

# Accelerated Domestication of New Crops: Yield is Key

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(Received 29 January 2022; Accepted 17 May 2022)

**Sustainable agriculture in the future will depend on crops that are tolerant to biotic and abiotic stresses, require minimal input of water and nutrients and can be cultivated with a minimal carbon footprint. Wild plants that fulfill these requirements abound in nature but are typically low yielding. Thus, replacing current high-yielding crops with less productive but resilient species will require the intractable trade-off of increasing land area under cultivation to produce the same yield. Cultivating more land reduces natural resources, reduces biodiversity and increases our carbon footprint. Sustainable intensification can be achieved by increasing the yield of underutilized or wild plant species that are already resilient, but achieving this goal by conventional breeding programs may be a long-term prospect. *De novo* domestication of orphan or crop wild relatives using mutagenesis is an alternative and fast approach to achieve resilient crops with high yields. With new precise molecular techniques, it should be possible to reach economically sustainable yields in a much shorter period of time than ever before in the history of agriculture.**

**Keywords:** Accelerated domestication • Breeding

• CRISPR/Cas • Mutagenesis • Orphan crops

• Sustainable intensification

## Introduction

Several strategies have been devised to meet the increasing demand for food posed by a growing population estimated to surpass 9 billion people by 2050 (Brescaglio and Guedes Coelho 2013, Crossa et al. 2021, Gao 2021). One argument is that agricultural production must increase globally to supply the food required (The Government Office for Science 2011; Tilman et al. 2011). Supply can expand either via

increasing the yield over the same area (intensification) or via bringing more land into agricultural production (extensification) (Gabriel et al. 2013). Intensification is challenging in highly productive areas, where yield increases have already plateaued (Ray et al. 2012), which leaves us with extensification, a solution that threatens nature and biodiversity.

Still, increasing supply may not be a solution at all. Increasing food supply typically generates increasing demand, creating a vicious circle (Benton and Bailey 2019). For example, providing more food for more people may result in a positive feedback loop, potentially delaying the problem rather than solving it (Rull 2010). Consequently, for future food production to be sustainable, an alternative theory is that demand must decrease, for example, through dietary changes like refraining from certain foods, particularly beef, in parallel with efforts to reduce food waste (Benton et al. 2021). However, even if projected demand is reduced, just maintaining supply is challenging. Ongoing and predicted climate change, the negative impact of agriculture on the environment and shrinking natural resources are likely to exacerbate existing yield gaps (Fisher 2015) and may even decrease yields (Godfray et al. 2010, Foley et al. 2011, FAO et al. 2018).

Sustainable intensification aims to increase agricultural productivity without negatively impacting natural resources or the integrity of associated systems (Cassman and Grassini 2020). This approach depends on high-yielding crops that require minimal input, tolerate abiotic and biotic stresses, have a low climate footprint and are nutritious. However, our current crops have been mainly selected for yield, while traits related to hardiness and stress tolerance, alongside others, either have not been selected for or have been lost (Østerberg et al. 2017). To reintroduce tolerance to unfavorable environments, one suggestion is to restore modern elite varieties with the lost properties that their landraces possessed (Palmgren et al. 2015).

Backcrossing with old cultivars or directly introducing specific genes can in some cases furnish a crop with a lost stress tolerance trait, but these approaches are challenging since most traits are multigenic (Wingen et al. 2017).

A possible alternative may be to direct breeding efforts toward plant species that are already adapted to harsh environments or contribute to naturally stable ecosystems (Østerberg et al. 2017). Potential candidates could be hardy orphan crops that have yet to be fully domesticated, for example, perennial grain crops (DeHaan et al. 2020), orphan legumes (e.g. *Lathyrus sativus*) (Lambein et al. 2019), or even wild plants, including nitrogen-fixing perennials (Table 1). Natural genetic diversity of plants is enormous, with plants found in almost every ecological niche. Thus, nature provides us with a rich source of novel crops, many of which are promising for use in sustainable agriculture. However, yields of wild plants or underutilized orphan crops are typically much lower compared to our current high-yielding crops. Therefore, to facilitate this switch, yields of these new crops must be improved to prevent dramatic reductions in food supply. Such domestication efforts should focus on increasing yield, while maintaining innate stress tolerance. While such a yield increase will not increase the current food supply, the additional resilience could secure food production and limit soil degradation under increasingly erratic climate conditions.

Crop domestication involved phenotypic transformation that rendered species dependent on humans for propagation, whereas the breeding and crop improvement efforts that followed produced high-yielding crops that are adapted to high-input agricultural practices (Hufford et al. 2012). Traits showing clear dimorphisms (e.g. brittle vs. non-brittle rachis in cereals) between domesticated and wild forms represent the domestication process, whereas traits with a continuous distribution (e.g. seed size) between domesticated and wild relatives mostly reflect post-domestication evolution directed by local farmers (Abbo et al. 2014). During domestication, yield and harvestability were simultaneously enhanced; thereafter, crop improvement has focused largely on yield improvement (Abbo et al. 2014, Haas et al. 2019).

Accelerating domestication on a timeline to meet the urgent need for sustainable intensification (Cassman and Grassini 2020) now seems possible with the advent of improved molecular screening methods and new breeding technologies. Here, we review strategies that could be targeted for the domestication of underutilized or even wild plants as well as ways to transform wild-type genes into domestication genes. The result could be a new repertoire of crops for environmentally friendly, sustainable and high-yielding agriculture.

### What Is Meant by Yield?

Yield is essentially the amount of edible biomass that can be harvested per area of land. We must consider not only the size of seeds and grains but seed number per plant and planting density, alongside crop harvestability, losses during transport and processing, nutritional value and the presence of toxic defense

compounds that reduce edibility. All these elements should be considered in strategies to increase the yield.

### Defense Compounds Also Affect Yield

Plants produce secondary metabolites for defense against herbivores and pathogens, which must be considered during breeding (Kaiser et al. 2020). Plants naturally protected this way are beneficial from a sustainability perspective but may be inedible. Toxic secondary metabolites in plants include cyanogenic glucosides, terpenes, glycoalkaloids, quinolizidine alkaloids, saponins and erucic acid (Kaiser et al. 2020). For example, alkaloids and glycoalkaloids, many of which are toxic to humans, are common to Solanaceae, including pepper, tomato, eggplant and potato. The selection against bitter tubers helped to domesticate potato by reducing tuber glycoalkaloids (Kozukue et al. 1987, Friedman et al. 2003). Meanwhile, domestication of wild mustard (*Brassica oleracea*) to domesticated cabbage resulted in a significant decrease of both constitutive and induced chemical defenses (e.g. aliphatic glucosinolates and indolic glucosinolates) in the leaves (Moreira et al. 2018). Secondary metabolites are common domestication traits (Meyer et al. 2012) originating either through natural evolution (e.g. zoochory) or in response to human appetites (e.g. human-directed cultivation toward increased sugar content, pleasant aroma or favorable colors, while reducing bitterness and acidity) (Alseekh et al. 2021).

### The Origin of Agriculture and Plant Breeding

Human agricultural activities date back to the Neolithic Period, about 10,000–15,000 years ago. Agriculture was associated with a major change in human history: a culture of hunting and gathering seeds shifted to one of farming and establishing settlements. The time and place of origin of agriculture have been extensively explored using improved bioarchaeological methods. Accumulated data from archaeobotany and plant genomic studies have clarified where this transition happened, identifying several centers of agricultural origin around the world (Lev-Yadun et al. 2000, Price and Bar-Yosef 2011, Gepts et al. 2012, Larson et al. 2014, Jones et al. 2021). However, what prompted our ancestors to change their lifestyle in this way remains debated (Cohen 1977, Jones et al. 2021, Martin-Merino 2021).

During the practice of agriculture, humans accumulated extensive knowledge about plants, allowing them to identify and benefit from important yield-related traits (Harlan 1975) and some plant species were selected based on their increased yield (Fig. 1).

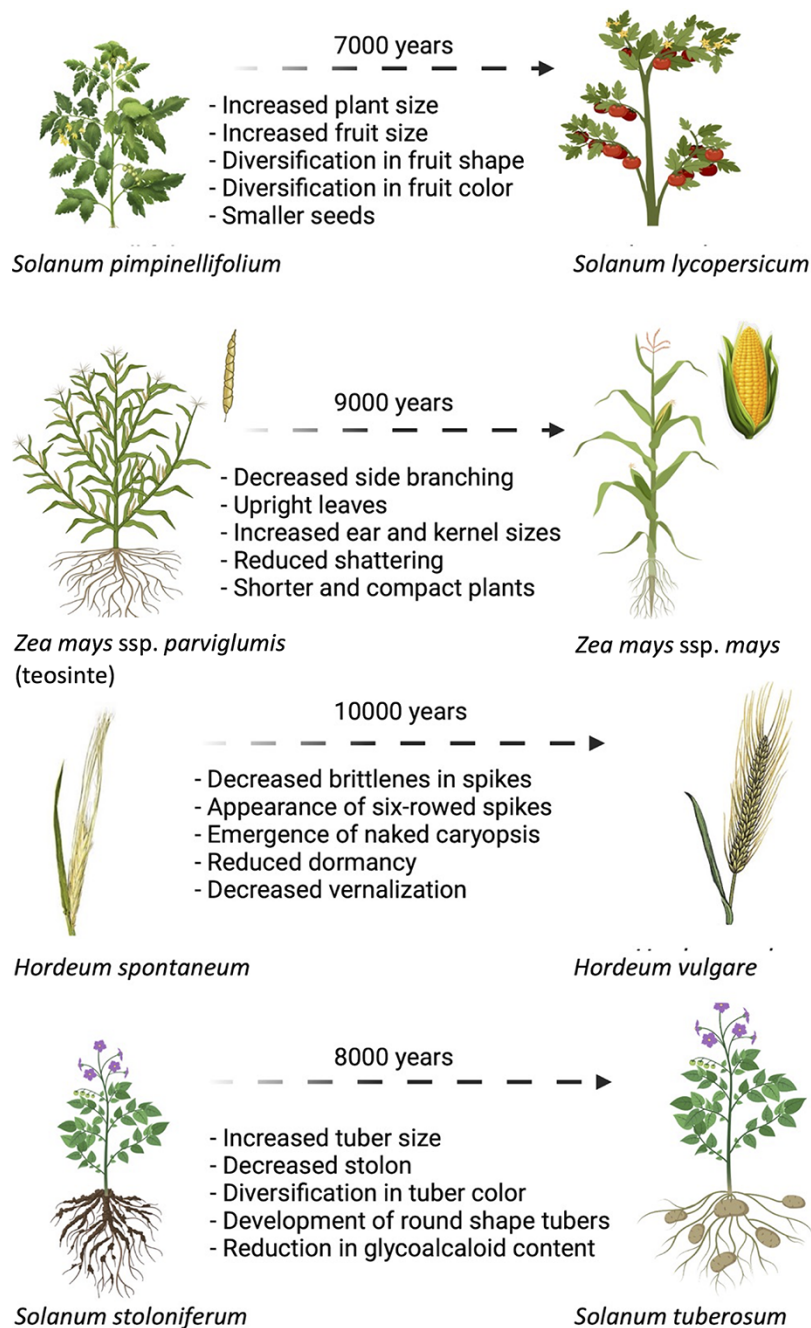
The first scientific works to unveil the principles of trait inheritance and effects of selection in plants and animals were conducted by Charles Darwin (1802–1889) (Darwin 1868) and Gregor Mendel (1822–1884), and a combination of their works founded the basis of genetics, evolution and modern plant breeding. Rediscovery of Mendel's work in the early 20th

