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Crop biotechnology and the future of food

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The global population continues to rise, as does the likelihood of reduced yields of major food crops due to the changing climate, thus making the development of genetically improved, stress-resilient crops a research priority. The convergence of low-cost genome sequencing with improved computational power and high-throughput molecular phenotyping technologies has accelerated the identification of genes underlying important agronomic traits relevant to food production and quality. Here, we discuss the evolution of plant improvement, and how researchers leverage genomic analyses and revolutionary new plant breeding technologies like site-directed nucleases to enhance food crop traits through agricultural biotechnology. Deployment of these products from the laboratory to the field remains hindered by biological and regulatory bottlenecks that require further development.

The production of food via agriculture began approximately 10,000 years ago, and its history is punctuated by strides in progress both technological and biological. During the relatively recent Green Revolution of the 1960s, international research investment in agricultural improvement of the cereal grains wheat, rice and maize resulted in new high-yielding varieties that saw widespread cultivation and gave greater food security in many parts of the world[1]. The subsequent rise in molecular genetic tools has ushered in the era of genomic breeding, wherein molecular breeding and genetic engineering have gained prominence[2].

By 2050, the global population is predicted to reach 9.7 billion. Meeting this higher food demand, if consumption practices and food waste do not change, requires estimated food production increases of 25 to 100 per cent[3,4]. At the same time, crop yield is stagnating in many parts of the world[5], and climate change threatens the worldwide agricultural system[6,7] with yields and nutritional content predicted to decline for major crops[8–10]. Additionally, crop pathogen and insect pest ranges are shifting into new territory towards the global poles[11]. These challenges to sustained food security will require multiple solutions encompassing social, technological and economic change. One part of the solution is intrinsic improvement of cultivated crops[2].

Increasing genomic and phenotypic information is becoming available as technology improves and costs decrease. New plant breeding techniques reduce the time necessary to enhance agronomic traits relative to conventional breeding. These techniques have potential to deliver improvements such as greater abiotic and biotic stress tolerance to minimize yield losses, and the improvement of food nutrition and quality. Underutilized and regionally important crops, often adapted to grow on marginal lands, can be further improved and grown more widely to diversify the global diet. We discuss how the application of new biomolecular and mechanistic tools provides greater understanding of the genetics and physiology underlying crop plant performance, and we address how those new tools are being applied to innovations in food production and quality.

Evolution of plant breeding in the computational era

Humans have manipulated plant genomes for millennia, long before the modern understanding of DNA underlying heritable genetics[12]. Early domestication of wild annual species occurred via selection for characteristics including upright vegetative structure, uniform flowering, seed retention on the plant for easier harvest, and reductions in seed dormancy and toxic chemicals in edible tissues[13]. This suite of traits, termed the 'domestication syndrome', typically renders domesticated crops poorly adapted to growth outside of human cultivation. Through iterative selective breeding, specific versions of genes (alleles) underlying these traits and their surrounding DNA regions became uniformly present across the domesticated breeding population[14]. (Box 1 provides a glossary of key terms.) Examples include the *teosinte branched 1* allele that is a major contributor to modern-day maize plant architecture[15] and the *Bitter fruit* allele responsible for reducing cucumber fruit bitterness[16]. Continued selection of these domesticated species further optimized agronomic traits, and geographic dispersal established locally adapted landrace cultivars[17]. For some species, further breeding led to globally adapted elite cultivars produced by seed companies and research institutions. While substantial research has been invested in producing new varieties of select crops (for example, maize, wheat and rice), so-called orphan crops—often staple foods like cassava, millet and sweet potato for people in developing areas of the world—have received less international genomic breeding focus[18].

Genetic diversity is crucial for continued crop improvement. Many crops possess reduced genetic diversity relative to their wild ancestors, though generally this effect is more prevalent in annual plants (for example, soybean and wheat) than in long-lived perennial species that extensively outcross and are often clonally propagated[19]. Domesticated species may harbour elevated numbers of deleterious mutations (see ref. [20] and references within). Novel genetic diversity can be introduced via induced mutagenesis using irradiation (for example, X-rays and gamma rays) or chemical treatment, causing DNA breakages, deletions and/or base changes that result in new alleles; a subset will have effects on agronomic and quality traits[21]. This process creates random genome-wide mutations, necessitating a multigenerational screening and selection pipeline to identify high-performing mutant individuals. The global FAO/IAEA Mutant Variety Database (http://mvd.iaea.org) documents over 3,300 mutant varieties representing over 230 plant species that have been officially released since 1950. Examples include mutant alleles for the semi-dwarfing trait key in Green Revolution varieties of wheat, maize and rice[22], and seedless mandarin citrus (PAU Kinnow-1)[23]. Landrace varieties and related

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Box 1 | Glossary of terminology

Agrobacterium tumefaciens. A naturally occurring plant pathogenic bacterium that delivers DNA into host cells as part of its infection process. Modified lab strains deliver DNA of interest into susceptible plant cells, where it is randomly integrated into the host genome. This property was exploited to develop mutant populations in model systems for functional gene analysis, which has provided the basis of much fundamental plant research over the past decades.

