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Update

Pathways to de novo domestication of crop wild relatives

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The domestication of wild species led to a wide variety of crops adapted to a range of climatic and edaphic conditions, which allowed expansion of cultivation to larger areas and over longer periods. Subsequent crop breeding led to higher yields and facilitated population growth (Evans, 1998). Until recent times, both domestication and breeding occurred empirically with little understanding of the underlying biological mechanisms. Today, we know that genetic variation is created by mutation and that breeding operates by stacking favorable mutations in a single plant through recombination. Technical advances will eventually allow controlled manipulation of mutation and recombination (Taagen et al., 2020; Nasti and Voytas, 2021), and thus *de novo* domestication of wild species by targeted modification of their genomes (Gasparini et al., 2021). Such tools will also aid in accelerating the improvement of traditional, semi-domesticated "orphan" crops that perform poorly in modern agricultural systems (Tadele, 2019). The combination of these approaches will help broaden the narrow genetic basis of crops on which humankind currently relies (Milla and Osborne, 2021). Furthermore, recent breakthroughs have shown that targeted control of gene expression is an even faster avenue to produce desirable phenotypes (Pan et al., 2021a), thus bypassing the need for mutation and recombination. However, the deliberate effort to create new crops or improve existing ones requires a thorough understanding of the genetic basis of domestication (Kantar et al., 2017). The synergistic combination of classical archeaobotany and genetics (Denham et al., 2020) with high-throughput genomics (Purugganan and Jackson, 2021) is revealing that a variety of different processes may have operated in the domestication of crops. This knowledge, combined with increasingly powerful gene-editing toolkits, sets the stage for the continual domestication of crop wild relatives and other lesser-known plant species by defining an ideal plant type ("ideotype") that is more resilient, nutritious, and productive in a given environment (Zsögön et al., 2017). Wild plants that are naturally resistant to biotic (insects and diseases)

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Advances

- Our understanding of the molecular genetic events contributing toward crop domestication has been greatly enhanced since the advent of next-generation sequencing.
- A handful of studies have demonstrated that, in using our knowledge of domestication genes, it is possible to design multi-gene strategies to enhance the agronomic potential of less cultivated species via gene editing or introgression approaches.
- More recently, the utility of manipulating gene regulator sequences to modify the expression of suites of genes in tandem has been postulated, and proof-of-concept studies in this research area have been realized.

and tolerant of abiotic (drought and heat) stresses can be selected and manipulated by introducing mutations that mimic the domestication events that led to improved yield and agronomic performance of the major crops of today. Proof-of-concept for the potential of this *de novo* domestication approach was provided using gene editing to create agronomically important traits in wild relatives of the tomato (*Solanum lycopersicum*) (Li et al., 2018; Zsögön et al., 2018), the orphan crop *Physalis* (Lemmon et al., 2018) and, more recently, in a polyploid wild relative of rice (*Oryza* species) (Yu et al., 2021). Here, we review how recent progress in the understanding of crop domestication and technical breakthroughs in gene-editing technology could be combined to produce better crops for the future.