**Table 1** Perennial orphan crops and candidates for domestication from a sustainability point of view.

Common name	Scientific name	Beneficial traits	Life cycle	Reference
<b>Cereals</b>				
European beachgrass	<i>Ammophila arenaria</i>	Tolerance to abiotic stresses	Perennial	Zhang et al. 2020
Wild barley	<i>Hordeum bulbosum</i>	Tolerance to biotic and abiotic stresses	Perennial	Westerbergh et al. 2018
Intermediate wheatgrass	<i>Thinopyrum intermedium</i>	Tolerance to abiotic stresses	Perennial	DeHaan et al. 2020
<b>Legumes</b>				
Alfalfa	<i>Medicago sativa</i>	Nitrogen fixing, nutritious, intercropping	Perennial	The Land Institute, USA
Kura clover	<i>Trifolium ambiguum</i>	Nitrogen fixing, nutritious, intercropping	Perennial	The Land Institute, USA
Sainfoin	<i>Onobrychis viciifolia</i>	Nitrogen fixing, nutritious, intercropping	Perennial	The Land Institute, USA
Lupin	<i>Lupinus</i> spp.	Nitrogen fixing, nutritious, intercropping	Perennial	The Land Institute, USA
<b>Oil seeds</b>				
Rosinweed	<i>Silphium integrifolium</i>	Oil, long root	Perennial	The Land Institute, USA
<b>Root crops</b>				
Wild potato	<i>Solanum</i> spp.	Tolerance to biotic and abiotic stresses	Perennial	Cadima et al. 2014
<b>Legume trees</b>				
Reonja	<i>Acacia leucophloea</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Gum arabic tree	<i>Acacia nilotica</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Coojong	<i>Acacia saligna</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Gum acacia	<i>Acacia senegal</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Red acacia	<i>Acacia seyal</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Umbrella thorn acacia	<i>Acacia tortilis</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Red lucky seed	<i>Adenanthera pavonina</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Monkeypod	<i>Albizia saman</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Camel's foot	<i>Bauhinia thonningii</i>	Nitrogen fixing, edible	Perennial	Toensmeier 2016
Pink shower tree	<i>Cassia grandis</i>	Livestock fodder, some are nitrogen fixing	Perennial	Toensmeier 2016
Carob	<i>Ceratonia siliqua</i>	Livestock fodder, edible	Perennial	Toensmeier 2016
Guanacaste	<i>Enterolobium cyclocarpum</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Psonay	<i>Erythrina edulis</i>	Nitrogen fixing, livestock fodder, edible food	Perennial	Toensmeier 2016
Apple-ring acacia	<i>Faidherbia albida</i>	Nitrogen fixing, livestock fodder, edible food	Perennial	Toensmeier 2016
Honey locust	<i>Gleditsia triacanthos</i>	Livestock fodder, edible food	Perennial	Toensmeier 2016
Golden rain	<i>Laburnum anagyroides</i>	Nitrogen fixing, prolific in temperate climate, toxic seeds	Perennial	Zhang et al. 2020
East African newtonia	<i>Newtonia buchananii</i>	Livestock fodder	Perennial	Toensmeier 2016
African locust bean	<i>Parkia biglobosa</i>	Nitrogen fixing, livestock fodder, edible	Perennial	Toensmeier 2016
Palo verde	<i>Parkinsonia aculeata</i>	Livestock fodder, edible	Perennial	Toensmeier 2016
Fara tree	<i>Piliostigma thonjii</i>	Nitrogen fixing, livestock fodder, edible	Perennial	Toensmeier 2016
Manila tamarind	<i>Pithecellobium dulce</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
African mesquite	<i>Prosopis africana</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
White carob tree	<i>Prosopis alba</i>	Nitrogen fixing, livestock fodder, edible food	Perennial	Toensmeier 2016
Chilean mesquite	<i>Prosopis chilensis</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Ghaf	<i>Prosopis cineraria</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Honey mesquite	<i>Prosopis glandulosa</i>	Nitrogen fixing, livestock fodder, edible	Perennial	Toensmeier 2016
Long-thorn kiawe	<i>Prosopis juliflora</i>	Nitrogen fixing, livestock fodder, edible	Perennial	Toensmeier 2016
American carob	<i>Prosopis pallida</i>	Nitrogen fixing, livestock fodder, edible	Perennial	Toensmeier 2016
Tamarugo	<i>Prosopis tamarugo</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Rain tree	<i>Samanea (=Albizia) saman</i>	Nitrogen fixing, livestock fodder	Perennial	Toensmeier 2016
Wild Cassia	<i>Senna singueana</i>	Livestock fodder	Perennial	Toensmeier 2016

century was exploited widely by plant breeders to study traits and improve varieties through controlled crosses in different crop species such as maize and self-pollinating plants (East 1910,

Roberts 1919, Brink 1921). Plant breeding has been defined as a deliberate manipulation of plants to improve the quality and quantity of yield in a permanent and heritable way



**Fig. 1** Phenotypic alterations of different plant species during domestication directed by human selection toward increasing yield. Despite common belief, most of the gained traits are the result of mutations that result in the loss of natural functions. See text for details.

(Acquaah 2007, Kingsbury 2009). Plant breeding depends on genetic variation, and the aim of plant breeding is thus to identify and exploit genetic variations (Holme et al. 2019).

Modern breeding programs consist of several general steps, beginning with objectives, sourcing of germplasm, identification of target traits and environments and trait improvement and assessment, before the ultimate release of new improved cultivars. Design of breeding strategies and objectives are dictated by multiple factors, relating to mating type, trait genetic

complexity, heritability and gene action, while the breeders' equation helps determine both the selection pressure and response (Udall 2003, Mackay et al. 2009). Central to plant breeding activities is the selection of the best individuals for the desired traits from a population. Breeding procedures and techniques have been developed over many years and range from directed crosses and simple bulk selection for a trait to marker-assisted and genomic selection. These latter techniques have been significantly aided by improved phenotyping capabilities



and the advent of low-cost genetic markers. Presently, hundreds to thousands of DNA markers can be used to genotype large populations and determine trait associations, identifying both trait genetic associations and individuals with superior breeding values (Meuwissen et al. 2001, Sleper and Poehlman 2006, Breseghello and Guedes Coelho 2013, Crossa et al. 2021).

Diversity is the raw material for selection and assures the productivity, resilience and adaptive capacity of agricultural systems (Renard and Tilman 2019, Khoury et al. 2022). In traditional agroecosystems, genetically heterogeneous orphan crops, selected by local farmers and traded among communities, are cultivated in a mosaic format (polyculture) of different varieties and species (Jarvis et al. 2008, Liebman 2018). Conversely, modern crop cultivars are bred to be genetically homogeneous and are cultivated over large geographic areas as monocultures, resulting in the gradual elimination of locally adapted landraces and their wild relatives. Furthermore, long-term farming and selection for a few desired traits in newly introduced cultivars leads to loss of diversity, termed genetic erosion (van de Wouw et al. 2010).

Over a century ago, concerns were raised about the consequences of the disappearance of traditional landraces and its effects on the future of plant breeding (Baur 1914). However, the Green Revolution of the 1960s accelerated genetic erosion by intensifying directional selection, whereupon elite high-yielding cultivars replaced well-adapted local landraces. Three-quarters of crop diversity is estimated to have been lost in the 20th century (FAO Annual Report 2004), coincidental with repeated reports of the susceptibility of modern cultivars to pests and disease outbreaks and abiotic stresses due to genetic homogeneity (Keneni et al. 2012). For example, the Irish potato famine can be attributed to the large-scale cultivation of the potato cultivar 'Aran Banner', which although very productive was susceptible to fungal disease late blight (*Phytophthora infestans*). The resultant famine had catastrophic socio-economical impacts in Ireland and the rest of Europe during the 1840s (Goodwin et al. 1994).

The term 'genetic erosion' is used to denote different degrees of gene pool shrinkage: an absolute loss of cultivars or landraces (Gao 2003, Tsegaye and Berg 2007), reduction in richness (reduction in the total number of crops, varieties or alleles) (Hammer et al. 1996, Dyer et al. 2014) and a reduction of specific rare alleles (Khlestkina et al. 2004). While overwhelming evidence indicates that crop diversity has declined across multiple scales, studies indicate a wide array of trends in particular instances, with uncertainty remaining about the significance of changes observed (Khoury et al. 2022).

### How Can Modern Crops be Refurbished with the Traits They Have Lost?

Traditionally, introducing new variation into crops has been performed through backcross breeding (Vogel 2009). Breeders have attempted to tackle the issue of genetic erosion through crossing cultivars with landraces and wild relatives harboring

desired traits to introduce novel variation (Breseghello and Guedes Coelho 2013, Sharma et al. 2021). However, this strategy has its limitations. First, it can only be used to introduce traits for which genetic variation already exists. Second, the outcome of a cross is often associated with undesirable traits obtained from the donor parent due to linkage drag, which need to be eliminated through multiple backcrosses over several cycles. Third, the strategy is largely limited to monogenic traits, whereas most agronomically important traits are polygenic and difficult to maintain after a cross to non-elite material (Kushwah et al. 2020). Backcross breeding has been utilized most successfully to introduce single genes that provide new resistance or control other qualitative traits. However, the majority of yield-related traits are polygenic, i.e. composed of multiple genes of small-effect size with accumulative effects (Cooper et al. 2009).

Another more recent approach to improve the hardiness of major crops utilizes transgenesis. This approach is independent of the ability of plants to form crosses with each other; therefore, the possibilities are, at least theoretically, greater. For example, a resistance trait found in a tolerant species can be transferred to an unrelated elite variety of a crop to confer disease resistance (Bailey-Serres et al. 2019). It has been proposed that transgenic technologies will inevitably be deployed for most major crops in the future (Tester and Langridge 2010). However, although transgenesis became possible approximately 40 years ago, there are few examples where it has been commercially viable as a technique to move a trait from one plant to another, with only a few transgenic crops successfully entering the market (Raman 2017). Single stress-responsive genes can be overexpressed to confer salt and drought tolerance, but this comes with a cost as transgenic plants may have reduced fitness in the absence of stress (Wani et al. 2017). Even simple traits are the result of many different genes that work together in a coordinated fashion, and establishing a new trait furthermore requires another layer of regulatory genes. Ultimately, the same problems exist with backcross breeding, as the gene 'package' to be transferred increases in size. For example, it is estimated that about 200 genes function together to govern the ability of legumes to utilize atmospheric nitrogen (Roy et al. 2020). Additionally, the limited success of transgenic crops is associated with public resistance against the use of genetically modified organisms (GMOs), which in many countries are considered on a case-by-case basis under strict regulatory systems (Raman 2017).

### Prime Candidates for Rapid Domestication Will Meet Urgent Agricultural and Ecological Needs

Rapid domestication may address issues such as free-threshing, seed shattering, lodging, seed size or antinutritional compounds. However, grain, or seed, yield remains a highly polygenic trait likely requiring decades of breeding to deliver improvement, as has been the case for modern grain crops.

Therefore, in the near term, we do not expect newly domesticated crops to replace the dominant crops, but *de novo* domestication of alternative crops could help solve critical human and environmental challenges within a decade.

First, domestication of new crops combined with breeding improvement of orphan crops has the potential to address nutrient deficiencies and stabilize food supply by increasing crop diversity. In Africa, orphan crops have the potential to address vitamin and mineral deficiencies and produce food even under low-input conditions, but these crops often lack full domestication (Jamnadass et al. 2020). For example, fonio millet (*Digitaria exilis*) has the potential to produce reliable yields of edible grains under harsh conditions, but shattering, lodging and small seed size pose significant agronomic challenges (Abrouk et al. 2020). Similarly, the orphan crop tef (*Eragrostis tef*) has a beneficial nutritional profile but is limited by lodging, shattering and tiny seeds (Girija et al. 2021). Salinization of land is also dramatically limiting yields in many regions, for which direct domestication of salt-tolerant species has the potential to bring these degraded lands back into production (Razzaq et al. 2021). Consequently, for this strategy to be successful, domestication and yield improvement must go hand in hand, as was the case with the introduction of wheat to Canada in the mid-1800s. Here, wheat breeding efforts trace their origin to the variety 'Red Fife', which most likely originated in Ukraine and was introduced by Scottish settlers. Compared to other varieties tested, 'Red Fife' tolerated the short season and intense continental climate of the Canadian prairies, was rust resistant and ripened before the onset of autumn frosts (Symko 1999). Similarly, if orphan crops are to be domesticated for cultivation outside of their natural range, breeding efforts should consider how to adapt plants to new climatic zones, soil types and biotic interactions, alongside yield traits.

Depending on the plants chosen, direct domestication has the potential to produce new crops with unprecedented potential to protect against soil erosion, sequester atmospheric carbon dioxide, build soil quality and protect ground and surface waters from agricultural runoff as perennial grain-producing crops are developed (Glover et al. 2010). For example, intermediate wheatgrass (*Thinopyrum intermedium*) is being produced on a small scale and marketed under the name Kernza™. While the grain yield of intermediate wheatgrass remains much lower than other annual grain crops, the ability of a perennial grain to protect soil and scavenge nitrate from deep in the soil profile makes the crop highly attractive. For example, the crop can be used as part of landscape restoration, with plantings used to protect wellheads and streams from nitrate contamination or surface runoff (Muckey 2019). Intermediate wheatgrass has already undergone some improvements in later breeding cycles, and, as breeding solves lodging and increases seed size, the crop is expected to be grown on expanded acres with widespread environmental benefits (Crain et al. 2021).

Finally, accelerated domestication could expand the role of long-lived woody species in agriculture. Agroforestry has a

unique potential to facilitate both climate change mitigation and adaptation. For example, tree crops meet human food and fiber needs while building soil quality, reducing runoff and tapping into water and nutrients beyond the reach of typical crops (Duguma et al. 2014). Unfortunately, due to their extended life cycle, most trees have experienced little attention from plant breeders (Molnar et al. 2013). Hybrid poplar has undergone genetic improvement as a biofuel crop but also has the potential as a fodder crop (Charlton et al. 2003, Davis 2008). With new approaches allowing rapid domestication of tree crops, benefits to sustainable food production could be profound.