Allele. Alternative forms of a gene at a chromosomal location that arise through mutation. Different alleles may vary by small DNA changes, encode multiple forms of a protein or possess structural changes that change when and how a gene is active. The diversity of alleles for a trait in a population is often greater than those present in any single individual; breeding is the directed accumulation of desired alleles from a population into an elite variety.

Clonal propagation. Asexual propagation that retains specific combinations of alleles present in the parent plant. This process can be used for rapid generation of additional crop plants like those that reproduce infrequently or are sterile (for example, bananas), or in grafting to fuse plants with desired traits to a genetically dissimilar rootstock.

Epigenetics. Heritable chemical markers on DNA that influences how genes are read, causing activation or repression of gene function. These markers are enzymatically added or removed, and typically vary based on cell identity and response to external cues.

Genomic selection. An accelerated breeding approach that utilizes models trained on genomic and phenotypic data to computationally predict breeding values for candidate lines based on their genotype, to improve varieties.

Haploid/diploid. The number of complete genome copies an organism possesses is termed ploidy. Gamete cells like pollen and ovules typically are haploid, containing just one copy of each chromosome, one genome copy. Adult animals and many plant species are typically a fusion of two gametes and possess one pair of each chromosome, termed diploid. Additional copies of genomes can arise through whole genome duplication or hybridization. For example, bread wheat is hexaploid (six genomic copies), while strawberries are octoploid.

Hybrid vigour. The improved growth and yield displayed by specific hybrid offspring relative to either parent line. This performance is due to a unique combination of alleles created by crossbreeding specific genetically distinct inbred parents and becomes variable in subsequent generations due to further segregation of these alleles.

Nanomaterials. A broad class of chemically and structurally diverse synthetic materials with at least one dimension between 1 and 100 nanometres, such as gold particles or carbon nanofilaments, which can be used as delivery systems for biological molecules into plant and animal cells. Nanomaterials can be designed for cell and organelle specificity, making them applicable to the agricultural and biomedical fields.

Omics. The generic term for study of large-scale data of a biological class, such as the total complement of genes or chemical metabolites present in an organism. Detection technologies vary by type of molecular component being assessed, may quantitate total or relative abundance, or provide other specific information.

Orphan crops. Also termed underutilized crops, underinvested crops, or crops for the future. Orphan crops encompass all food types: cereals, root and tubers, legumes, and vegetables. Although they provide nutrition to large numbers of people, these crops have historically received little attention by the international research community, and thus genomic data and molecular tools remain limited.

Particle bombardment. Also termed biolistics or gene gun, this delivery system uses helium under high pressure to propel microparticles bonded with DNA, RNA and/or protein into cells. These particles break through cell walls and penetrate to the interior, where the biomolecules dissociate and perform biological functions.

Selective breeding. The reiterative process of assessing genetically heterogeneous organisms for desired traits and combining these advantageous characteristics to improve cultivars. Genetic diversity can originate from multiple sources, including DNA recombination, sexual outcrossing or induced mutations.

Site-directed nuclease. A protein enzyme that cleaves DNA at locations specified by chemical interactions between cellular DNA and protein molecules (for example, zinc-finger nuclease or TALEN) or RNA (for example, CRISPR-Cas) sequences. Canonical nucleases break both strands of double-stranded DNA, while modified enzyme variants can cleave only one strand, or perform other biochemical functions.

Transgenic. Plants that have integrated genetic material artificially introduced from a foreign source are classified as transgenic. This transgene DNA is commonly from related plant species, bacteria or synthetic origin, and includes regulatory sequences that can specify cell- or tissue-specific gene expression.

wild populations also serve as sources to introduce diversity, such as desired stress-adaptive traits, using genetic markers strongly linked to or underlying the desired phenotype to guide breeding selection[24,25]. For example, the landrace rice cultivar Flood Resistant (FR) 13A possesses the ability to survive in complete water submergence for multiple weeks, but lacks the desired high yield and high-quality grain of other commercial cultivars[26]. Severe flooding and prolonged, complete submergence of rice plants results in crop and yield losses for most commercial varieties. Crossbreeding generated an FR13A-derived rice population, and subsequent genetic mapping identified the gene underlying its submergence tolerance as *Sub1A-1*, a gene absent from intolerant varieties[26–28] (Fig. 1b). Precise genetic-marker-assisted breeding was used to introduce the *Sub1A-1* gene into several farmer-preferred rice cultivars throughout South and Southeast Asia[29], while selecting against introduction of undesired genes of the donor parent, accruing benefits to generationally disadvantaged farmers[30].