The evolving understanding of crop domestication

Understanding the dynamics of crop domestication is essential to discover the genomic signature of the phenotypic changes operated in the wild ancestral species. Until recently, it was widely believed that domestication was long and protracted, produced exclusively by human agency, and that convergent traits of the domestication syndrome (the suite of traits common to many crops that are not found in their respective wild ancestors, see Table 1 for examples) would have a similar genetic basis (Purugganan, 2019). Growing evidence is painting a more complex picture. First, although genomic analyses confirmed that in some cases, the domestication process took hundreds or even thousands of years, there are many examples of fairly recent and rapid domestication events: sugar beet (*Beta vulgaris*) (Dohm et al., 2014), kiwifruit (*Actinidia chinensis*) (Ferguson, 2013), and African oil palm (*Elaeis guineensis*) (Zeven, 1972). There are also notable cases of "second cycle domestication", whereby existing domesticates were quickly adapted to new uses, for instance, changing soybean (*Glycine max*) from a vegetable to a protein and oil crop (Kofsky et al., 2018) and lupines (*Lupinus* species) from green manure to a seed crop (Gulisano et al., 2019). Second, operational selection, which occurs mostly because of human operations (hence the name) may have occurred after a first round of genetic changes created by the attraction of animals and plants to human habitations and their adaptation to this human-made environment (Spengler and Mueller, 2019). Pre-domestication of plants by grazing and foraging animals would also have contributed to early selection on and seed and horticultural crops and fruit trees (Zonneveld et al., 2018). Thus, it is possible that at least some traits of the domestication syndrome represent exaptations ("preadaptations") for farming that were initially fixed by mutualism with animals (Spengler et al., 2021). Finally, studies dissecting the function of "domestication genes" point to a highly diversified genetic basis for convergent domestication traits in crops (Lai et al., 2018; Chen et al., 2021a). For instance, the nonshattering trait selected during domestication was achieved via widely divergent mechanisms in legumes and cereals: pod indehiscence in the former and rachis flexibility in the latter, which are mechanisms controlled by different developmental pathways and genes (Parker et al., 2021). Similarly, other fundamental domestication and breeding traits such as changes in growth habit, altered photoperiodic response for flowering or tuberization, reduced seed or fruit dispersal, and reduced seed dormancy are controlled by a multiplicity of genes from different families (Table 1).

The diversified genetic basis for domestication traits in plants is quite unlike the findings for animals, in which strong loss-of-function mutations in pluripotent embryonic cells of the neural crest lead to multiple traits of the domestication syndrome (Wilkins, 2020). This is not unexpected considering the marked differences in the general system of development between plants and animals (Meyerowitz, 2002). Most animals lack persistent stem cells, whereas plants retain embryonic cells in their meristems (Périlleux et al., 2019). Animals, therefore, have a closed developmental program and respond to environmental cues mainly through movement. Plants, on the other hand, are sessile, and their open and modular developmental pattern is highly flexible (Zsögön and Peres, 2018). The flexibility of the genetic circuitry controlling phenotypic outcomes is a consequence of the enormous environmental variability to which plants are subjected over the course of their lifetime. Thus, the plethora of genes underlying plant domestication traits, compared to the relatively few in animals, may reflect the higher developmental plasticity of plants. Plasticity itself is a quantitative trait strongly shaped by environmental conditions (Stotz et al., 2021), and the initial degree of plasticity could have determined which early domesticants were advanced and which were abandoned (Vilela and González-Paleo, 2015; Piperno et al., 2019). The potential for domestication of a species may thus be directly related to its genomic (Dubcovsky and Dvorak, 2007), physiological (Matesanz and Milla, 2018), and developmental (Chen et al., 2021b)

Table 1 The diversified genetic basis of some relevant crop domestication and breeding traits