### What We have Learned: Domestication Generally Results from Loss of Function, not from Gain of Function

Domestication genes are genes that when mutated result in a domestication phenotype. Identification of domestication genes in current crops has led to interesting findings regarding the nature of such mutations. In an analysis of 60 domestication genes, Meyer and Purugganan (2013) found that in 28 cases such mutations resulted in the loss of function (e.g. frameshifts and splicing defects that lead to premature truncations). The second largest class were cis-regulatory mutations (15 of 60 genes). In 10 domestication genes, the domestication phenotype resulted from either loss of function or cis-regulatory mutations. These results suggest that loss-of-function mutations or ones that alter gene expression are the basis for human-driven crop evolution. Additional examples are given by Gross and Olsen (2010) and Østerberg et al. (2017).

Loss-of-function variants of transcription factors regulating the biosynthesis of toxic metabolites may give rise to less toxic plants. In the Andes, domestication of quinoa (*Chenopodium quinoa*) produced many cultivars with various bitter and sweet grain phenotypes (Bazile et al. 2016). Genomic analysis of bitter and sweet cultivars suggests mutation events in the *TSARL1* gene, expressed mainly in seeds, are associated with a reduction in saponin concentration of seed pericarps (Jarvis et al. 2017). *TSARL1* encodes a basic helix-loop-helix transcription factor, and in sweet cultivars tested, the expression of several genes in the saponin biosynthesis pathway was downregulated (Jarvis et al. 2017).

Cis-regulatory mutations are in gene regulatory regions of DNA and may affect the amount of expressed protein but not its nature. Although it can be argued that cis-regulatory mutations do not lead to loss of function (Gross and Olsen 2010), they result in loss of natural control, likely important for survival in nature. Loss-of-function alleles can also be dominant, as exemplified in einkorn wheat (*Triticum boeoticum*, wild ancestor of the domesticated form of einkorn, *Triticum monococcum*), where seeds are more firmly attached to the mother plant, making them easy to harvest (Fans et al. 2005). The observed trait is due to a single-base mutation in a gene named *q*, which gives rise to the dominant *Q* allele (MacKey 1954).

From a natural selection perspective, this mutation is not actually beneficial, as it reduces the ability of plants to shatter and readily disperse their seed. However, the early farmers selected and multiplied these seeds, maximizing both threshability and the harvest index. Indeed, selection for this phenotype may be one of the first human plant breeding activities, which eventually led to the domestication of modern einkorn wheat, *T. monococcum* (Schlegel 2018). The levels of the wild-type *q* gene product are regulated by a microRNA (microRNA172 or miR172). This binds to the *q* mRNA, and the resulting double-stranded RNA is subjected to rapid degradation. The single point mutation underpinning the two different alleles, converting *q* to *Q*, occurs in the sequence that miR172 anneals to and weakens the interaction between *Q* mRNA and miR172. As a result, fewer gene transcripts are degraded and more protein is synthesized. In this way, the loss of regulation results in greater accumulation of the *Q* protein and a dominant domestication trait: loss of seed shattering.

The difference between domestication and breeding is sometimes contentious, for example, in the *GPC-1* locus (*NAM-1*) in wheat. Haudry et al. (2007) and Luo et al. (2007) argued that the *GPC-1* locus is under domestication and of monophyletic origin, whereas Feldman and Kislev (2007) and Thanh et al. (2014) argued the opposite. Meanwhile, Dubcovsky and Dvorak (2007) suggested indirect fixation of *GPC-B1* occurred due to its pleiotropic effect on increasing grain size. In the majority of hexaploid bread wheat (*Triticum aestivum*) cultivars, the *NAM-B1* homoeolog is nonfunctional, except for a group of Fennoscandian varieties (Hagenblad et al. 2012). Adoption of the functional *NAM-B1* copy under these environments is thought to be advantageous, due to accelerating senescence under short growing seasons, alongside grain protein content improvement (Asplund et al. 2013). Meanwhile, haplotype variation has also been reported for the *NAM-A1* homoeolog (Cormier et al. 2015) and is present in several Australian cultivars, where it is associated with differences in grain maturation and nitrogen use efficiency (Alhabbar et al. 2018).

A common misconception is that the high-yielding crops we grow today have gained something (i.e. an improved gene) compared to their wild progenitors. However, domestication can be seen as human-driven evolution that selects for the loss of traits or functions that are superior for plant survival and propagation in nature. Upon fixation of these domestication traits, subsequent or parallel breeding efforts have also selected loss-, or alteration-, of-function genes that confer important adaptive, agronomically important or quality traits (Haas et al. 2019). In wheat, this includes the selection of promoter deletions of *Ppd-A1a* and *Ppd-D1a*, which are associated with earlier flowering under increasing daylengths (Shaw et al. 2013). Other examples include loss-of-function mutations in the granule-bound starch synthase I (*GBSSI*) *Waxy* gene in millet, which results in glutinous grains (Hunt et al. 2010); *PsMLO1* in pea, which confers mildew resistance (Humphry et al. 2011); and *OsBadh2* in rice, which contributes to fragrance (Chen et al. 2008).

## Loss-of-Function Strategies Cannot Establish Traits that Require New Genes

Genes are the basic units of heritable traits. For example, nitrogen-fixing legumes are equipped with multiple genes essential for the symbiosis with *Rhizobium* that cereal crops do not possess. Thus, it is obvious that for a cereal crop to become nitrogen fixing it requires new genes (Mus et al. 2016). In contrast, even complex traits can disappear if just a single gene in the pathway is mutated. Breeding strategies that only require loss-of-function mutations are much more likely to succeed than those that require gain-of-function genes. However, they are unlikely to provide new beneficial traits that allow the plant to survive environmental challenges.

It thus follows that a breeding strategy for the development of new crops tolerant of adverse environmental conditions must be based on plants already adapted to the different target breeding environments or stresses (Østerberg et al. 2017). Thus, if an extremely drought-resistant crop is needed, efforts could focus on identifying and domesticating an edible desert plant, instead of introgressing novel traits into a previously domesticated species. Even when taking into consideration that resilience has a cost, growth potential and drought tolerance need not be trade-offs (Fernández and Reynolds 2000). However, the potential trade-off between stress tolerance and yield must be considered (Gambino and Vilela 2011, Koziol et al. 2012) due to the associated production of costly proteins, stomatal closure or large storage organs, which may reduce the yield (Ferreles et al. 2014).

## How to Identify Orthologs of Domestication Genes in a Wild Plant?

Genetic variants of natural gene orthologs underpin plant domestication events, resulting in novel domestication phenotypes. Thus, the strategy for accelerated domestication is to introduce mutations in such orthologous genes. Genes, which when mutated in progenitors of our major crops, result in domestication phenotypes, as listed by Østerberg et al. (2017). However, how would one identify true orthologs of domestication genes in orphan crops or wild unrelated plants?

Homology search of domestication genes through mining databases is a useful method for the identification of potential target genes but usually produces many candidates. A problem with a strategy that depends on loss-of-function mutagenesis is that even diploid plants may have multiple versions of a gene possessing the same function, termed functional redundancy. This problem is even more significant in polyploid species due to the presence of homoeologous gene copies. We suggest performing functional complementation of already domesticated crops as a screening method to identify true orthologs of domestication genes in polyploid species. Pourkheirandish et al. (2015) demonstrated that introduction of functional *Brittle rachis1* (*Btr1*) and *Btr2* from wild *Hordeum spontaneum* could revert a domestication phenotype to wild type when transferred into domesticated *Hordeum vulgare*.



An example of an interesting domestication gene is the rice semi-dwarfing gene (*sd-1*), known as the 'green revolution gene'. This allele provides rice cultivars with short, thick culms that are associated with increasing harvest index, improving lodging resistance and responsiveness to nitrogen fertilizer (Hedden 2003). The dwarfing gene originated from a Chinese cultivar, Dee-geo-woo-gene, which encodes a 383-bp deletion in the GA20ox gene (*OsGA20ox2*), leading to deficiency in gibberellin (GA) synthesis (Monna et al. 2002, Spielmeier et al. 2002). If the introduction of an *sd-1* homolog from a wild grass in a semi-dwarf rice can revert the domesticated phenotype, this gene would be a true ortholog of *sd-1*, confirming it as a domestication gene. Such a complementation strategy for the identification of true orthologs of domestication genes should take advantage of manageable diploid domesticated crops, such as rice and barley, which are readily transformable (Nishimura et al. 2007, Harwood and Smedley 2009, Sahoo et al. 2011, Marthe et al. 2015) and for which extensive genomic resources are available (Matsumoto et al. 2005, Mayer et al. 2012).

### Approaches for Achieving Genetic Variation by Mutagenesis

Two major techniques are available that create genetic variation through mutagenesis: random mutagenesis (mutation breeding) and precision mutagenesis (genome editing) (Chen et al. 2019, Gao 2021) (Fig. 2).

#### Random mutagenesis

Until the last century, spontaneous mutations were the only source of genetic diversity. However, since the discovery in the late 1920s that experimentally induced mutagenesis had the potential to accelerate plant breeding, induced mutagenesis has been widely used as a tool in practical breeding programs (Oladosu et al. 2015). Mutation breeding relies on mutagens such as chemicals or radiation that randomly induce mutations in the genome (Holme et al. 2019, Ma et al. 2021). Each affected genome may carry hundreds or thousands of mutations, mainly with unknown consequences (Holme et al. 2019). Nevertheless, mutagens can increase genetic variation, and new varieties of crops resulting from random mutagenesis are readily released (Halford 2019).

Application of random mutagenesis was adopted by the Joint Food and Agriculture Organization (FAO)/International Atomic Energy Agency (IAEA) after the 1960s (Oladosu et al. 2015). Crop varieties originating from mutagenesis are reported to the Joint FAO/IAEA program and recorded in the searchable Mutant Variety Database (MVD; available from: <http://mvd.iaea.org/>, Fig. 3). Physical and chemical mutagenesis leads to different types of mutations. Physical mutagenesis, induced by X-rays, UV and gamma radiation, mainly causes large chromosomal deletions, rearrangements and point mutations (single-base substitutions or deletions) (Shu et al. 2012). Most of the gene mutations induced by physical mutagenesis effectively generate either knockouts or nonfunctional genes,

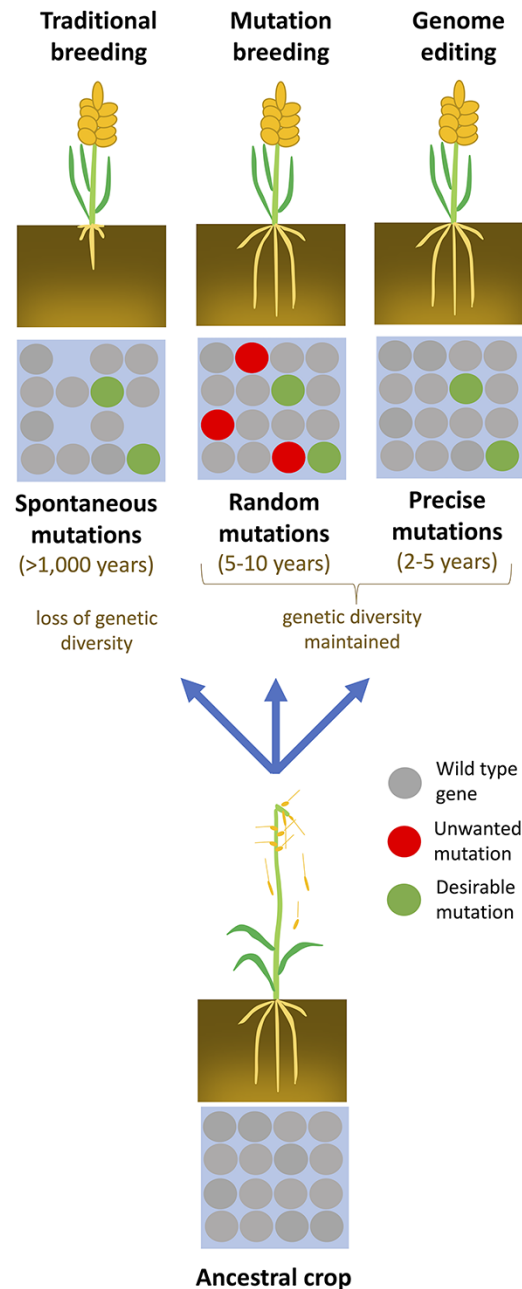
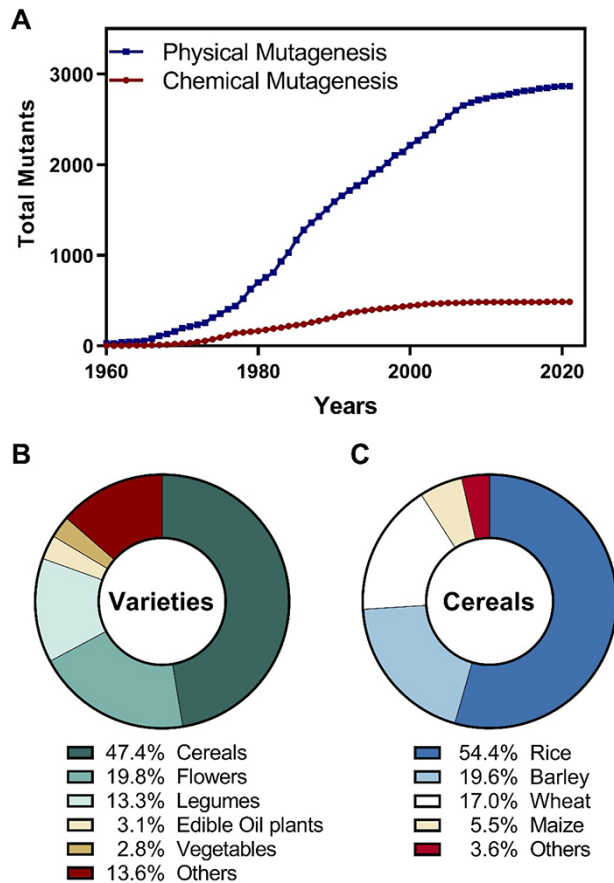


Fig. 2 Overview of different strategies for domestication of plants. Traditional breeding depends on spontaneous mutations and is associated with the loss of genetic diversity due to inbreeding. Genetic diversity can be gained by mutagenesis techniques. Random mutagenesis may result in many unwanted mutations. These can be removed by repeated rounds of backcrossing. Genome editing techniques can induce specific mutations without the loss of genetic diversity.

although the majority are recessive (90–100%) (Rakszegi et al. 2010, Mba et al. 2012).