Using omics technology to augment traditional breeding programmes. Recent technological advances have increased the ubiquity of molecular '-omics' studies in plant science, a term denoting the total complement of a biological unit like genes (genome) or proteins (proteome). With modern high-throughput genome sequencing technology more accessible at lowering costs

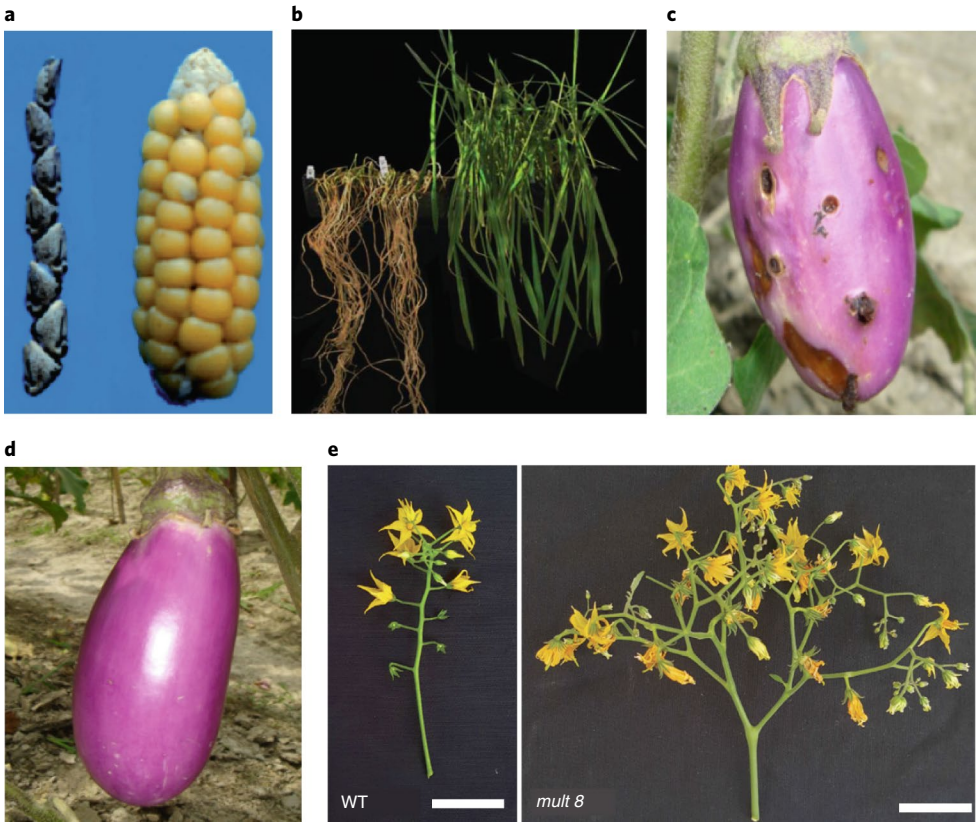

Fig. 1 | Examples of genetically altered agricultural species. a, Inflorescence with fruits from maize progenitor teosinte (left) and primitive domesticated maize (right). **b**, Submergence tolerance is conferred by the presence of the *Sub1a-1* gene (right) compared with control rice (left) after two weeks of submergence, followed by two weeks of recovery[30]. **c**,**d**, Eggplant variety Uttara showing injury from eggplant fruit and shoot borer (**c**) and lack of injury in Bt eggplant variety[131] (**d**). **e**, Wild tomato *S. pimpinellifolium* flower WT morphology (left) and after Cas9 targeted mutagenesis of the *MULTIFLORA* locus (right) during de novo domestication[113]. Scale bars, 2 cm. Figure reproduced with permission from John Doebley (**a**); ref. [30], Laboratory of Pamela Ronald (**b**); ref. [131], Cold Spring Harbor Laboratory Press (**c**,**d**); ref. [113], Springer Nature America, Inc (**e**).

(US$1,000–10,000s), the depth of crop variety and breadth of species genomic data is quickly expanding. For example, collaborative sequencing efforts have produced whole genome sequences for 3,000 rice varieties from 89 countries[31], and the African Orphan Crops Consortium aims to sequence 101 genomes of important native crops, including finger millet (*Eleusine coracana*) and Bambara groundnut (*Vigna subterranea*)[32]. To capture the diversity of specific gene families within a large group, genomic DNA samples can be preferentially enriched prior to sequencing. This method has been used to define genetic variation in disease resistance gene repertoires in Solanaceae and Triticeae (RenSeq[33,34]), and gluten gene families in bread wheat (GlutEnSeq[35]).