Trait	Species	Gene	Type of variant	References
Changes in growth habit (dwarfism, determinate growth and/or side branching)	Wheat (*Triticum* spp)	*Rht* genes (gibberellin insensitivity)	C	Peng et al., 1999
	Rice (*Oryza* spp)	*sd1* (gibberellin biosynthesis)	C, ID	Sasaki et al., 2002
	Common beans (*Phaseolus vulgaris*)	*TFL1* (*CETS*)	C, ID, S, T	Kwak et al., 2012
	Pea (*Pisum sativum*)	*TFL1* (*CETS*)	C, ID, R	Foucher et al., 2003
	Soybean (*Glycine max*)	*Dt1* (*CETS*) and *Dt2* (*MADS-box*)	C (*Dt1*) R (*Dt2*)	Tian et al., 2010; Ping et al., 2014
	Tomato (*Solanum lycopersicum*)	*SP*/*TFL1* (*CETS*)	C	Pnueli et al. 1998; Silva et al. 2018
	Cotton (*Gossypium* spp)	*GbAF* (*CETS*)	C	Si et al., 2018
Altered photoperiodic response	Barley (*Hordeum vulgare*)	*pseudo-response regulator PpdH1*	C	Turner et al., 2005
	Maize (*Zea mays*)	*CMF* transcription factor	R	Yang et al., 2013; Huang et al., 2018
	Sorghum (*Sorghum bicolor*)	*pseudo-response regulator 37* (*PRR37*)	C	Murphy et al., 2011
	Sunflower (*Helianthus annuus*)	*FT* (*CETS*)	C	Blackman et al., 2010
	Soybean (*Glycine max*)	*pseudo-response regulator 37* (*PRR37*)	C	Lu et al., 2020*a*; Wang et al., 2020
	Common beans (*Phaseolus vulgaris*)	*PHYTOCHROME A3* (*PHYA3*) and *CONSTANS-like 2*	C (*PHYA3*) C, ID (*COl-2*)	Weller et al., 2019; González et al., 2021
	Beet (*Beta vulgaris*)	*pseudo-response regulator BTC1*	R	Pin et al., 2012
	Tomato (*Solanum lycopersicum*)	*SP5G and SP11b* (*CETS*)	R (*SP5G*) S (*SP11b*)	Soyk et al., 2017; Song et al., 2020b
	Cassava (*Manihot esculenta*)	*FT* (*CETS*)	R	Adeyemo et al., 2019
	Potato (*Solanum tuberosum*)	*CYCLING DOF FACTOR*	ID	Kloosterman et al., 2013
	Cotton (*Gossypium* spp)	*FT* (*CETS*)	R	Prewitt et al., 2018
	Rose (*Rosa chinensis*)	*CONSTANS and CONSTANS-like 4*	R	Lu et al., 2020a
Reduced seed or fruit dispersal	Barley (*Hordeum vulgare*)	*btr1* and *btr2*	C	Pourkheirandish et al., 2015
	Foxtail millet (*Setaria italica*)	*Les1* (*MYB*)	T	Mamidi et al., 2020
	Rice (*Oryza* spp)	*qSH1* (homeodomain TF), *sh4* (*MYB3* binding domain), *SH3* (*YABBY*)	C (*qSH1*, *sh4*) D (*SH3*)	Konishi et al., 2006; Li et al., 2006; Lv et al., 2018
	Sorghum (*Sorghum bicolor*)	*Sh1* (*YABBY*)	D, S	Lin et al., 2012
	Wheat (*Triticum* spp)	*Q* (*APETALA2*)	C, R	Simons et al., 2006
	Soybean (*Glycine max*)	*Shatt1-5*; *PDH1* (dirigent-like protein)	R (*Shatt1-5*) C (*PDH1*)	Dong et al., 2014; Funatsuki et al., 2014
	Tomato (*Solanum lycopersicum*)	*JOINTLESS-2* (*MADS-box*)	C, T	Roldan et al., 2017
Reduced seed dormancy	Barley (*Hordeum vulgare*)	*qsd1* (alanine aminotransferase) *Qsd2* (*MKK3*)	C	Sato et al., 2016; Nakamura et al., 2016
	Rice (*Oryza* spp)	*seed dormancy 4* (Zn-finger transcription factor), *G* (CAAX amino-terminal protease)	C, D	Sugimoto et al., 2010; Wang et al., 2018
	Wheat (*Triticum* spp)	*Phs1* (MKK3)	C	Torada et al., 2016
	Common beans (*Phaseolus vulgaris*)	*pectin acetylesterase 8*	ID	Soltani et al., 2021
	Soybean (*Glycine max*)	*G* (CAAX amino-terminal protease)	C	Wang et al., 2018
	Cotton (*Gossypium* spp)	*GhMFT1* and *GhMFT2* (*CETS*)	R	Yu et al., 2019

Type of variant refers to the allele changes found in domesticated versus wild relatives.
C, non-synonymous coding sequence change; ID, insertion–deletion; R, regulatory variant (that alters gene expression); S, splicing variant; T, transposon insertion in coding sequence.

plasticity. Identification of the causal genes for plasticity and functional characterization of their roles in plant fitness will inform future domestication studies and provide new valuable targets for manipulation (Laitinen and Nikoloski, 2019; Liu et al. 2021b).