Chemical mutagens [e.g. *N*-methyl-*N*-nitrosourea (MNU), sodium azide ( $\text{NaN}_3$ ), hydrogen fluoride, methyl methanesulfonate, ethyl methanesulfonate (EMS)] almost exclusively cause single-nucleotide polymorphisms, mostly GC-to-AT base





**Fig. 3** Mutant varieties officially registered in the Mutant Variety Database (MVD; available from: <http://mvd.iaea.org/>). (A) Evolution of the release of mutant varieties produced by physical or chemical mutagenesis, from 1960 to the present. (B) Relative proportion of mutants released in the principal groups of crops. (C) Relative proportion of cereal mutant varieties.

pair transitions. Currently, EMS and MNU are the most used mutagens due to their high efficiency and ease of application without the need for specialized equipment. Notably, they are being used to create large, highly mutagenized populations to screen for phenotypically relevant changes and specific genomic sequences (Jankowicz-Cieslak and Till 2016) and are widely used in many crops, including rice and barley (Till et al. 2007, Talamè et al. 2008, Uauy et al. 2009, Krasileva et al. 2017).

Due to promotion by the FAO/IAEA agency, some important cultivars have been developed by utilizing physical mutagenesis. Among them are the stiffed-strawed barley varieties (Diamond and Golden Promise) carrying mutant dwarfing genes (*sdw1* and *DEP1*, respectively) and the rice semi-dwarf varieties (Calrose 76, Reimei), carrying a mutation in the gene *sd1*, previously selected during the Green Revolution (Forster et al. 2001, Hedden 2003, Wendt et al. 2016). In the minor crop lentil (*Lens culinaris*), chemical mutagenesis has been used to generate tolerance to imidazolinone herbicides (Canadian Food Inspection Agency 2004, Slinkard et al. 2007). Additionally, chemically induced mutations in the barley *Mlo* gene, rendering

the gene nonfunctional and the plant less susceptible to powdery mildew (*Blumeria graminis* sp. *hordei*) infection, have been implemented in barley breeding programs (Jørgensen 1992). Varieties developed from the use of random mutagenesis have been used in breeding programs and subsequent variety release.

Notwithstanding the high number of mutant varieties registered in MVD (Fig. 3A) and some commercial success, most of the random mutagenesis methodologies were used to improve already domesticated crops (Fig. 3B-C), including the four major cereal crops (rice, barley, wheat and maize) (Ahloowalia et al. 2004). Applications include tailoring barley for industrial needs, such as for malting and brewing (Knudsen et al. 2020). However, the use of random mutagenesis in orphan or crop wild relatives has been neglected.

### Precision mutagenesis

Methods of genome editing techniques have been developed to introduce precise and predictable genome modifications in plants, giving rise to precision breeding (Chen et al. 2019, Gao 2021). To date, the most precise method of mutagenesis in plants is gene editing via site-directed nucleases. Among different techniques, clustered regularly interspaced short palindromic repeats/CRISPR-associated protein9 (CRISPR/Cas9) has become a widely used tool due to its higher efficiency, reduced number of off-target mutations and methodological simplicity (Voytas 2013, Chen et al. 2019). CRISPR/Cas systems provide an unprecedented possibility for precise targeting of homologs of domestication genes in virtually any plant, providing it is amenable to transformation (Wolter et al. 2019). Thus, CRISPR/Cas technology offers an unmatched opportunity to stimulate domestication of neglected, semi-domesticated or wild plants. However, despite the advantages of CRISPR/Cas, new varieties produced by precision mutagenesis are considered GMOs in many countries, making them subject to strong regulatory regimes.

## Genome Editing as a Promising Tool to Accelerate Orphan Crop Domestication

### Multiplex genome editing and trait stacking

Numerous orphan crops and neodomesticates, such as intermediate wheatgrass (*T. intermedium*), quinoa (*C. quinoa*) and wild potato (*Solanum stoloniferum*), are polyploids (DeHaan et al. 2020, López-Marqués et al. 2020, Del Mar Martínez-Prada et al. 2021). In polyploids, each gene has multiple copies associated with their multiple genomes (homoeologs). Homoeologous genes often demonstrate functional redundancy, and depending on their expression patterns, mutations in multiple homoeologs are often required to obtain the expected phenotype (Adamski et al. 2020). Moreover, when genome data from 16 fully sequenced plant genomes were analyzed, 72% of protein-coding genes belonged to paralogous gene families (Hyams et al. 2018). Many paralogs share similar structures and functions, requiring the simultaneous mutation of numerable paralogs to acquire the desirable phenotype. Genome editing

strategies can be multiplexed for simultaneously editing several homoeologous or paralogous copies through allowing multiplex single guide RNAs (sgRNAs) to be transcribed in a single cell (Minkenberg et al. 2017). Using this strategy, Wang and colleagues edited all three *MLO* homoeologs in bread wheat to create powdery mildew-resistant mutants (Wang et al. 2014). Similarly, using a CRISPR/Cas9 approach, up to 35  $\alpha$ -gliadin paralogs were knocked out in a bread wheat mutant to reduce celiac disease immunoreactivity (Sánchez-León et al. 2018).

### Editing of quantitative trait variation

A trait may result from the combined effect of several genes, each of which may have a minor effect and may interact with the environment. Quantitative variation may also be the result of different alleles of the same gene. A domestication quantitative trait was created in tomato via CRISPR/Cas9 targeting of a promoter in tomato (Rodríguez-Leal et al. 2017). Thus, multiplex editing of the *cis*-regulatory motif in the *SICLV3* gene generated an array of locule number variations in the fruit.

### Editing of QTLs

One of the challenges in orphan crop domestication is the improvement of a trait controlled by a quantitative trait locus (QTL), which refers to a specific genetic region to which a mutation(s) causing a quantitative trait has been mapped. However, due to the varying degree of accuracy associated with QTL mapping, a single mapped QTL may cover hundreds of genes that might also have been mutated but are not responsible for the phenotype under question. Thus, the actual genes and mutations responsible for most QTLs are not known, making it extremely difficult to modify these loci by gene editing. QTLs were targeted in maize via multiplex CRISPR/Cas9, which allowed for high-throughput targeting of over 1,000 candidate genes derived from genetic mapping and comparative genomic analysis (Liu et al. 2020).

### Haploid induction and artificial apomixis

Domestication of orphan crops via traditional breeding takes six to seven generations of self-pollination to produce homozygotes. To accelerate domestication of orphan crops, doubled haploid technology, which rapidly fixes recombinant haploid genomes within two generations, is promising. Haploid inducer lines can be created via the editing of endogenous genes. For example, one *Arabidopsis* haploid inducer line was successfully produced using CRISPR/Cas9 to induce a deletion in the N terminus of *CENH3* (Kuppu et al. 2020). Similarly, a maternal haploid was induced in maize, rice and wheat via knockout of the *ZmPHOSPHOLIPASE-A1* (*ZmPLA1*) gene, also known in other species as *MATRILINEAL* (*OsMTL*) and *NOT LIKE DAD* (*TaNLD*) (Zhong et al. 2019).

Another strategy to accelerate the breeding of orphan crops is artificial apomixis, which rapidly produces genetically identical seeds (Sailer et al. 2016). Apomixis seldom occurs naturally in major crops (Willmann 2019) but can be induced by

CRISPR/Cas9. For example, apomeiosis was induced in rice via CRISPR/Cas9-mediated knockout of the meiotic genes *REC8*, *PAIR1* and *OSD1* (Khanday et al. 2019, Wang et al. 2019). Genome editing of these genes in orphan crops might induce apomixis as these genes are highly conserved.

### Large-scale screening for trait discovery

Elucidation of the genetic regulation of agronomically important traits is the prerequisite of orphan crop domestication. CRISPR/Cas9 screening is a promising reverse genetic approach to characterize the relationships between genotypes and phenotypes at the genome scale (Gaillochet et al. 2020). During CRISPR/Cas9 screening, a library of sgRNAs capable of targeting almost all genes in a single plant genome are co-expressed with Cas9. Subsequently, edited plants are screened for the trait of interest, with the genes controlling a trait of interest further identified based on the sgRNA corresponding sequences. CRISPR/Cas9 screening has been successfully applied to identify genes controlling agronomically important traits genome-wide in maize, rice, tomato and soybean (Jacobs et al. 2017, Lu et al. 2017, Meng et al. 2017, Bai et al. 2020, Liu et al. 2020). With the advent of the genome era, increasing numbers of orphan crops have been genome sequenced, which makes CRISPR/Cas9 screening possible as sgRNAs targeting all genes in the genome can be designed based on the genomic information.

### Transport engineering

Accelerating *de novo* domestication of wild toxin-produced plants by genome editing requires the identification of several target genes. These can be genes encoding biosynthetic enzymes or transcription factors, which regulate the expression of such enzymes in target organs, or transport proteins involved in translating the synthesized metabolites to target organs. Genomic sequences of genes of interest in wild plants first need to be established by sequencing tools and bioinformatics analysis. Sequencing and expression analysis of mRNA transcripts could specify the genes expressed, mainly, in the target organs or tissues in the wild plants. Large-scale functional screens can be employed to identify the transport proteins of specific metabolites (Jørgensen et al. 2017).

The challenge is to breed for plants that have edible grains (or other plant parts) but are otherwise naturally protected. In this way, some parts of the domesticated plant are palatable and safe for human consumption, while the rest of the plant is protected against biotic and abiotic factors. If the biosynthetic pathway of a secondary metabolite is disrupted, this secondary metabolite will disappear in all parts of the plant. However, a plant unable to generate its own protective metabolites will need to be protected by other means, e.g. by application of pesticides, which can be environmentally damaging. In this context, it is noteworthy that secondary metabolites are often synthesized in specialized cells whereupon they are transported to other plant parts (Jørgensen et al. 2015). For example, in *Nicotiana* species, nicotine is synthesized in the root and then

transferred to leaves (Hashimoto and Yamada 2003). In contrast, in cassava (*Manihot esculenta*), cyanogenic glucosides are synthesized in leaves before being transferred to roots (Gleadow and Møller 2014), while seeds of *Brassica napus* import glucosinolates from maternal tissues (Nour-Eldin et al. 2017). An alternative strategy would be to leave the defense compound biosynthesis pathway intact, enabling the plant to be protected, while neutralizing specific transporters involved in their allocation to harvested tissues. Therefore, genes encoding metabolite transporters could be promising targets for domestication of wild plants in the future.

Considering the advances in plant genome editing, many countries are discussing easier regulatory pathways for approving edited crops. Present regulatory systems remain a critical limitation for applying gene editing to improve major commodity crops and specialty crops and to neodomesticate new species. These regulatory systems limit the potential of edited crops to secure not only food but also sustainability of cropping systems.

### Challenges for Genome Editing of Orphan Crops Include Improving Their Regeneration Efficiency

Genome editing has been used successfully to accelerate the domestication of orphan crops. For example, wild tomato (*Solanum pimpinellifolium*) was partially domesticated using multiplex editing of several domestication-related genes (Li et al. 2018; Zsögön et al. 2018). Similarly, allotetraploid wild rice *Oryza alta* was *de novo* domesticated using genome editing (Yu et al. 2021). Below, we focus on some of the challenges associated with genome editing of orphan crops.