Robotics and other technologies are speeding acquisition of data on macrophysiology of crop plants[36], while an assortment of high-throughput detection technologies can be employed to produce physiological data on RNA, protein, metabolites and other molecular metrics shaped by the plant's genomics and its environment. Network-based analyses using a broad array of transcriptomic data can identify major regulatory hubs underlying adaptive responses to environmental stress (for example, refs. [37–39]). Plant proteomic analysis may detect context-dependent protein abundance and the presence of transient chemical modifications that alter their biological function[40]. Recently, a proteomic survey of 13 species across the green plant lineage has been reported, identifying known and novel conserved multi-protein complexes and protein interactions; genes encoding proteins in these complexes likely play a role in important agronomic traits[41].

Computational correlative association studies synthesize the information contained in these agronomic, proteomic, transcriptomic and/or metabolomic datasets to find genes with large and small effects in crop phenotype to guide manipulation of complex traits[42] (Fig. 2). Recent research for tomato flavour improvement exemplifies this application. Flavour is a complex combination of the taste of numerous sugars, acids and bitter chemical metabolites and the smell of volatile aromatic compounds, governed by numerous genetic determinants that make it a difficult breeding goal[43]. Indeed, historic selection for a gene underlying pale green, evenly ripening tomato fruits also had a deleterious effect on total sugar content[44]; breeding for increased fruit size or pink colouration also altered its chemical profile[45]. Multiple studies paired quantification of a select number of tomato metabolites (~20–80) with genetic markers in wild and cultivated tomato fruits to identify genes underlying specific potential flavour determinants[46–48]. Consumer flavour preference surveys were paired with analysis of 398 modern, heirloom and wild accessions to identify which compounds and genetic markers were correlated with positive taste preference[49]. Work by Zhu et al. expanded the metabolite panel to quantify 980 distinct fruit chemical metabolites in 610 wild and commercial red-fruited tomato accessions and paired that information with genetic sequence and gene expression network analysis, describing how domestication and breeding have altered tomato metabolites[45]. Most recently, the first meta-analysis of previous association studies in tomato[46,47,49] compiled data from 775 tomato accessions and identified 305 genes tied to sugars, acids and flavour-related volatiles[50].

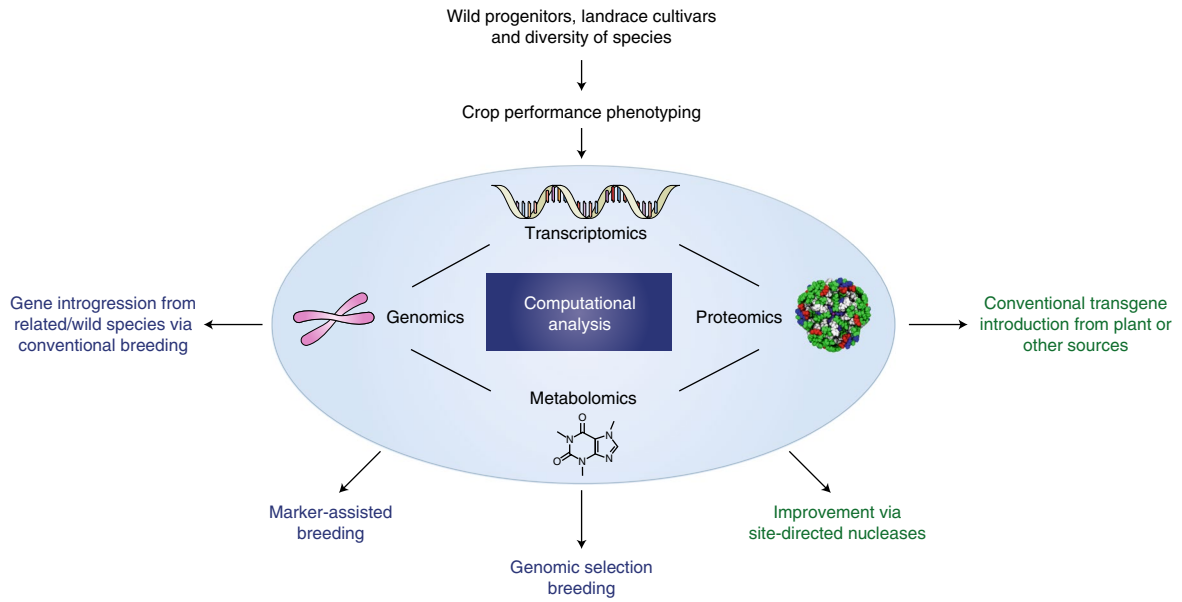

Fig. 2 | Omics data can be used to inform marker-assisted breeding, genomic selection and site-directed nuclease targets. Plant population diversity can be characterized for agronomic traits through field phenotyping and using omics technologies that capture genome sequence (genomics) and RNA (transcriptomics), protein (proteomics) and metabolite abundance (metabolomics) under a number of conditions. Computational algorithms and correlation analysis of these different data sets can uncover linkages between genes and macro and molecular phenotypes and identify novel genes with potential agronomic value. These data can be used to generate markers for use in marker-assisted selection breeding, to produce predictive models for use in genomic selection or guide introgression of genes from wild sources without the use of biotechnology (blue text). Use of omics data and phenotypic analysis can also identify gene candidates for manipulation via site-directed nucleases (including targeted mutagenesis and targeted gene insertion) and inform conventional introduction of genes or multi-gene clusters for creation of stable transgenic lines using biotechnology tools (green text). Credit: Scott Camazine / Alamy Stock Photo (protein structure)

The genetic locations identified in these studies represent potential targets for manipulation, either through conventional breeding or biotechnological approaches.