The molecular mechanisms underlying crop domestication and breeding

Plant breeding is the selection of optimal phenotypes, i.e. the best possible combinations of genotype and environment. The work of breeders began long before it was understood that genotypic variation is created by mutation, whereby new traits arise, and recombination, which allows the stacking of useful traits into a single plant (Evans, 1998). Since both of these biological processes happen by chance, breeders create new varieties by crossing parents with traits of interest and selecting the best individuals among the progenies. The incorporation of powerful genotyping (Bevan et al., 2017), phenotyping (Atefi et al., 2021), and growth acceleration methods (Ghosh et al., 2018) represent valuable contributions to the crop breeding enterprise. However, they still rely on a strong component of chance, as the phenotypes are not predictable and ought to be found among large numbers of variants. The first step to achieve full predictability in the generation of novel phenotypes is by controlling the basic processes that give rise to new genotypes: mutation and recombination.

A mutation is the ultimate source of genetic variation and occurs spontaneously at low frequencies. Soybean (*Glycine max*), for instance, has a genome of 1.15 Gbp and suffers a spontaneous mutation rate of one bp per $\sim 5 \times 10^{-8}$ bp per year. Assuming for simplicity that all mutations will be single-nucleotide polymorphisms (SNPs), one specific SNP of interest will occur in only one out of 100 million plants in one growing season. If the plants are grown at conventional density, this would represent a field area of 400 hectares. One of the major breakthroughs to accelerate the creation of genetic variation was the discovery of artificial mutagenesis by Hermann Müller (1890–1967). Application of physical or chemical treatments increases the mutation rate by many orders of magnitude, for instance, treating soybean seeds with ethyl methanesulfonate can generate one mutation per 74 kb of the genome (Tsuda et al., 2015). Thus, from the early 20th century on, agronomists incorporated mutagenic treatments to increase genetic variation in their breeding programs.

The first crop variety produced via induced mutagenesis was "Chlorina" tobacco (*Nicotiana tabacum*) in the 1930s (Jankowicz-Cieslak et al., 2017). Today, over 3,200 mutant varieties in 170 species are grown commercially (https://mvd.iaea.org/). However, mutagenesis is an undirected and random process, and in many cases, the identity of the variants and their developmental and physiological bases remained largely unexplored. This lack of knowledge precluded targeted manipulation and extrapolation between different crop species. Furthermore, creating or discovering mutations that produce desirable agronomic traits is only the first step. The next challenge is to combine or stack different beneficial mutations into a single plant (e.g. Chopra et al., 2020). This is achieved through crossing and selection in a segregating population, and the chances of finding the appropriate mutant combinations depend on the frequency of genetic recombination during meiosis (Choi and Henderson, 2015). Unlike the mutation rate, however, the rate of meiotic recombination is highly plastic and notoriously difficult to even determine accurately (Stumpf and McVean, 2003).

There is evidence that the domestication process itself may have favored the selection of increased recombination rates (Ross-Ibarra, 2004). In the laboratory model Arabidopsis (*Arabidopsis thaliana*), crossovers occur in higher frequency at specific regions of the genome ("hotspots") (Choi and Henderson, 2015), and they can be influenced by environmental parameters, for instance, temperature (Lloyd et al., 2018). Wide crosses to wild crop relatives have been instrumental to regain genetic variation lost during the domestication process (Hajjar and Hodgkin, 2007). However, the introduction of foreign genome segments into crops may also reduce or altogether impair recombination, leading to breeding "blind spots" in the genome, with the additional downside that potentially deleterious alleles may become fixed in these regions (Lenormand and Otto, 2000). A classic example is the introduction of root-knot nematode resistance in tomato from its wild relative *S. peruvianum* (controlled by the *Mi-1* gene) and of resistance to yellow leaf curl virus from *S. chilense* (via the *Ty-1* gene). The loci are located in close proximity on chromosome 6 in a region of suppressed recombination, so only commercial hybrid cultivars harbor both resistance traits (Lin et al., 2014).