#### Transformation and regeneration of plants

Transformation is the prerequisite for genome editing in plants. However, most orphan crops are transformation recalcitrant, with low regeneration efficiency remaining the bottleneck of genome editing in plants. In particular, legumes, which are key to perennial agricultural systems built around neodomesticates, are notoriously recalcitrant to both regeneration and transformation (Choudhury and Rajam 2021). To solve this problem, boosters, which are developmental regulators, are used in plant transformation. For example, *WUSCHEL* (*WUS*) and *BABY BOOM* (*BBM*) improved the regeneration frequencies in a variety of transformation recalcitrant crops including maize, sorghum (*Sorghum bicolor*), sugarcane (*Saccharum officinarum*) and indica rice (*Oryza sativa* ssp. *indica*) (Lowe et al. 2016). The use of tissue-specific promoters to drive the spatiotemporal expression of *WUS* and *BBM* can circumvent their adverse effects (Lowe et al. 2018). In addition, growth regulating factors (GRFs), GRF-interacting factors (GIFs) and GRF–GIF chimeras were also used to boost the regeneration of numerous monocot and dicot plant species without any side effects (Debernardi et al. 2020; Kong et al. 2020; Luo and Palmgren 2021). *TRA1* is a candidate gene determining

*Agrobacterium*-mediated transformability of barley (Orman-Ligeza et al. 2020). Recently, *TaWOX5* was proven to improve wheat regeneration and hence bypassed genotype dependency in wheat transformation (Wang et al. 2022). Combined with *de novo* meristem induction, boosters can also help regenerate dicots, which bypasses tissue culture (Maher et al. 2020). Collectively, the identification and application of booster homologs are important for facilitating genome editing in orphan crops.

An additional challenge of transformation and regeneration is the number of unintended mutations introduced during tissue culture, known as somaclonal variation (Neelakandan and Wang 2012). Therefore, although genome editing techniques result in a low number of off-target effects themselves, the transformation system often needed for their application can introduce mutations in the produced plants (Graham et al. 2020). Furthermore, even when transformation protocols for difficult-to-transform crops have been developed, they are largely confined to specific lines (Do et al. 2018; Che et al. 2021), limiting the capacity of genome editing in grower-preferred varieties.

#### Poor sequence information

Unannotated or partly annotated genomes complicate the identification of all relevant target genes. Furthermore, the lack of complete genome sequence information complicates the design of specific sgRNAs, which increases the off-target activity of CRISPR/Cas.

#### Complicated genetics

As mentioned above, orphan crops are often polyploids, making genome editing difficult. Allopolyploid plants have only one or a maximum of two alleles per plant for each homoeologous gene, which simplifies genotyping and inheritance to some degree, although it is still complicated by the presence of homoeologous gene copies. Additionally, polyploids can have more than two copies of a given gene (paralogs), which further complicates genotyping and inheritance. Most perennial species, such as intermediate wheatgrass, are self-incompatible, meaning creation of inbred (true breeding) lines, as is commonly performed in other domesticated cereals such as maize, rice or wheat, is not an option. Additionally, many, if not most, genes are heterozygous with at least two different alleles for each gene in each plant. In self-compatible plants, low-frequency alleles are usually found in a heterozygous state, meaning recessive traits are usually hidden by dominant alleles unless present at a relatively high frequency in the population. If the frequency of a recessive allele is 0.01 (one in 100), then the frequency of the recessive homozygote (recessive phenotype) is  $0.01 \times 0.01 = 0.0001$  (one in 10,000) in a randomly mating diploid population. The situation is even more complicated in self-incompatible polyploid plants.

#### Screening for Mutations in Large Populations

As regulatory regimes for genome-edited plants are uncertain, and because the technology may not be applicable in countries



hit hardest by climate change, there is an urgent need for alternative approaches for innovative plant breeding (Anders *et al.* 2020). Previously, mutation breeding depended on selection for visible, or readily quantifiable, traits observable during forward screens of large mutant populations. However, if the domestication gene is recessive, which is the norm, the domestication phenotype is only exhibited in plants that are homozygous for the domestication gene, and such plants are rare. However, this problem was circumvented with the development of TILLING (targeting-induced local lesions in genomes) techniques (McCallum *et al.* 2000, Henikoff *et al.* 2004) (Fig. 4). TILLING made it possible to move from screening for a phenotype (forward genetics) to screening for a genotype (reverse genetics) (Holme *et al.* 2019). Thus, the technique allows for the detection of mutations in a specific, known gene in a mutant population, even though it is not associated with a phenotype. The only requirements are that the DNA sequence of the target

gene is known and the mutant population is suitably large (Jankowicz-Cieslak *et al.* 2017, Holme *et al.* 2019). A variant of TILLING called EcoTILLING allows for the detection of natural polymorphisms in natural populations (Comai *et al.* 2004).

Although TILLING and EcoTILLING enable genome-wide scanning of mutations, they can only screen population sizes of fewer than 10,000 individuals because of technical obstacles and low detection sensitivity. To improve the screening efficiency, the Carlsberg Research Laboratory in Denmark has established the 'Fast Identification of Nucleotide variants by DigiTal PCR' (FIND-IT) method (Knudsen *et al.* 2021). Due to the combination of systematic sample pooling-and-splitting and high sensitivity of droplet digital polymerase chain reaction (PCR), FIND-IT can screen a mutant population containing 500,000 individuals and isolate desired variants within 2 weeks. FIND-IT has been proven to identify knockout mutants, non-synonymous codon mutations and mutations in promoter

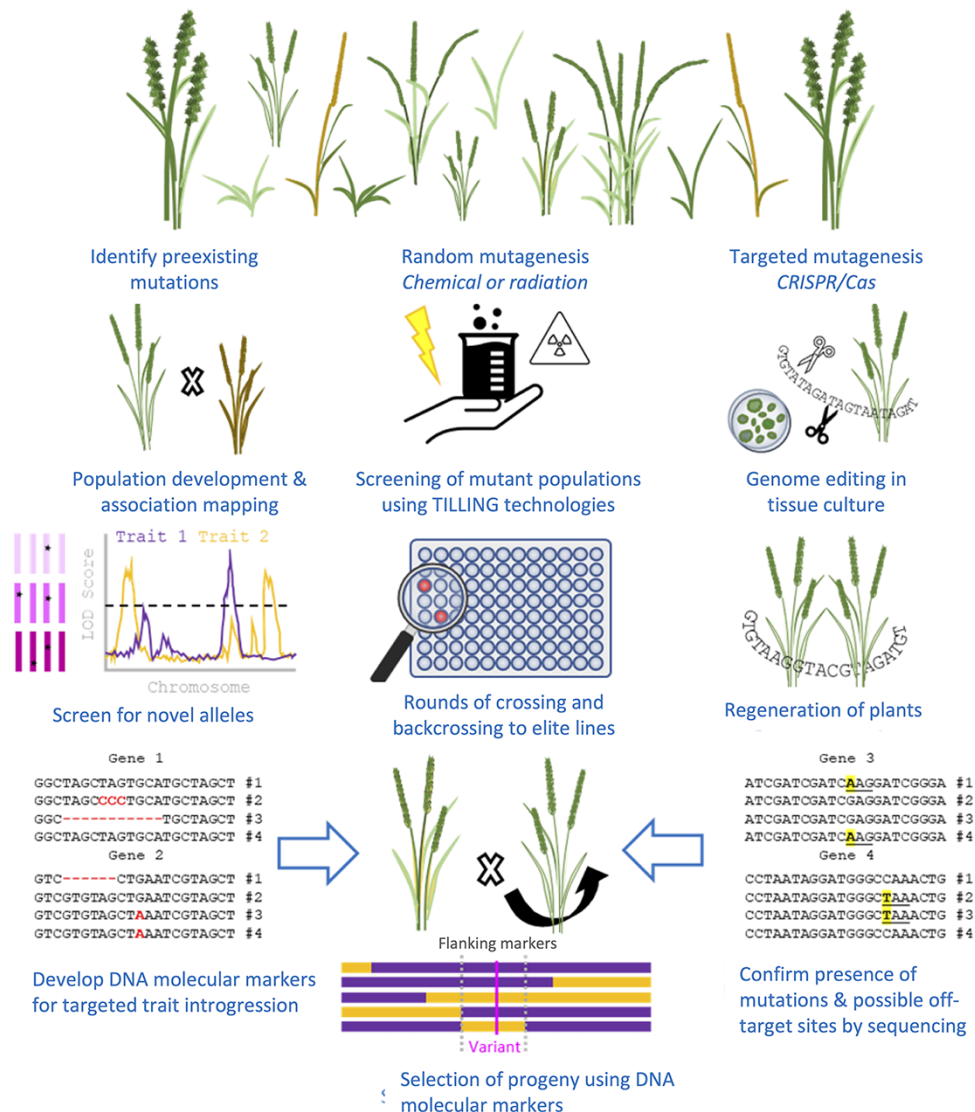


Fig. 4 Outline of the procedure for mutagenesis and subsequent identification of plants with mutations in genes of interest. See text for details.



regions and miRNA binding sites with a single-nucleotide resolution. This method has been tested for mutant screening in barley and wheat but has also been successful in other organisms such as yeast (*Saccharomyces cerevisiae*) and bacteria (*Lactobacillus pasteurii*). Considering the above advantages, FIND-IT is a promising technique to screen chemically induced mutant populations of orphan crops and wild relatives to create non-GMO crops for the future.

New varieties of plants that result from conventional methods of random mutagenesis are easy to release to the market and, in some regions, such as the European Union, are much easier to release than those resulting from precise targeted mutagenesis (Halford 2019). In these regions, random mutagenesis combined with TILLING techniques or FIND-IT may be preferable to accelerate domestication (Holme et al. 2019, Knudsen et al. 2021).

## Conclusion

Nature provides a plethora of plants that have the traits required for crops in a future sustainable agriculture. The challenge now is to increase the yields of these orphan crops so they can compete with present-day elite cultivars. By traditional breeding, this would take an unacceptably long period of time, but with new breeding techniques, it has become possible to accelerate their domestication. The future of green solutions to our future food supply therefore looks bright.

## Data Availability

No new datasets were generated or analyzed in this study.

## Funding

NovoCrops (Novo Nordisk Foundation; project number 2019OC53580 to M.P.); the Innovation Fund Denmark (LESSIS-MORE to M.P.; DEEPROOTS to M.P. and S.K.); the Carlsberg Foundation (RaisingQuinoa, project number CF18-1113 to M.P.; 'Crops for the future—tackling the challenges of changing climates', project number CF20-0352 to S.K.).

## Disclosures

The authors have no conflicts of interest to declare.