Additionally, data from genome sequencing, gene association studies and phenotypic data can be used to generate and train machine-learning predictive models for use in genomic selection plant breeding (reviewed in ref. [51]). This accelerated process uses a trained computational model to select lines based on their genetic markers without repeated phenotyping during breeding cycles. Such models can be repeatedly improved with data from high-throughput phenotyping technologies in both research and field settings, utilizing stress-phenotyping via imaging sensors to detect disease, drought and nutrient deficiency, among others[52].

Gene-editing tools enable customization of germplasm

The discovery and application of targetable site-directed nuclease (SDN) enzymes ushered in a new era of plant mutagenesis breeding, giving researchers powerful tools for precision manipulation of crop genomes (broadly termed 'gene editing') to leverage the wealth of genomic data. Among these, RNA-guided CRISPR-Cas technology has become a dominant tool since 2013, when gene editing capacity was demonstrated in plant cells. The gene editing toolkit has bloomed in the short period that has followed, with Cas enzyme variants discovered or developed that enable A/T to G/C DNA base-pair swapping, direct gene repression or activation, directed DNA methylation and RNA targeting. For specific details of current CRISPR technology, Cas variants and gene-editing methods, readers are referred to a recent review[53].

Application of site-directed nucleases to molecular breeding of food crops. Site-directed nucleases, also called sequence-specific nucleases, create a targeted breakage in DNA. Two distinct mechanisms within the plant cell exist to repair this damage: non-homologous end joining, in which broken DNA strands are reattached, often creating small deletions or insertions of DNA bases on either end of the break, and homology directed repair, in which the gap is bridged using a template that shares sequence with the DNA region surrounding the break[54]. These repair processes can be exploited to enable the introduction of several genetic changes of different classifications, including: mutation of gene function through random error-prone DNA repair via end joining (classified as site-directed nuclease application 1 (SDN1), see Fig. 3); allele replacement through repair mechanisms using supplied short DNA fragments as a template (SDN2); or targeted insertion of DNA at the cut site using supplied long template DNA (SDN3). In non-reproductive plant cells, non-homologous end joining repair occurs at high efficiency, while homology directed repair occurs with low efficiency[55]. Introduced DNA breakages can also stimulate targeted recombination between homologous chromosomes. Cas9-induced cuts were used to trigger recombination in tomato somatic cells to alter fruit colour[56], suggesting the potential application of the technology to break inheritance linkages between physically close genes or combine favourable alleles onto one chromosome.

Improvement of cultivar performance and product quality can be achieved via deactivation of genes with undesirable effects, or modification of regulatory regions governing when and how genes are expressed[57]. Targeted mutagenesis without inserting foreign DNA at the target site (SDN1) accounts for roughly 90 per cent of SDN usage thus far in published agricultural research[58] due to its relative ease and can be viewed as an evolution of mutation breeding previously discussed. Cas cutting specificity is determined by an associated guide RNA molecule that can bind to the target DNA sequence. Supplying the enzyme with multiple guide RNAs allows for simultaneous changes at multiple locations across chromosomes within a single cell[59] or allows for large deletions of DNA[60] when

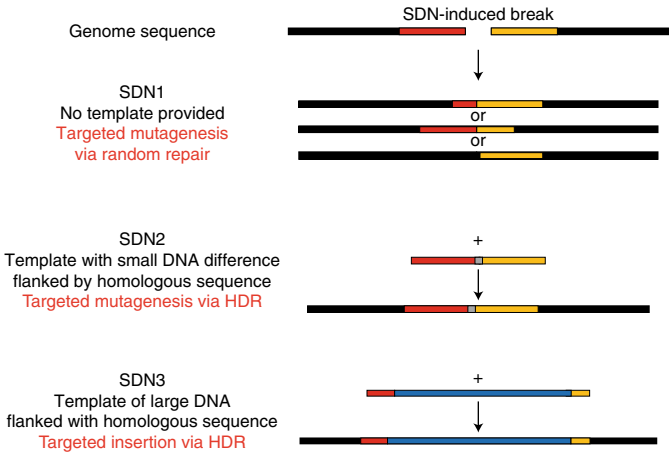

Fig. 3 | Classification of possible products produced via site-directed nuclease technology. Three classifications for application of site-directed nucleases (SDN) are established based on European Union working group guidelines[186], resulting in end products possibly governed by differing regulations. Recovery of plants with targeted mutations that result from only the random non-homologous end joining repair mechanism of the cell with no donor template are classified as SDN1—the most commonly applied SDN approach in plants to date. Such edits are largely insertions or deletions that occur surrounding the double stranded DNA break. Plants classified as SDN2 or SDN3 both utilize a supplied repair template and the homology directed repair (HDR) process but vary in degree of the introduced change. SDN2 is typically classified by templates that induce small alterations (<20 base pairs), while SDN3 templates can include one or multiple genes flanked by homologous regions and result in either gene replacement or targeted insertion of foreign DNA at DNA cut sites. Two cut sites can be cleaved simultaneously to excise larger DNA fragments to achieve complete gene deletion (SDN1) or to effect gene replacement via SDN3.