The difficulty in combining favorable mutations in a single plant during the domestication process was partially bypassed by selecting genes with pleiotropic effects. For instance, mutations of the *Q* gene in wheat allows free threshing (separation of the grain from the straw), a key domestication trait in cereals, but also simultaneously confers increased grain weight, roundness and yield (Xie et al., 2018). The *Q* gene encodes an APETALA2-family transcription factor, and the wild and domesticated alleles diverge by a nonsynonymous SNP that leads to an amino acid substitution and a synonymous SNP in a microRNA target site that leads to higher gene expression (Simons et al., 2006). Classical QTL mapping studies contributed to narrowing down and identifying chromosome regions associated with specific domestication traits in crops (Paterson, 2002). However, both this approach for a causal association of genotype and phenotype and its most modern iteration, genome-wide association studies (GWAS), while very powerful in defining the genes underlying a wide range of agronomic and metabolic traits (Fernie and Gutierrez-Marcos, 2019; Liu and Yan, 2019; Alseekh et al., 2021) have had limited impact in uncovering pleiotropy in domestication traits: most studies unfortunately only addressed a single, or

various very similar, phenotypes at a time. The development of multi-phenotype databases such as those developed for Arabidopsis should fill this gap (Togninalli et al., 2020). High-throughput phenomics platforms will allow the creation of similar databases for other crops, with the result that the potential for gene discovery will increase proportionally.

Controlling mutation and recombination to produce predictable phenotypes

A recurrent theme of the plant biology literature in the late 20th century was the attempts of physiologists and geneticists to uncover the mechanisms underlying agronomic gains produced by breeders in crops (Fischer et al., 2014). Even with the improved technical platforms available today, dissecting the genetic and physiological basis of advantageous phenotypes is a challenging endeavor (Korte and Farlow, 2013). However, the improved understanding of plant function gathered in the last decades constitutes a valuable repertoire that can inform a new, knowledge-based breeding pipeline (Li and Yan, 2020). Traits can now be designed, modeled, engineered, and stacked using a priori knowledge by targeted manipulation of the fundamental processes of mutation and, potentially, very soon also recombination (Taagen et al., 2020). This suggests that the mechanistic understanding of the connection between genotype and phenotype will increasingly be used to inform and direct rather than learn from plant breeding (Figure 1).

Targeted DNA modifications with a level of precision that was not thought possible even a few years ago are now routinely carried out in laboratories around the world (Gao, 2021; Pan et al., 2021a). Tools that modify DNA sequence with specific and predictable changes have undergone rapid improvements over the past decade. Beginning with customized zinc-finger nucleases and closely followed by TAL-effector nucleases, the technology has quickly expanded to multiple CRISPR/Cas platforms (Čermák, 2021). Today, these Cas systems include four variants: nucleases, transposases/recombinases, base editors (BEs), and prime editors (PEs) (Anzalone et al., 2020). Both BE and PE are promising tools for genetic manipulation of crop wild relatives (Molla et al., 2021), as they can perform a variety of tasks including the disruption of specific enhancer or transcription factor motif sequences, microRNA (miRNA) target sites, and precise modification of coding sequences to alter specific amino acid residues of proteins, such as those associated with pathogen resistance. BE works by enzymatically converting DNA nucleotide bases to generate point mutations without generating a double-strand break or requiring a DNA repair template (Koblan et al., 2021). PE is the most recent addition to the gene-editing toolkit and is essentially a *Streptococcus pyogenes* Cas9 nickase (SpCas9n; a nickase is an enzyme capable of introducing a single-strand cut in the DNA molecule) protein fused to a reverse transcriptase (RT) module (Anzalone et al., 2019). An editing template from a modified guide RNA (gRNA) called "pegRNA" (prime-editing guide RNA) is reverse transcribed by the SpCas9n–RT complex and incorporated into the nicked target locus. Unlike the BE machinery, PE reagents can introduce most types of edits, including short insertions and deletions (Anzalone et al 2019). The reports demonstrating the use of this