## References

- Abbo, S., Van-oss, R.P., Gopher, A., Saranga, Y., Ofner, I. and Peleg, Z. (2014) Plant domestication versus crop evolution: a conceptual framework for cereals and grain legumes. *Trends Plant Sci.* 19: 351–360.
- Abrouk, M., Ahmed, H.I., Cubry, P., Šimoníková, D., Cauet, S., et al. (2020) Fonio millet genome unlocks African orphan crop diversity for agriculture in a changing climate. *Nat. Commun.* 11: 4488.
- Acquaah, G. (2007) *Principles of Plant Genetics and Breeding*. Blackwell, Oxford, UK.
- Adamski, N.M., Borrill, P., Brinton, J., Harrington, S.A., Marchal, C., Bentley, A.R., et al. (2020) A roadmap for gene functional characterisation in crops with large genomes: lessons from polyploid wheat. *Elife* 9: e55646.
- Ahloowalia, B.S., Maluszynski, M. and Nichterlein, K. (2004) Global impact of mutation-derived varieties. *Euphytica* 135: 187–204.
- Alhabbar, Z., Yang, R., Juhasz, A., Xin, H., She, M., Anwar, M., et al. (2018) NAM gene allelic composition and its relation to grain-filling duration and nitrogen utilisation efficiency of Australian wheat. *PLoS One* 13: e0205448.
- Alseikh, S., Scossa, F., Wen, W., Luo, J., Yan, J., Beleggia, R., et al. (2021) Domestication of crop metabolomes: desired and unintended consequences. *Trends Plant Sci.* 26: 650–661.
- Anders, S., Pareek, A., Singla-Pareek, S.L., Gupta, K.J. and Foyer, C.H. (2020) Innovative plant breeding could deliver crop revolution. *Nature* 577: 622.
- Asplund, L., Bergkvist, G., Meino, M.W., Westerbergh, A. and Weih, M. (2013) Swedish spring wheat varieties with the rare high grain protein alleles of NAM-B1 differ in leaf senescence and grain mineral content. *PLoS One* 8: e59704.
- Bai, M., Yuan, J., Kuang, H., Gong, P., Li, S., Zhang, Z., et al. (2020) Generation of a multiplex mutagenesis population via pooled CRISPR-Cas9 in soya bean. *Plant Biotechnol. J.* 18: 721–731.
- Bailey-Serres, J., Parker, J.E., Ainsworth, E.A., Oldroyd, G.E.D. and Schroeder, J.I. (2019) Genetic strategies for improving crop yields. *Nature* 575: 109–118.
- Baur, E. (1914) Die Bedeutung der primitiven Kulturrassen und der wilden Verwandten unserer Kulturpflanzen für die Pflanzenzüchtung. pp. 104–110. *Jahrbuch der DLG* 29.
- Bazile, D., Jacobsen, S.E. and Verniau, A. (2016) The global expansion of quinoa: Trends and limits. *Front. Plant Sci.* 7: 622.
- Benton, T.G. and Bailey, R. (2019) The paradox of productivity: agricultural productivity promotes food system inefficiency. *Global Sustainability* 2: 1–8.
- Benton, T.G., Bieg, C., Harwatt, H., Pudasaini, R. and Wellesley, L. (2021) Food system impacts on biodiversity loss. Three levers for food system transformation in support of nature. Chatham House, the Royal Institute of International Affairs, London. <https://www.ciwf.com/media/7443948/food-system-impacts-on-biodiversity-loss-feb-2021.pdf> (January 29, 2022, date last accessed).
- Breseghello, F. and Guedes Coelho, A. (2013) Traditional and modern plant breeding methods with examples in rice (*Oryza sativa* L.). *J. Agric. Food Chem.* 61: 8277–8268.
- Brink, R.A. (1921) The genetic basis for improvement in self-fertilized crops. *Sci. Agric.* 2: 83–87.
- Cadima, X., van Zonneveld, M., Scheldeman, X., Castañeda, N., Patiño, F., Beltran, M., et al. (2014) Endemic wild potato (*Solanum* spp.) biodiversity status in Bolivia: reasons for conservation concerns. *J. Nat. Conserv.* 22: 113–131.
- Canadian Food Inspection Agency. (2004) Decision document DD2004-46: determination of the safety of the BASF Canada imidazolinone-tolerant lentil line RH44. [Online]. <http://www.inspection.gc.ca/english/plaveg/bio/dd/dd0446e.shtml> (March 6, 2022, date last accessed).
- Cassman, K.G. and Grassini, P. (2020) A global perspective on sustainable intensification research. *Nat. Sustainability* 3: 262–268.
- Charlton, J.F.L., Douglas, G.B., Wills, B.J. and Prebble, J.E. (2003) Farmer experience with tree fodder. In *Using Trees on Farms*. Vol. 10, Edited by Charlton, J.F.L. pp. 7–16. Grassl. res. pract. ser.
- Che, P., Chang, S., Simon, M.K., Zhang, Z., Shaharyar, A., Ourada, J., et al. (2021) Developing a rapid and highly efficient cowpea regeneration, transformation and genome editing system using embryonic axis explants. *Plant J.* 106: 817–830.
- Chen, K., Wang, Y., Zhang, R., Zhang, H. and Gao, C. (2019) CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annu. Rev. Plant Biol.* 70: 667–697.
- Chen, S., Yang, Y., Shi, W., Ji, Q., He, F., Zhang, Z., et al. (2008) Badh2, Encoding betaine aldehyde dehydrogenase, inhibits the biosynthesis of

- 2-acetyl-1-pyrroline, a major component in rice fragrance. *Plant Cell* 20: 1850–1861.
- Choudhury, A. and Rajam, M.V. (2021) Genetic transformation of legumes: an update. *Plant Cell Rep.* 40: 1813–1830.
- Cohen, M.N. (1977) The food crises in prehistory: overpopulation and the origins of agriculture. Yale University Press, New Haven.
- Comai, L., Young, K., Till, B.J., Reynolds, S.H., Greene, E.A., Codomo, C.A., et al. (2004) Efficient discovery of DNA polymorphisms in natural populations by Ecotilling. *Plant J.* 37: 778–786.
- Cooper, M., van Eeuwijk, F.A., Hammer, G.L., Podlich, D.W. and Messina, C. (2009) Modeling QTL for complex traits: detection and context for plant breeding. *Curr. Opin. Plant Biol.* 12: 231–240.
- Cormier, F., Throude, M., Ravel, C., Le Gouis, J., Leveugle, M., Lafarge, S., et al. (2015) Detection of NAM-A1 natural variants in bread wheat reveals differences in haplotype distribution between a worldwide core collection and European elite germplasm. *Agronomy* 5: 143–151.
- Crain, J., DeHaan, L. and Poland, J. (2021) Genomic prediction enables rapid selection of high-performing genets in an intermediate wheatgrass breeding program. *Plant Genome* 14: e20080.
- Crossa, J., Fritsche-Neto, R., Montesinos-Lopez, O.A., Costa-Neto, G., Dreisigacker, S., Montesinos-Lopez, A., et al. (2021) The modern plant breeding triangle: Optimizing the use of genomics, phenomics, and enviromics data. *Front. Plant. Sci.* 12: 651480.
- Darwin, C. (1868) The Variation of Animals and Plants under Domestication. John Murray, London.
- Davis, J.M. (2008) Genetic improvement of poplar (*Populus* spp.) as a bioenergy crop. In Genetic Improvement of Bioenergy Crops. Edited by Vermerris, W. pp. 397–419. Springer, New York, NY.
- Debernardi, J.M., Tricoli, D.M., Ercoli, M.F., Hayta, S., Ronald, P., Palatnik, J.F., et al. (2020) A GRF-GIF chimeric protein improves the regeneration efficiency of transgenic plants. *Nat. Biotechnol.* 38: 1274–1279.
- DeHaan, L., Larson, S., López-Marqués, R.L., Wenkel, S., Gao, C. and Palmgren, M. (2020) Roadmap for accelerated domestication of an emerging perennial grain crop. *Trends Plant Sci.* 25: 525–537.
- Del Mar Martínez-Prada, M., Curtin, S.J. and Gutiérrez-González, J.J. (2021) Potato improvement through genetic engineering. *GM Crops Food* 12: 479–496.
- Do, P.T., Lee, H., Nelson-Vasilchik, K., Kausch, A. and Zhang, Z.J. (2018) Rapid and efficient genetic transformation of sorghum via agrobacterium-mediated method. *Curr. Protoc. Plant Biol.* 3: e20077.
- Dubcovsky, J. and Dvorak, J. (2007) Genome plasticity a key factor in the success of polyploid wheat under domestication. *Science* 316: 1862–1866.
- Duguma, L.A., Minang, P.A. and van Noordwijk, M. (2014) Climate change mitigation and adaptation in the land use sector: from complementarity to synergy. *Environ. Manage.* 54: 420–432.
- Dyer, G.A., López-Feldman, A., Yúnez-Naude, A. and Taylor, J.E. (2014) Genetic erosion in maize's center of origin. *Proc. Natl. Acad. Sci. U.S.A.* 111: 14094–14099.
- East, E.M. (1910) A Mendelian interpretation of variation that is apparently continuous. *Am. Nat.* 44: 65–82.
- Fans, J.D., Simons, K.J., Zhang, Z. and Gill, B.S. (2005) The wheat super domestication gene. *Q. Front. Wheat Biosci. Memorial Issue, Wheat Inf. Serv.* 100: 129–148.
- FAO Annual Report. (2004) Agrobiodiversity? Food and Agriculture Organization of the United Nations (FAO). What is happening to agrobiodiversity?
- FAO, IFAD, UNICEF, WFP & WHO. (2018) The State of Food Security and Nutrition in the World 2018. Building climate resilience for food security and nutrition. Rome, FAO. [www.fao.org/3/I9553EN/i9553en.pdf](http://www.fao.org/3/I9553EN/i9553en.pdf) (January 29, 2022, date last accessed).
- Feldman, M. and Kislev, M.E. (2007) Domestication of emmer wheat and evolution of free-threshing tetraploid wheat. *Israel J. Plant Sci.* 55: 207–221.
- Fereres, E., Orgaz, F., Gonzalez-Dugo, V., Testi, L. and Villalobos, F.J. (2014) Balancing crop yield and water productivity tradeoffs in herbaceous and woody crops. *Funct. Plant Biol.* 41: 1009–1018.
- Fernández, R.J. and Reynolds, J.F. (2000) Potential growth and drought tolerance of eight desert grasses: lack of a trade-off? *Oecologia* 123: 90–98.
- Fisher, R.A. (2015) Definitions and determination of crop yield, yield gaps, and of rates of change. *Field Crops Res.* 182: 9–18.
- Foley, J.A., Ramankutty, N., Brauman, K.A., Cassidy, E.S., Gerber, J.S., Johnston, M., et al. (2011) Solutions for a cultivated planet. *Nature* 478: 337–342.
- Forster, P., Torroni, A., Renfrew, C. and Röhl, A. (2001) Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution. *Mol. Biol. Evol.* 18: 1864–1881.
- Friedman, M., Roitman, J.N. and Kozukue, N. (2003) Glycoalkaloid and calystegine contents of eight potato cultivars. *J. Agricult. Food Chem.* 51: 2964–2973.
- Gabriel, D., Sait, S.M., Kunin, W.E. and Benton, T.G. (2013) Food production vs. biodiversity: comparing organic and conventional agriculture. *J. Applied Ecol.* 50: 355–364.
- Gaillochet, C., Develtere, W. and Jacobs, T.B. (2020) CRISPR screens in plants: approaches, guidelines, and future prospects. *Plant Cell* 33: 794–813.
- Gambino, P. and Vilela, A. (2011) Morphological traits and allocation patterns related to stress-tolerance and seed-yield in wild and domesticated evening primrose (*Oenothera* L. Onagraceae). *Ind. Crops Prod.* 34: 1269–1276.
- Gao, C. (2021) Genome engineering for crop improvement and future agriculture. *Cell* 184: 1621–1635.
- Gao, L. (2003) The conservation of Chinese rice biodiversity: genetic erosion, ethnobotany and prospects. *Genetic Res. Crop Evol.* 50: 17–32.
- Gepts, P., Famula, T.R., Bettinger, R.L., Brush, S.B., Damania, A.B., McGuire, P.E., et al. (eds.) (2012) Biodiversity in Agriculture: Domestication, Evolution, and Sustainability. Cambridge Univ. Press, Cambridge.
- Girija, A., Jifar, H., Jones, C., Yadav, R., Doonan, J. and Mur, L.A.J. (2021) Tef: a tiny grain with enormous potential. *Trends Plant Sci.* 27: 220–223.
- Gleadow, R.M. and Möller, B.L. (2014) Cyanogenic glycosides: synthesis, physiology, and phenotypic plasticity. *Annu. Rev. Plant Biol.* 65: 155–185.
- Glover, J.D., Reganold, J.P., Bell, L.W., Borevitz, J., Brummer, E.C., Buckler, E.S., et al. (2010) Agriculture. Increased food and ecosystem security via perennial grains. *Science* 328: 1638–1639.
- Godfray, H.C., Beddington, J.R., Crute, I.R., Haddad, L., Lawrence, D., Muir, J.F., et al. (2010) Food security: the challenge of feeding 9 billion people. *Science* 327: 812–818.
- Goodwin, S.B., Cohen, B.A. and Fry, W.E. (1994) Panglobal distribution of a single clonal lineage of the Irish potato famine fungus. *Proc. Natl. Acad. Sci.* 91: 11591–11595.
- Graham, N., Patil, G.B., Bubeck, D.M., Dobert, R.C., Glenn, K.C., Gutsche, A.T., et al. (2020) Plant genome editing and the relevance of off-target changes. *Plant Physiol.* 183: 1453–1471.
- Gross, B.L. and Olsen, K.M. (2010) Genetic perspectives on crop domestication. *Trends Plant Sci.* 15: 529–537.
- Haas, M., Schreiber, M. and Mascher, M. (2019) Domestication and crop evolution of wheat and barley: genes, genomics, and future directions. *J. Integr. Plant Biol.* 61: 204–225.
- Hagenblad, J., Asplund, L., Balfourier, F., Ravel, C. and Leino, M.W. (2012) Strong presence of the high grain protein content allele of NAM-B1 in Fennoscandian wheat. *Theor. Appl. Genet.* 125: 1677–1686.

