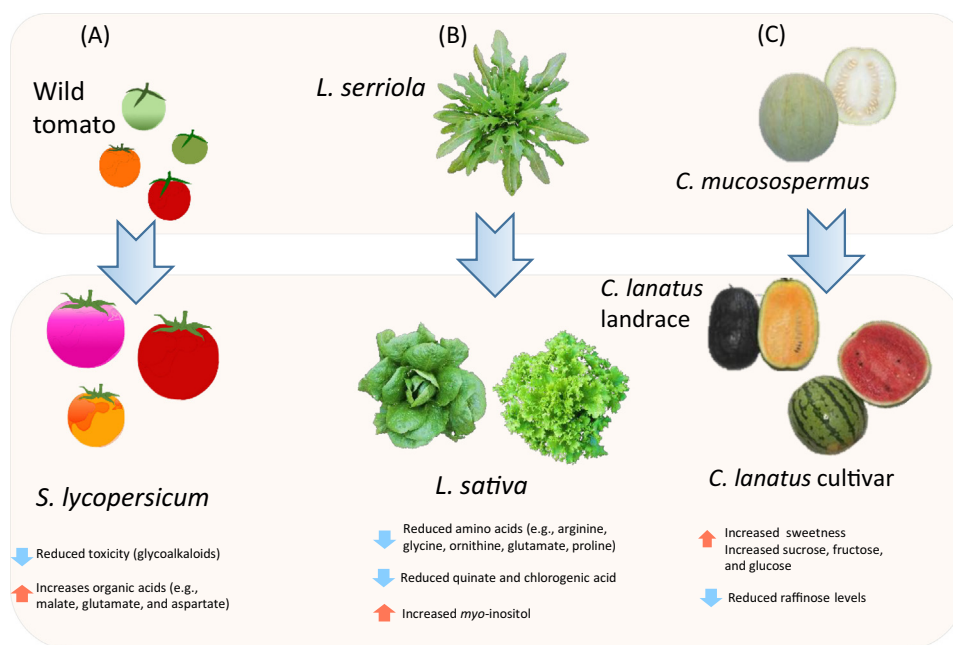
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Figure 1. Metabolic Changes Concurrent with the Domestication and Improvement of Major Cereals. The top part of the lower panel shows morphological changes in the crops (A) maize, (B) rice, (C) wheat, and (D) barley. The lower part of the lower panel shows the metabolic changes associated with primary and secondary domestication in wheat alongside domestication in maize, rice, and barley. Data from [24,49,90,92].

emphasis that breeders have put on yield-related traits, with consequent erosion of the genetic diversity that is important for quality traits (color, flavor, taste, and nutritional potential [95,96]). In another study, the metabolic divergence between maize and its wild ancestor teosinte was analyzed [91]. This study revealed that evolutionary transitions in the levels of alkaloids, terpenoids, and lipids were targeted at the divergence between teosinte and tropical maize, whereas benzoxazinoids were targeted at the divergence between tropical and temperate maize. This study and another like it additionally examined either maize–teosinte cross populations or maize and teosinte populations separately, and identified candidate genes underlying differences in metabolite composition [90,91] or sulfur-rich storage proteins [97], respectively. The changes associated with domestication in rice, as compared to maize, were recently addressed in a metabolomic study [89], revealing that these species displayed different metabolomic shifts during their evolution, and moreover suggests that those shifts were different from those reported in wheat. Two recent studies assessed changes in the metabolome during the domestication of barley – again showing diversity in their domestication-driven changes. In the first of these studies, the levels of secondary metabolites, particularly those known to confer UV-tolerance, were found to be considerably higher in quinke, a hull-less naked grained barley grown on the Tibetan plains [93]. The second study revealed differences in the allelopathic alkaloids gramine and hordenine in cultivars that likely had different domestication histories [92].

Although many studies have been carried out in cereals, a wide range of non-grass studies have been performed, providing insight into the metabolic changes that occurred during domestication