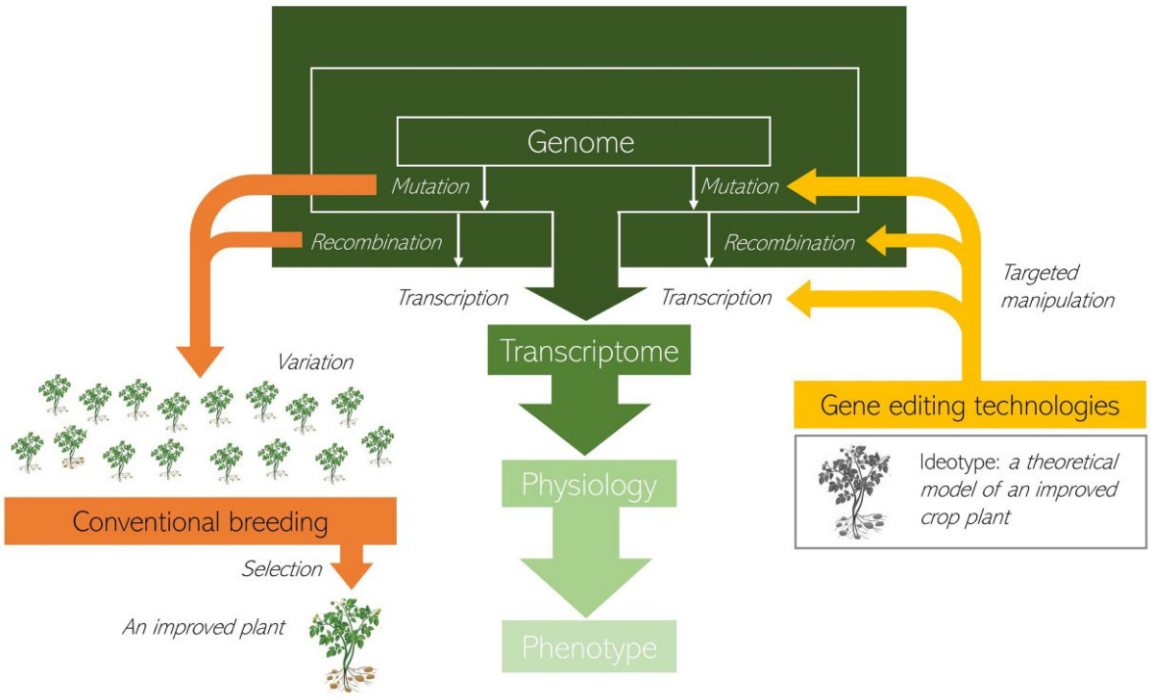

Figure 1 A pipeline that combines recently developed gene-editing technologies and conventional breeding to allow domestication and breeding of orphan crops and crop wild relatives.

platform in plants suggest that its efficacy is hitherto limited (Lin et al., 2020), and further optimization is required before it can become a routine tool. Recent encouraging results reported improved efficiency of PEs in maize (*Zea mays*) from zero to 71.7% by increasing pegRNA expression with a composite 35S:CmYLCV:U6 promoter (Jiang et al., 2020).

The main bottlenecks preventing the widespread implementation of a platform for de novo domestication of wild species is not, however, a lack of gene-editing tools but methods for efficient delivery of the gene-editing reagents into the plant cells and the need for *in vitro* plant regeneration (Atkins and Voytas, 2020). Highly efficient DNA-free systems to deliver gene-editing reagents using RNA viruses have recently been described and hold promise to avoid the need for transgenes entirely (Ellison et al., 2020; Ma et al., 2020). However, these methods still require plant regeneration from explants using protocols that are time-consuming and labor-intensive and are feasible only for a handful of species (Anjanappa and Gruissem, 2021).

Research that focuses on improved transformation methods, especially for wild and recalcitrant exotic cultivars of certain crops, is paramount for effective gene editing. Two alternative approaches are emerging to overcome this hurdle. The first is the optimization of existing plant transformation and regeneration protocols. For example, using the embryonic axis as explant tissue has seen improved cultivar-independent transformation of legumes including soybean (*Glycine max*), cowpea (*Vigna unguiculata*), and common bean (*Phaseolus vulgaris*) (Paes de Melo et al., 2020; Song et al., 2020a; Che et al., 2021). In addition, regenerated shoots can be produced rapidly in 3–6 weeks and screened for the targeted edits. These protocols are likely to become increasingly more efficient with the introduction of developmental regulator technology that has recently transformed monocot transformation (Lowe et al., 2018). The second aims at avoiding *in vitro* regeneration steps altogether by delivering gene-editing reagents to somatic cells of whole plants: *Fast-T*reated *A*grobacterium *C*o-*C*ulture (Fast-TrACC) followed by de novo meristem induction. Fast-TrACC is a protocol whereby an Agrobacterium culture harboring a plasmid with plant developmental regulators (e.g. *WUSCHEL*, *SHOOTMERISTMLESS*) that induce meristem formation and a reporter gene (e.g. luciferase, GUS) that may be included to monitor and calibrate the efficiency of reagent delivery (Nasti et al., 2021). The method was demonstrated in *Nicotiana benthamiana*, a Solanaceae species, and required only 5–15 plants to create gene-edited shoots (Maher et al., 2020). When optimized and extended to other species, this platform will allow gene-edited somatic cells to form meristems that yield seed-producing shoots, increasing throughput and shrinking timescales for creating edited plants. As these approaches are refined and other new ones developed, they will allow crop breeding, metabolic engineering and creation of useful traits in crop wild relatives and orphan crops in a highly predictable and controlled manner (Nasti and Voytas, 2021).