- Halford, N.G. (2019) Legislation governing genetically modified and genome-edited crops in Europe: the need for change. *J. Sci. Food Agric.* 99: 8–12.
- Hammer, K., Knüpffer, H., Xhuvelli, L. and Perrino, P. (1996) Estimating genetic erosion in landraces—two case studies. *Genet. Resour. Crop Evol.* 43: 329–336.
- Harlan, J.R. (1975) Crops and Man. American Society of Agronomy and Crop Science Society of America, Madison, WI.
- Harwood, W.A. and Smedley, M.A. (2009) Barley transformation using biolistic techniques. *Methods Mol. Biol.* 478: 125–136.
- Hashimoto, T. and Yamada, Y. (2003) New genes in alkaloid metabolism and transport. *Curr. Opin. Biotechnol.* 14: 163–168.
- Haudry, A., Cenci, A., Ravel, R., Batallion, T., Brunel, D., Poncet, C., et al. (2007) Grinding up wheat: a massive loss of nucleotide diversity since domestication. *Mol. Biol. Evol.* 24: 1506–1517.
- Hedden, P. (2003) The genes of the green revolution. *Trends Genet.* 19: 5–9.
- Henikoff, S., Till, B.J. and Comai, L. (2004) TILLING. Traditional mutagenesis meets functional genomics. *Plant Physiol.* 135: 630–636.
- Holme, I.B., Gregersen, P.L. and Brinch-Pedersen, H. (2019) Induced genetic variation in crop plants by random or targeted mutagenesis: convergence and differences. *Front. Plant Sci.* 10: 68.
- Hufford, M.B., Xu, X., van Heerwaarden, J., Pyhäjärvi, T., Chia, J.M., Cartwright, R.A., et al. (2012) Comparative population genomics of maize domestication and improvement. *Nat. Genet.* 44: 808–811.
- Humphry, M., Reinstädler, A., Ivanov, S., Bisseling, T. and Panstruga, R. (2011) Durable broad-spectrum powdery mildew resistance in pea *er1* plants is conferred by natural loss-of-function mutations in PsML01. *Mol. Plant Pathol.* 12: 866–878.
- Hunt, H.V., Denyer, K., Packman, L.C., Jones, M.K. and Howe, C.J. (2010) Molecular basis of the waxy endosperm starch phenotype in broomcorn miller (*Panicum miliaceum* L.). *Mol. Biol. Evol.* 27: 1478–1494.
- Hyams, G., Abadi, S., Lahav, S., Avni, A., Halperin, E., Shani, E., et al. (2018) CRISPRs: optimal sgRNA design for editing multiple members of a gene family using the CRISPR system. *J. Mol. Biol.* 430: 2184–2195.
- Jacobs, T.B., Zhang, N., Patel, D. and Martin, G.B. (2017) Generation of a collection of mutant tomato lines using pooled CRISPR libraries. *Plant Physiol.* 174: 2023–2037.
- Jamnadas, R., Mumm, R.H., Hale, I., Hendre, P., Muchugi, A., Dawson, I.K., et al. (2020) Enhancing African orphan crops with genomics. *Nat. Genet.* 52: 356–360.
- Jankowicz-Cieslak, J., Mba, C. and Till, B.J. (2017) Mutagenesis for crop breeding and functional genomics. In *Biotechnologies for Plant Mutation Breeding*. Edited by Jankowicz-Cieslak, J., et al. pp. 3–18. International Atomic Energy Agency, Springer, Cham.
- Jankowicz-Cieslak, J. and Till, B.J. (2016) Chemical mutagenesis of seed and vegetatively propagated plants using EMS. *Curr. Protoc. Plant Biol.* 1: 617–635.
- Jarvis, D.E., Ho, Y.S., Lightfoot, D.J., Schmöckel, S.M., Li, B., Borm, T.J.A., et al. (2017) The genome of *Chenopodium quinoa*. *Nature* 542: 307–312.
- Jarvis, D.I., Brown, A.H., Cuong, P.H., Collado-Panduro, L., Latournerie-Moreno, L., Gyawali, S., et al. (2008) A global perspective of the richness and evenness of traditional crop-variety diversity maintained by farming communities. *Proc. Natl. Acad. Sci. U.S.A.* 105: 5326–5331.
- Jones, G., Kluyver, T., Preece, C., Swarbrick, J., Forster, E., Wallace, M., et al. (2021) The origins of agriculture: Intentions and consequences. *J. Archaeol. Sci.* 125: 105290.
- Jørgensen, I.H. (1992) Discovery, characterization and exploitation of Mlo powdery mildew resistance in barley. *Euphytica* 63: 141–152.
- Jørgensen, M.E., Crocoll, C., Halkier, B.A. and Nour-Eldin, H.H. (2017) Uptake assays in *Xenopus laevis* oocytes using liquid chromatography-mass spectrometry to detect transport activity. *Bio. Protoc.* 7: e2581.
- Jørgensen, M.E., Nour-Eldin, H.H. and Halkier, B.A. (2015) Transport of defense compounds from source to sink: lessons learned from glucosinolates. *Trends Plant Sci.* 20: 508–514.
- Kaiser, N., Douches, D., Dhirra, A., Glenn, K.C., Herzig, P.R., Stowe, E.C., et al. (2020) The role of conventional plant breeding in ensuring safe levels of naturally occurring toxins in food crops. *Trends Food Sci. Technol.* 100: 51–66.
- Keneni, G., Bekele, E., Imtiaz, M. and Dagne, K. (2012) Genetic vulnerability of modern crop cultivars: causes, mechanism and remedies. *Int. J. Plant Res.* 2: 69–79.
- Khanday, I., Skinner, D., Yang, B., Mercier, R. and Sundaresan, V. (2019) A male-expressed rice embryogenic trigger redirected for asexual propagation through seeds. *Nature* 565: 91–95.
- Khlestkina, E.K., Huang, X.Q., Quenum, F.J., Chebotar, S., Röder, M.S. and Börner, A. (2004) Genetic diversity in cultivated plants—loss or stability? *Theor. Appl. Genet.* 108: 1466–1472.
- Khoury, C.K., Brush, S., Costich, D.E., Curry, H.A., de Haan, S., Engels, J.M.M., et al. (2022) Crop genetic erosion: understanding and responding to loss of crop diversity. *New Phytol.* 233: 84–118.
- Kingsbury, N. (2009) Hybrid: The History and Science of Plant Breeding. University of Chicago Press, Chicago, IL.
- Knudsen, S., Lok, F. and Braumann, I. (2020) Developing barley crops for improved brewing quality. In *Achieving Sustainable Cultivation of Barley*. Edited by Fox, G. and Li, C. Burleigh Dodds Science, Cambridge. ISBN 978-1-78676-308-2.
- Knudsen, S., Wendt, T., Dockter, C., Thomsen, H.C., Rasmussen, M., Jørgensen, M.E., et al. (2021) FIND-IT: Ultrafast mining of genome diversity. *BioRxiv*.
- Kong, J., Martin-Ortigosa, S., Finer, J., Orchard, N., Gunadi, A., Batts, L.A., et al. (2020) Overexpression of the transcription factor GROWTH-REGULATING FACTORS improves transformation of dicot and monocot species. *Front. Plant Sci.* 11: 572319.
- Kozioł, L., Rieseberg, L.H., Kane, N. and Bever, J.D. (2012) Reduced drought tolerance during domestication and the evolution of weediness results from tolerance-growth trade-offs. *Evolution* 66: 3803–3814.
- Kozukue, N., Kozukue, E. and Mizuno, S. (1987) Glycoalkaloids in potato plants and tubers. *Hortic. Sci.* 22: 294–296.
- Krasileva, K.V., Vasquez-Gross, H.A., Howell, T., Bailey, P., Paraiso, F., Clissold, L., et al. (2017) Uncovering hidden variation in polyploid wheat. *Proc. Natl. Acad. Sci. U.S.A.* 114: E913–E921.
- Kuppu, S., Ron, M., Marimuthu, M.P.A., Li, G., Huddleson, A., Siddeek, M.H., et al. (2020) A variety of changes, including CRISPR/Cas9-mediated deletions, in CENH3 lead to haploid induction on outcrossing. *Plant Biotechnol. J.* 18: 2068–2080.
- Kushwah, A., Gupta, S., Bindra, S., Johal, N., Singh, I., Bharadwaj, C. et al. (2020) Gene pyramiding and multiple character breeding. In *Chickpea: Crop Wild Relatives for Enhancing Genetic Gains*. Edited by Singh, M. pp. 131–165. Academic Press, Cambridge, MA.
- Lambein, F., Travella, S., Kuo, Y.H., Van Montagu, M. and Heijde, M. (2019) Grass pea (*Lathyrus sativus* L.): orphan crop, nutraceutical or just plain food? *Planta* 250: 821–838.
- Larson, G., Piperno, D.R., Allaby, R.G., Purugganan, M.D., Andersson, L., Arroyo-Kalin, M., et al. (2014) Current perspectives and the future of domestication studies. *Proc. Natl. Acad. Sci.* 111: 6139–6146.
- Lev-Yadun, S., Gopher, A. and Abbo, S. (2000) The cradle of agriculture. *Science* 288: 1602–1603.
- Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., et al. (2018) Domestication of wild tomato is accelerated by genome editing. *Nat. Biotechnol.* 36: 1160–1163.
- Liebman, M. (2018) Polyculture cropping systems. In *Agroecology. The science of sustainable agriculture*. Edited by Altieri, M.A. pp. 205–218. CRC Press, Boca Raton, FL.



- Liu, H.J., Jian, L., Xu, J., Zhang, Q., Zhang, M., Jin, M., et al. (2020) High-throughput CRISPR/Cas9 mutagenesis streamlines trait gene identification in maize. *Plant Cell* 32: 1397–1413.
- López-Marqués, R.L., Nørrevang, A.F., Ache, P., Moog, M., Visintainer, D., Wendt, T., et al. (2020) Prospects for the accelerated improvement of the resilient crop quinoa. *J. Exp. Bot.* 71: 5333–5347.
- Lowe, K., La Rota, M., Hoerster, G., Hastings, C., Wang, N., Chamberlin, M., et al. (2018) Rapid genotype “independent” *Zea mays* L. (maize) transformation via direct somatic embryogenesis. *Vitro Cell. Dev. Biol. Plant.* 54: 240–252.
- Lowe, K., Wu, E., Wang, N., Hoerster, G., Hastings, C., Cho, M.J., et al. (2016) Morphogenic regulators *Baby boom* and *Wuschel* improve monocot transformation. *Plant Cell* 28: 1998–2015.
- Lu, Y., Ye, X., Guo, R., Huang, J., Wang, W., Tang, J., et al. (2017) Genome-wide targeted mutagenesis in rice using the CRISPR/Cas9 system. *Mol. Plant* 10: 1242–1245.
- Luo, G. and Palmgren, M. (2021) GRF-GIF chimeras boost plant regeneration. *Trends Plant Sci.* 26: 201–204.
- Luo, M.-C., Yang, Z.-L., You, F.M., Kawahara, R., Waines, J.G. and Dvorak, J. (2007) The structure of wild and domesticated emmer wheat populations, gene flow between them, and the site of emmer domestication. *Theor. Appl. Genet.* 114: 947–959.
- Ma, L., Kong, F., Sun, K., Wang, T. and Guo, T. (2021) From classical radiation to modern radiation: past, present, and future of radiation mutation breeding. *Front. Public Health* 9: 768071.
- Mackay, T.F.C., Stone, E.A. and Ayroles, J.F. (2009) The genetics of quantitative traits: challenges and prospects. *Nat. Rev. Genet.* 10: 565–577.
- MacKey, J. (1954) Neutron and X-ray experiments in wheat and a revision of the speltoid problem. *Hereditas* 40: 65–180.
- Maher, M.F., Nasti, R.A., Vollbrecht, M., Starker, C.G., Clark, M.D. and Voytas, D.F. (2020) Plant gene editing through de novo induction of meristems. *Nat. Biotechnol.* 38: 84–89.
- Marthe, C., Kümlehn, J. and Hensel, G. (2015) Barley (*Hordeum vulgare* L.) transformation using immature embryos. *Methods Mol. Biol.* 1223: 71–83.
- Martin-Merino, M. (2021) The Neolithic revolution: agriculture, sedentary lifestyle and its consequences. Cambridge Open Engage. [10.33774/coe-2021-2589h](https://doi.org/10.33774/coe-2021-2589h).
- Matsumoto, T., Wu, J., Kanamori, H., Katayose, Y., Fujisawa, M., Namiki, N., et al. (2005) The map-based sequence of the rice genome. *Nature* 436: 793–800.
- Mayer, K.F.X., Waugh, R., Langridge, P., Close, T.J., Wise, R.P., Graner, A., et al. (2012) A physical, genetic and functional sequence assembly of the barley genome. *Nature* 491: 711–716.
- Mba, C., Guimaraes, E.P. and Ghosh, K. (2012) Re-orienting crop improvement for the changing climatic conditions of the 21st century. *Agric. Food Secur.* 1: 7.
- McCallum, C.M., Comai, L., Greene, E.A. and Henikoff, S. (2000) Targeted screening for induced mutations. *Nat. Biotechnol.* 18: 455–457.
- Meng, X., Yu, H., Zhang, Y., Zhuang, F., Song, X., Gao, S., et al. (2017) Construction of a genome-wide mutant library in rice using CRISPR/Cas9. *Mol. Plant* 10: 1238–1241.
- Meuwissen, T.H.E., Hayes, B.J. and Goddard, M.E. (2001) Prediction of total genetic value using genome-wide dense marker maps. *Genetics* 157: 1819–1829.
- Meyer, R.S., DuVal, A.E. and Jensen, H.R. (2012) Patterns and processes in crop domestication: an historical review and quantitative analysis of 203 global food crops. *New Phytol.* 196: 29–48.
- Meyer, R.S. and Purugganan, M.D. (2013) Evolution of crop species: genetics of domestication and diversification. *Nat. Rev. Genet.* 14: 840–852.
- Minkenberg, B., Wheatley, M. and Yang, Y. (2017) CRISPR/Cas9-enabled multiplex genome editing and its application. *Prog. Mol. Biol. Transl. Sci.* 149: 111–132.
- Molnar, T.J., Kahn, P.C., Ford, T.M., Funk, C.J. and Funk, C.R. (2013) Tree crops, a permanent agriculture: concepts from the past for a sustainable future. *Resources* 2: 457–488.
- Monna, L., Kitazawa, N., Yoshino, R., Suzuki, J., Masuda, H., Maehara, Y., et al. (2002) Positional cloning of rice semidwarfing gene, *sd-1*: rice “Green Revolution Gene” encodes a mutant enzyme involved in gibberellin synthesis. *DNA Res.* 9: 11–17.
- Moreira, X., Abdala-Roberts, L., Gols, R. and Francisco, M. (2018) Plant domestication decreases both constitutive and induced chemical defenses by direct selection against defensive traits. *Sci. Rep.* 8: 12678.
- Muckey, E. (2019) Kernza® in Southern Minnesota: Assessing local viability of intermediate wheatgrass. Co-publication of the University of Minnesota’s Center for Urban and Regional Affairs (CURA), Southwest Regional Sustainable Development Partnership (RSDP), and Rural Advantage.
- Mus, F., Crook, M.B., Garcia, K., Garcia Costas, A., Geddes, B.A., Kouri, E.D., et al. (2016) Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes. *Appl. Environ. Microbiol.* 82: 3698–3710.
- Neelakandan, A.K. and Wang, K. (2012) Recent progress in the understanding of tissue culture-induced genome level changes in plants and potential applications. *Plant Cell Rep.* 31: 597–620.
- Nishimura, A., Aichi, I. and Matsuoka, M. (2007) A protocol for *Agrobacterium*-mediated transformation in rice. *Nat. Protoc.* 1: 2796–2802.
- Nour-Eldin, H.H., Madsen, S.R., Engelen, S., Jørgensen, M.E., Olsen, C.E., Andersen, J.S., et al. (2017) Reduction of antinutritional glucosinolates in *Brassica* oilseeds by mutation of genes encoding transporters. *Nat. Biotechnol.* 35: 377–382.
- Oladosu, Y., Rafii, M.Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H.A., et al. (2015) Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnol. Biotechnol. Equip.* 30: 1–16.
- Orman-Ligeza, B., Harwood, W., Hedley, P.E., Hinchcliffe, A., Macaulay, M., Uauy, C., et al. (2020) TRA1: A locus responsible for controlling *Agrobacterium*-mediated transformability in barley. *Front. Plant Sci.* 11: 355.
- Østerberg, J.T., Xiang, W., Olsen, L.I., Edenbrandt, A.K., Vedel, S.E., Christiansen, A., et al. (2017) Accelerating the domestication of new crops: feasibility and approaches. *Trends Plant Sci.* 22: 373–384.
- Palmgren, M.G., Edenbrandt, A.K., Vedel, S.E., Andersen, M.M., Landes, X., Østerberg, J.T., et al. (2015) Are we ready for back-to-nature crop breeding? *Trends Plant Sci.* 20: 155–164.
- Pourkheirandish, M., Hensel, G., Kilian, B., Senthil, N., Chen, G., Sameri, M., et al. (2015) Evolution of the grain dispersal system in barley. *Cell* 162: 527–539.
- Price, T.D. and Bar-Yosef, O. (2011) The origins of agriculture: new data, new ideas: an introduction to supplement 4. *Curr. Anthropol.* 52: S163–S174.
- Rakszegi, M., Kisgyörgy, B.N., Tearall, K., Shewry, P.R., Láng, L., Phillips, A., et al. (2010) Diversity of agronomic and morphological traits in a mutant population of bread wheat studied in the Healthgrain program. *Euphytica* 174: 409–421.
- Raman, R. (2017) The impact of Genetically Modified (GM) crops in modern agriculture: a review. *GM Crops Food* 8: 195–208.
- Ray, D.K., Ramankutty, N., Mueller, N.D., West, P.C. and Foley, J.A. (2012) Recent patterns of crop yield growth and stagnation. *Nat. Commun.* 3: 1293.
- Razzaq, A., Saleem, F., Wani, S.H., Abdelmohsen, S.A.M., Alyousef, H.A., Abdelbacki, A.M.M., et al. (2021) De-novo domestication for improving salt tolerance in crops. *Front Plant Sci.* 12: 681367.
- Renard, D. and Tilman, D. (2019) National food production stabilized by crop diversity. *Nature* 571: 257–260.
- Roberts, H.F. (1919) The founders of the art of breeding. *J. Heredity* 10: 99–106.