narrowly targeted to one location. This multiplexing allows the stacking of multiple altered traits in a single generation[59] and can streamline trait development in species like wheat and potato, which contain complex genomes with many copies of redundant genes (summarized in ref. [61]). Because these mutations do not directly incorporate foreign genetic material, it is possible to produce transgene-free plants carrying these edited traits that are virtually indistinguishable from their conventionally produced counterparts, and in some countries these products are exempt from transgenic regulation[62]. Application of this technology can accelerate innovation in both annual crops and perennial woody crops. For example, gene editing techniques can reduce the time for coffee cultivar improvement from thirty years in a traditional breeding scheme to as few as six[63]. Here, we highlight the diversity of potential agricultural and food trait improvement with select examples.

Breeding to increase crop yield performance remains a high priority. Yield is a complex trait representing the cumulative output of plant architecture, nutrient acquisition, and resource allocation limited by disease, pests and abiotic stress encountered throughout the growing season per area of land. Developing new allelic variation in genes modulating plant developmental pathways, among others, holds potential for improving crop productivity[64] and land usage, and is particularly important for genomic regions where domestication selectively reduced the allele diversity of the breeding population. Rodríguez-Leal and colleagues demonstrated this approach in tomato, targeting DNA controlling when and how developmental genes underlying fruit size, flower number, or plant shoot architecture are active[60]. The resulting edited populations possessed new alleles with a spectrum of plant changes, reiterating previously isolated, naturally occurring phenotypes and creating new ones that can be incorporated into breeding programmes. In *Brassica napus* (canola), leaf and seed number[65] and seed yield per plant[66] were increased by mutating development and plant architecture genes. In cereal crops such as rice, genes restraining grain size and seed number[67–69] are promising targets. New alleles for yield genes *Grain number 1a* and *DENSE AND ERECT PANICLES 1* were created that each had superior performance relative to naturally occurring high-yield alleles[68] in field trials, and combining alleles generated in elite rice lines had additive effects on grain size and grain number[67]. Altering the response to plant growth regulating hormones also increased plant size and flower number, resulting in 30 per cent greater grain yield in field trials[70].

Plant diseases and pests, including fungi, bacteria, oomycetes and nematodes, reduce global yield of major crops by an estimated 17 to 30 per cent[71], with higher losses found in food-insecure regions. One method to engineer enhanced disease resistance is to remove plant genes that facilitate disease susceptibility, either because they suppress plant immune responses or they are required by the plant pathogen for its growth and proliferation[72]. Such susceptibility genes have been identified widely in crop species in relation to many disease-causing organisms of agronomic importance (reviewed in ref. [73]) and are often conserved between species. For example, breeders have used a naturally occurring mutant allele of the *MILDEW RESISTANCE LOCUS O* (*MLO*) gene to confer heritable broad-spectrum immunity against powdery mildew races in susceptible barley cultivars for decades; researchers used SDNs to edit the corresponding *MLO* genes in tomato[74] and wheat[75] to generate similar broad resistance against the powdery mildew species infecting these crops. The first application of CRISPR technology in the cocoa tree *Theobroma cacao* deactivated a conserved immune suppression gene to hinder development of *Phytophthora* pathogen infection[76]. Editing of susceptibility gene promoter regions needed by bacterial leaf blight and citrus canker pathogens significantly reduced disease symptoms in rice[77,78] and orange[79]. Broad viral resistance in cucumber was deployed through editing of the cucumber *eIF4E* gene necessary for viral spread[80], while viral infection symptoms were attenuated in *eIF4E*-edited cassava[81]. Because susceptibility genes may possess necessary function in the host plant in the absence of disease, not all may be acceptable targets for modification in this manner.