of a range of other species including tomato [54,98], lettuce [99], tea [100], watermelon [101], soybean [102], strawberry (*Fragaria × ananassa*) [103], and citrus [104]. The evaluation of the tomato metabolome was carried out at a previously unprecedented level in a large-scale multi-omic study including 610 tomato accessions, extending work on a previous population assembled to gain insight into the processes of tomato domestication and improvement [105]. This study demonstrated that selection of alleles of genes associated with larger fruits altered the metabolome as a consequence of linkage drag, whereas selection at five major loci reduced the accumulation of the anti-nutritional steroidal glycoalkaloids (SGAs) in ripe fruits. This study concurrently identified a second gene cluster for SGAs in addition to that previously reported by Itkin *et al.* [106]. Moreover, the study by Zhu *et al.* revealed that breeding for pink fruits, favored by Asian consumers, also modified the content of >100 metabolites, whereas the introgression of resistance genes from wild relatives also caused unexpected disturbance to the metabolome (Figure 2). Study of the primary metabolite complement of the lettuce population described previously revealed that metabolites in the wild species *L. serriola* differed from those of all evaluated cultivated lettuces, supporting a single domestication event for this species [99]. Moreover, galactinol, raffinose, malate, quinate, and threonate were affected by the domestication and cultivar differentiation processes, with the first two metabolites likely being selected during stem lettuce cultivation as an adaptation to the environment in China. Furthermore, quinate and chlorogenic acid levels were strongly reduced upon domestication of lettuce, probably as a consequence of the desire to reduce bitterness. Similarly, melon domestication was characterized by negative selection of cucurbitacins and flavonoids but also by elevated levels of sugars, carotenoids, and citrate:malate ratios [107], soybean domestication by negative selection of isoflavones [102], strawberry by shifts in ripening regulated metabolites [103], and citrus by negative selection on terpenoids [104]. By contrast, a pedigree



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Figure 2. Metabolic Changes Concurrent with Domestication and Improvement of Major Fruit and Vegetables. The top part of the lower panel shows morphological changes in the crops (A) tomato, (B) lettuce, and (C) watermelon. The lower part of the lower panel shows the metabolic changes associated with increase in size, selection for color, and introgression of resistance genes in tomato, domestication in lettuce, and the stepwise domestication of watermelon. Data from [54,77,82,84,90].

and metabolite analysis in tea, which has a more complex domestication history, revealed virtually no long-term artificial directional selection for flavor-related metabolites [100].

Conclusion and Future Prospects

Although by no means comprehensive in terms of the crop species studied, the massive qualitative and quantitative changes that have been documented to occur during domestication are consistent. Intriguingly, unlike the changes that occur in the genome and transcriptome during domestication, there is little commonality within the changes of the metabolomes across species. Importantly, the study on changes in the tomato metabolome, which remains the largest in scale to date, suggests that many more changes occurred during the crop improvement process than during domestication *per se*. This study further indicated that many of the changes in metabolism were unintended, suggesting that metabolomic studies will be highly instructive in assessing the nutritional composition of our crops and their progenitors. The large-scale metabolic reprogramming underlying both domestication and improvement must also be taken into account when considering both the use of *de novo* domestication of wild crop relatives and approaches concerned with engineering metabolically valuable crops [3]. In this regard, the Genotype–Phenotype Database (www.gephebase.org) aims to collect data from eukaryotic organisms regarding the 'quantitative trait nucleotides' (QTNs) that are directly related to evolutionary changes. The database could thus provide an inventory of suitable genes, for which a clear genotype–phenotype link has been already established, to be engineered or introgressed into crops for the transfer of useful traits. Analysis of the Gephebase database also confirms that the largest majority of the mutations selected during the breeding process, both in animal and plants, are mostly loss-of-function mutations (nonsense, frameshift, or regulatory mutations abolishing gene expression). As such, the global view of evolution under crop diversification is consistent with fixation of large-effect loss-of-function mutations that generate evolutionary novelties in the human-controlled setting, but are then maladaptive under natural or highly variable environments [108]. This point aside, the database additionally contains several other metabolism-associated domestication genes of interest, including the *BADH2* gene in rice, inactivation of which has been demonstrated to enrich aromatic properties [109]. Taken together, from the multiple examples described previously it is safe to say that domestication and subsequent crop improvement have been characterized not only by substantial changes in transcription and visible phenotype but also by considerable changes in metabolism. In terms of utilizing characterized domestication genes for *de novo* domestications of new species (e.g., [110,111]), it is important to note that, aside from *tb1*, the metabolic consequences of key developmental genes associated with domestication have rarely been studied. Such research is a clear priority for better integration of metabolic data to understand and influence yield and quality traits of current and novel crops. With respect to understanding metabolic changes on domestication *per se* as we list in the Outstanding Questions, a crucial point will be to include more landraces in future studies to allow us to determine whether changes occurred during the domestication event or instead in the subsequent process of crop improvement.

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Declaration of Interests

The authors declare no conflicts of interest.

Outstanding Questions

Although the metabolic changes on domestication and improvement processes combined are divergent in diverse species, it will be interesting to dissect this at higher resolution by the inclusion of more landraces to see if the domestication event *per se* triggered similar changes in the metabolome.

Similarly, higher-level resolution of the type performed in tomato to establish the metabolomic consequences of wild species introgressions in a wider range of species will likely be highly informative.

Finally, more extensive studies concerning the role of adaptive changes when a crop is introduced into a novel habitat will be highly useful in understanding the historical expansion of particular crops as well as for planning future agricultural strategies.

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