- Rodríguez-Leal, D., Lemmon, Z.H., Man, J., Bartlett, M.E. and Lippman, Z.B. (2017) Engineering quantitative trait variation for crop improvement by genome editing. *Cell* 2: 470–480.e8.
- Roy, S., Liu, W., Nandety, R.S., Crook, A., Mysore, K.S., Pislariu, C.I., et al. (2020) Celebrating 20 years of genetic discoveries in legume nodulation and symbiotic nitrogen fixation. *Plant Cell* 32: 15–41.
- Rull, V. (2010) Food security: green revolution drawbacks. *Science* 328: 169.
- Sahoo, K.K., Tripathi, A.K., Pareek, A., Sopory, S.K. and Singla-Pareek, S.L. (2011) An improved protocol for efficient transformation and regeneration of diverse indica rice cultivars. *Plant Methods* 7: 1–11.
- Sailer, C., Schmid, B. and Grossniklaus, U. (2016) Apomixis allows the transgenerational fixation of phenotypes in hybrid plants. *Curr. Biol.* 26: 331–337.
- Sánchez-León, S., Gil-Humanes, J., Ozuna, C.V., Giménez, M.J., Sousa, C., Voytas, D.F., et al. (2018) Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnol. J.* 16: 902–910.
- Schlegel, R.H.J. (2018) History of Plant Breeding. CRC Press, Boca Raton, FL.
- Sharma, S., Schulthess, A.W., Bassi, F.M., Badeaeva, E.D., Neumann, K., Graner, A., et al. (2021) Introducing beneficial alleles from plant genetic resources into the wheat germplasm. *Biology* 10: 982.
- Shaw, L.M., Turner, A.S., Henry, L., Griffiths, S. and Laurie, D.A. (2013) Mutant alleles of Photoperiod-1 in wheat (*Triticum aestivum* L.) that confer a later flowering phenotype in long days. *PLoS One* 8: e79459.
- Shu, Q.Y., Forster, B.P. and Nakagawa, H. (2012) Plant Mutation Breeding and Biotechnology. p. 608. CABI, Wallingford, UK.
- Sleper, D.A. and Poehlman, J.M. (2006) Breeding Field Crops. p. 424. Blackwell Publishing, Oxford.
- Slinkard, A.E., Vanderberg, A. and Holm, F.A. (2007) Lentil plants having increased resistance to imidazolinone herbicides. U.S. Patent, 2007. <http://www.uspto.gov/patft/index.html> (March 6, 2022 date last accessed).
- Spielmeier, W., Ellis, M.H. and Chandler, P.M. (2002) Semidwarf (*sd-1*), “green revolution” rice, contains a defective gibberellin 20-oxidase gene. *Proc. Natl. Acad. Sci. U.S.A.* 99: 9043–9048.
- Symko, S. (1999) From a Single Seed. Tracing the Marquis Wheat Success Story in Canada to Its Roots in the Ukraine. Agriculture and Agri-Food Canada. Strategic Promotion, Research Branch, Agriculture and Agri-Food Canada, Ottawa, Canada.
- Talamè, V., Bovina, R., Sanguineti, M.C., Tuberosa, R., Lundqvist, U. and Salvi, S. (2008) TILLMore, a resource for the discovery of chemically induced mutants in barley. *Plant Biotechnol. J.* 6: 477–485.
- Tester, M. and Langridge, P. (2010) Breeding technologies to increase crop production in a changing world. *Science* 327: 818–822.
- Thanh, P.T., Vladutu, C.I., Kianian, S.F., Thanh, P.T., Ishii, T., Nitta, M., et al. (2014) Molecular genetic analysis of domestication traits in emmer wheat. I: map construction and QTL analysis using an F2 Population. *Biotechnol. Biotechnol. Equip.* 27: 3627–3637.
- The Government Office for Science. (2011) Foresight: the future of food and farming. Final project report. The Government Office for Science, London.
- Till, B.J., Cooper, J., Tai, T.H., Colowit, P., Greene, E.A., Henikoff, S., et al. (2007) Discovery of chemically induced mutations in rice by TILLING. *BMC Plant Biol.* 7: 19.
- Tilman, D., Balzer, C., Hill, J., and Befort, B.L. (2011) Global food demand and the sustainable intensification of agriculture. *Proc Natl Acad Sci U.S.A.* 108: 20260–20264.
- Toensmeier, E. (2016) The Carbon Farming Solution: A Global Toolkit of Perennial Crops and Regenerative Agriculture Practices for Climate Change Mitigation and Food Security. p. 512. Chelsea Green Publishing, White River Junction, VT.
- Tsegaye, B. and Berg, T. (2007) Genetic erosion of Ethiopian tetraploid wheat landraces in Eastern Shewa, Central Ethiopia. *Genet. Res. Crop Evol.* 54: 715–726.
- Uauy, C., Paraiso, F., Colasuonno, P., Tran, R.K., Tsai, H., Berardi, S., et al. (2009) A modified TILLING approach to detect induced mutations in tetraploid and hexaploid wheat. *BMC Plant Biol.* 9: 1–14.
- Udall, J.A. (2003) Breeding for quantitative traits in plants. *Crop Sci.* 43: 1578–1580.
- van de Wouw, M., Kik, C., van Hintum, T., van Treuren, R. and Visser, B. (2010) Genetic erosion in crops: concept, research results and challenges. *Plant Genet. Res.* 8: 1–15.
- Vogel, K.E. (2009) Backcross-breeding. *Methods Mol. Biol.* 526: 161–169.
- Voytas, D.F. (2013) Plant genome engineering with sequence-specific nucleases. *Annu. Rev. Plant Biol.* 64: 327–350.
- Wang, C., Liu, Q., Shen, Y., Hua, Y., Wang, J., Lin, J., et al. (2019) Clonal seeds from hybrid rice by simultaneous genome engineering of meiosis and fertilization genes. *Nat. Biotechnol.* 37: 283–286.
- Wang, K., Shi, L., Liang, X., Zhao, P., Wang, W., Liu, J., et al. (2022) The gene *TaWOX5* overcomes genotype dependency in wheat genetic transformation. *Nat. Plants* 8: 110–117.
- Wang, Y., Cheng, X., Shan, Q., Zhang, Y., Liu, J., Gao, C., et al. (2014) Simultaneous editing of three homoeoalleles in hexaploid bread wheat confers heritable resistance to powdery mildew. *Nat. Biotechnol.* 32: 947–951.
- Wani, S.H., Dutta, T., Neelapu, N.R.R. and Surekha, C. (2017) Transgenic approaches to enhance salt and drought tolerance in plants. *Plant Gene* 11: 219–231.
- Wendt, T., Holme, I., Dockter, C., Preu, A., Thomas, W., Druka, A., et al. (2016) *HvDep1* is a positive regulator of culm elongation and grain size in barley and impacts yield in an environment-dependent manner. *PLoS One* 11: e0168924.
- Westerbergh, A., Lerceteau-Köhler, E., Sameri, M., Bedada, G. and Lundquist, P.-O. (2018) Towards the development of perennial barley for cold temperate climates—evaluation of wild barley relatives as genetic resources. *Sustainability* 10: 1969.
- Willmann, M.R. (2019) Seeds without sex: clonal seed production in rice. *CRISPR J.* 2: 11–14.
- Wingen, L.U., West, C., Leverington-Waite, M., Collier, S., Orford, S., Goram, R., et al. (2017) Wheat landrace genome diversity. *Genetics* 205: 1657–1676.
- Wolter, F., Schindele, P. and Puchta, H. (2019) Plant breeding at the speed of light: the power of CRISPR/Cas to generate directed genetic diversity at multiple sites. *BMC Plant Biol.* 19: 176.
- Yu, H., Lin, T., Meng, X., Du, H., Zhang, J., Liu, G., et al. (2021) A route to de novo domestication of wild allotetraploid rice. *Cell* 184: 1156–1170.e14.
- Zhang, Y., Pribil, M., Palmgren, M. and Gao, C. (2020) A CRISPR way for accelerating improvement of food crops. *Nat. Food* 1: 200–205.
- Zhong, Y., Liu, C., Qi, X., Jiao, Y., Wang, D., Wang, Y., et al. (2019) Mutation of *ZmDMP* enhances haploid induction in maize. *Nat. Plants* 5: 575–580.
- Zsögön, A., Čermák, T., Naves, E.R., Notini, M.M., Edel, K.H., Weinl, S., et al. (2018) De novo domestication of wild tomato using genome editing. *Nat. Biotechnol.* 36: 1211–1216.