A neat example of the potential of gene editing for crop breeding is provided by the history of canola, the improved varieties of rapeseed (*Brassica napus*) and turnip rape (*B. rapa*) that revolutionized Canadian agriculture in the 1960s. Prior to the 1970s, the high level of toxic erucic acid and glucosinolates hampered the use of rapeseed oils and meals for human and animal consumption, respectively (Khachatourians et al., 2001). Low erucic acid varieties of rapeseed and turnip rape were identified in Germany and Poland in 1960 and were used as parental lines to introduce the trait into commercial varieties that were only released in 1968 (*B. napus* "Oro") and 1971 (*B. rapa* "Span"). Today, a thorough understanding of erucic acid biosynthesis in canola and its genetic basis (Chiron et al., 2015) allows creation of varieties with reduced erucic acid content via targeted gene editing (Okuzaki et al., 2018). Furthermore, altered fatty acid profiles in the seeds can be generated by precise mutation at a specific region of a gene in a much shorter timeframe *via* targeted gene manipulation (Huang et al., 2020). This case in point shows how a deeper understanding of metabolic pathways, in particular, and the genetic basis of agronomic traits in general can be harnessed by state-of-the-art DNA manipulation technologies to yield impressive breeding results. In contrast to the relatively simple goal of inactivating gene function using targeted mutagenesis (e.g. Zsögön et al., 2018), a more ambitious research challenge that will soon become feasible is to modify gene expression patterns in a predictable and controlled way (Pan et al., 2021a).

The next frontier: manipulation of gene expression as a tool to create predictable phenotypes

The coordination of gene expression is the foundation of cell identity and tissue function, and thus, ultimately determines the existence of differential traits between species. Engineering of *cis*-regulatory regions that control gene expression is increasingly gaining traction as an alternative to create desirable agronomic traits that closely resemble those produced by crop domestication and improvement (Rodríguez-Leal et al., 2017). Regulatory variants are the basis of many important domestication traits in crops such as maize (Chen et al., 2021a) and tomato (Alonge et al., 2020). However, two major hurdles exist for this approach. The first is that, in many cases, the causative changes reside in distant regions of functional genes, which poses a challenge to effective genome manipulation. For instance, reduced branching in the maize plant is controlled by a transposon insertion 60-kb upstream of *teosinte branched 1* (*tb1*) that enhances its expression in leaf axils and prevents outgrowth of axillary buds (Studer et al., 2011). The product of *tb1* is basic helix–loop–helix-containing transcription factor of the TEOSINTE BRANCHED1, CYCLOIDEA, PCF family. The second is the lack of sufficient knowledge about the relation between promoter sequence and function to allow deliberate manipulation with predictable phenotypic outcomes. Through variation in its expression pattern, the *fw2.2* gene controls

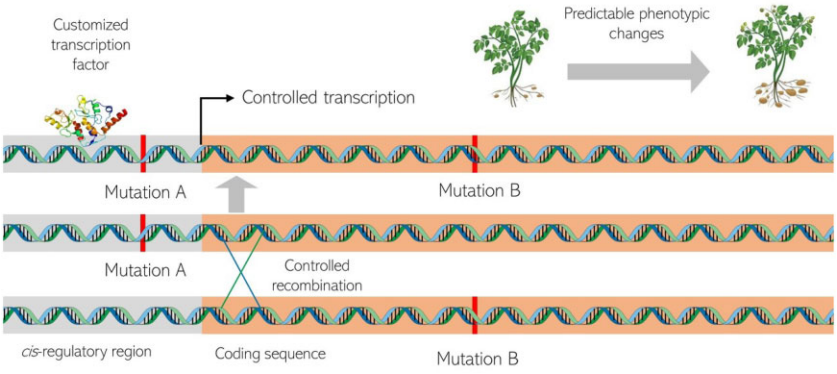

Figure 2 Schematic representation showing how manipulating mutation and recombination can create new traits predictably and reliably.