Targeted mutagenesis has also been used to enhance the quality of food, particularly related to removing undesired compounds and increasing nutritional value. For example, many plant food products contain allergens, often seed storage proteins, perceived by the human immune system in a small segment of the population[82]. The main therapeutic strategy for those with a food allergy is avoidance of these inciting compounds. Gene-editing tools allow removal of genes encoding these allergens in food products, potentially creating a more hypoallergenic product. Upon completion of the hexaploid bread wheat genome, genes encoding gluten proteins linked to wheat intolerance (for example, coeliac disease and baker's asthma) were identified and mapped across all chromosomes[83]; the large number and distribution of these genes has likely hindered breeding efforts to produce low-allergenic wheat. Gluten is a polymer largely composed of two classes of protein: glutenins and α-, γ- and ω-gliadins. Gene editing successfully disrupted ~30 α-gliadin genes in bread wheat[84], resulting in up to an 85 per cent reduction in detectable immunoreactive gluten in seed grains. Unlike previous efforts to reduce gluten proteins through RNA silencing methods that require a permanent transgene in the wheat[85], transgene-free lines were isolated with these heritable α-gliadin mutations. Genes encoding major allergens from apple[86], peanut[87], rice[88] and soy[89] are potential gene editing targets to ameliorate food allergy symptoms. Likewise, manipulation of innate metabolism genes can alter the nutritional profile of the food. The tomato antioxidant

compound lycopene, responsible for the fruit's red colour, and the health-promoting compound γ-aminobutyric acid were highly enriched[90–92] by manipulating biosynthesis genes to favour accumulation of these compounds. In lettuce, removal of regulatory DNA regions enhanced production of vitamin C in the leaf threefold[93]. In sorghum, deletion of multiple α-kafirin gene regions encoding seed storage proteins enhanced protein digestibility and increased overall essential amino acid lysine and total protein content of the grain[94]. Soybean seed oil genes were altered to increase fourfold the percentage of the polyunsaturated fat oleic acid[95], and oleic acid levels could also be modulated by different mutation combinations of redundant genes in *Camelina sativa*[96].

Gene editing has also been applied to traits that prolong the post-harvest shelf life in certain food crops, potentially reducing food waste along the supply chain[97]. Transgenic silencing of polyphenol oxidase genes reduces potato tuber browning after processing[98], and this trait was subsequently commercialized after application to transgenic non-browning Arctic apples[99]. Recently, targeted mutagenesis was used to create varieties of non-browning, transgene-free white button mushrooms[100], potatoes[101] and romaine lettuce[102,103]. Development of ripe tomatoes that remain firm longer has been a long-sought goal briefly realized in the Flavr Savr tomato, in which transgenic gene silencing reduced activity of cell-wall modifying enzyme that contributes to fruit softening[104]. Recent manipulation of a different gene has yielded firmer ripe fruits[105] with greater resistance to fungal rot[106] that retain flavour-contributing metabolite profiles[107].

Creating multi-generational hybrid vigour. Agriculture of the past century has widely exploited the genetic phenomenon of hybrid vigour, or heterosis, to produce high-performing, genetically uniform F_1 hybrid crops that possess variable yield in subsequent generations due to genes segregating during sexual reproduction. Mutagenesis of three genes creates the genotype *Mitosis instead of Meiosis* (*MiMe*) in diploid rice, which results in clonal diploid gametes instead of haploid gametes due to the doubling of the genome without two rounds of segregation and cell division[108]. When *MiMe* is paired with the pollen mutation *matrilineal* that prevents transfer of pollen-derived DNA to the embryo, self-fertilization in F_1 hybrids produced clonal seeds that retain the F_1 diploid genotype[109]. Another study in rice achieved a similar result by combining the *MiMe* genotype with transgenic cell-specific expression of the embryogenic gene *Baby Boom 1*, which stimulates embryo development without fertilization[110]. However, this application remains in the early phases and further optimization is necessary before commercial adoption, as clonal seed production was reported to occur at low rates ranging from 5 to 29 per cent.

Increase the diversity of cultivated species through rapid domestication. Another promising approach to expand the food palate is through de novo domestication, in which domestication alleles are introduced to wild species possessing desired stress tolerance or adaptation to marginal lands, compressing thousands of years of human mutagenesis and selective breeding to a few plant generations[111]. This strategy has been demonstrated in the wild tomato relative *Solanum pimpinellifolium*, as simultaneous editing via CRISPR of four[112] and six[113] known domestication genes was sufficient to remodel shoot and flower morphology (Fig. 1e) and increase the size and number of fruits to be similar to cultivated tomato *Solanum lycopersicum* within a single generation. De novo domesticated fruits contained 500 per cent higher levels of lycopene than a commercial tomato variety[113] and retained disease resistance and salinity tolerance traits of the wild parent[112]. Similarly, recent stacking of mutations modifying flowering time, seed retention, removal of undesired chemicals and enrichment of oleic acids in seeds brings the weed pennycress (*Thlaspi arvense* L.) closer to future use as a winter cover crop and source of oilseed for food[114].