fruit size in tomato. However, even though the gene was identified more than two decades ago, the causal variant upstream of the coding sequence remains unknown, precluding its manipulation for breeding (Beauchet et al., 2021). Targeted dissection of promoter regions is starting to reveal the complex relationships between *cis*-regulatory mutations, gene expression patterns and phenotypic outcomes (Hendelman et al., 2021; Liu et al., 2021a; Wang et al., 2021). Application of machine learning on these large datasets (Vo ngoc et al., 2020), coupled with genome-wide analysis through technologies such as ATAC-seq, which allow assaying chromatin accessibility (Buenrostro et al., 2015), make it likely that *cis*-element editing will soon be better designed to yield more predictable phenotypes (Figure 2).

Many relevant crop traits such as yield and drought tolerance are under complex, polygenic control. Thus, to achieve a desirable phenotype during crop improvement it is imperative to regulate the expression of multiple genes. Pinpoint control over endogenous gene expression would be the ultimate tool to design and deploy agronomic traits in plant species. Repurposing of the CRISPR toolkit now allows manipulation of gene expression using synthetic transcriptional activators or repressors. CRISPR activation (CRISPRa) and CRISPR interference (CRISPRi) are powerful technologies that allow for the simultaneous fine-tuning expression of multiple genes in plants (Lowder et al., 2015; Zhang et al., 2019; Pan et al., 2021a). For instance, with an improved CRISPRa system, termed CRISPR-Act3.0, multiple genes in the same metabolic pathway or in different biological processes could be upregulated at the same time (Pan et al., 2021b). Another promising application of CRISPRa and CRISPRi is their use in large-scale screening of a pool of genes or the whole genome for identifying a causal relationship between gene expression and a desirable phenotype. Once the gene target is identified, CRISPR-based genome editing tools can then be applied to edit *cis*-elements of the gene to achieve the expected expression level and create a predictable phenotype. However, exactly how *cis*-regulatory sequences, epigenetic modifications, and chromatin structure mediate transcription is insufficiently understood (Cramer, 2019). This area of research will benefit from advances in synthetic biology and studies using animal and human cell models (Pandelakis et al., 2020).

Conclusion

The ability to control mutation and recombination, the two fundamental biological processes underlying crop domestication and breeding, may soon make it possible to engineer novel plant phenotypes with agronomic potential. Technical advances in gene editing promise novel tools that will allow manipulating not only gene coding sequences and their combination into a single plant but also *cis*-regulatory elements, epigenetic regulation, and quantitative genetic variation. Simultaneously editing multigene families and controlling epistatic interactions between many genes should then be feasible (Soyk et al., 2020). Systems to customize gene expression under different environments will allow specific genetic control of developmental and physiological variation. However, it is important to bear in mind that all of these advances will ultimately rely on fundamental knowledge about the relationship between genotype and phenotype in crops and their wild relatives (see "Outstanding Questions"). Thus, the expanding knowledge about the genetic basis of complex agronomic traits in crops will represent a key asset to allow improvement of orphan crops and domestication of crop wild relatives. A synergistic combination of conventional breeding and gene-editing technology (Figure 1) will contribute to food security in the face of uncertain future conditions.

Outstanding questions

- Is our molecular genetic toolbox already sufficiently diverse to allow the facile development of new crops? More specifically, do we have the tools we need for regeneration/introgression of the genetic diversity which we will require?
- Is our understanding of wild or semi-domesticated species sufficient to allow us to predict which changes would be most likely to

produce an agronomic impact?
- Can increasing knowledge about the molecular basis of transcriptional control at the *cis*-regulatory, epigenetic, and chromatin structure levels be harnessed to produce predictable phenotypes through the manipulation of gene expression?

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