As costs further decline and genomic data and resources accrue, gene editing can facilitate rapid improvement and greater adoption of orphan crops and landrace cultivars[115]. Lemmon and co-workers generated whole genome sequence, gene expression data, and gene editing protocols for the groundcherry *Physalis grisea* with the goal of transforming this fruit into a mass-produced berry crop among the rank of strawberry and raspberry. Weedy, sprawling groundcherry plant growth was compacted, and fruit size and number increased[116,117]. Genome editing protocols have also proved successful in cassava, an orphan crop grown for uses in both food and manufacturing. The cassava tuber starch profile was altered through modification of amylose synthesis genes, conferring desirable cooking and food processing properties[118]. Careful elimination of genes responsible for toxic compound formation could also enhance nutritional quality and processing in cassava[119] and grass pea (*Lathyrus sativus*)[120]. As genomic information and biotech methods on wild and orphan species continues to develop, editing of known domestication genes[111,121] will further expand crop diversity and food options.

Insertion of transgenes and synthetic gene clusters

The process of plant transformation, in which genetic material is introduced and integrated into the heritable plant genome, has been a fundamental tool in the development of cultivated genetically engineered food, fibre and biofuel crops. Trait design can introduce single or multiple genes to affect a phenotype, or introduce DNA sequences that cause silencing of endogenous genes. Inclusion of regulatory DNA elements can produce selective effects that are difficult to achieve via targeted mutagenesis. For example, seed-specific gene silencing of widely expressed gossypol synthesis genes in cotton was used to engineer seed with minimal levels of the toxic compound, making them fit for human consumption while preserving gossypol content in vegetative parts as a necessary chemical deterrent against pathogens and insect pests[122]. Transgenic products have historically received higher scrutiny from government regulatory bodies, vastly increasing costs and time to develop and commercialize transgenics compared with conventional varieties[123,124]. In 2018, 26 countries cultivated 191.7 million hectares of genetically engineered crops, with the United States, Brazil, Argentina, Canada and India collectively representing 91 per cent of the global transgenic crop area[125].

One of the most prevalent engineered traits is insect resistance conferred by genes originating from the soil bacterium *Bacillus thuringiensis* (Bt). The Bt trait has been applied to many crops, including maize, soybean, cotton and eggplant[126], and the cumulative use of Bt maize, soybean and cotton crops has resulted in 37 per cent less global pesticide use[127] and potential insect pest-suppressive benefits to nearby non-Bt crops[128]. In India, Bt cotton pesticide application was reduced by 50 per cent and, accordingly, reduced acute pesticide poisonings were seen in cotton growers[129]. The Bt trait in maize has also led to increased consumer safety through reduced grain contamination with mycotoxins produced by fungal infections that can follow insect damage[130]. In Bangladesh, where introduction of four varieties of Bt eggplant (Fig. 1c,d) in 2014 marked the first genetically engineered food crop released in a developing country and first Bt vegetable, net returns for farmers increased sixfold in part due to a 61% reduction in pesticide costs[131].

The breadth of phenotypic change possible with transgenesis is far greater than can be achieved through gene editing alone in part due to the wide genetic diversity that can be utilized, including across species with innate reproductive barriers (for example, ref. [132]). Assembling a repertoire of disease-resistance genes from numerous accessions or species, as well as rationally designed novel resistance genes[133], could provide durable, broad-based resistance

against plant pathogens. Applications of synthetic biology, the creation of modular DNA components and rationale design of genetic circuits to confer novel traits, can accelerate improvement of fundamental plant biological processes (reviewed in ref. [134]). For example, the enzyme RuBisCo is responsible for atmospheric carbon fixation in photosynthesis but can also utilize atmospheric oxygen in a process called photorespiration to produce glycolate, which can inhibit photosynthesis. Photorespiration rate increases with temperature, thus reducing photosynthetic efficiency, and ultimately sacrificing growth of the plant. Introducing alternative biosynthetic pathways using bacterial or plant enzymes to convert glycolate into a usable form with introduction of three to five genes was sufficient to increase photosynthetic efficiency and vegetative biomass in field trials in tobacco[135] and laboratory tests in the seed oil crop *Camelina sativa*[136]. Bacterial-derived traits that allow rice and cotton to metabolize phosphite in addition to phosphate[137,138] permits phosphite's dual use as both weed control agent and fertilizer. Extending symbiotic nitrogen fixation capability (reviewed in ref. [139]) and production of nutritional and biopharmaceutical compounds are also likely routes to further augment agricultural productivity and product value. Examples of biofortification of foods with new nutrients not found in conventional varieties include the enhanced accumulation of the vitamin A precursor beta-carotene in edible tissue of rice (Golden Rice[140,141]) and Cavendish bananas (Banana21[142]) and the carotenoid antioxidant astaxanthin in rice grains[143].

Use of site-directed nucleases for targeted gene insertion or replacement. Commonly used methods of *Agrobacterium tumefaciens*-mediated and DNA-coated particle bombardment transformation introduce transgenic sequences randomly in the crop genome with potential for endogenous gene disruption or misexpression of neighbouring genes through *trans*- or *cis*-gene regulation. Another potential drawback of random DNA insertion is that, when multiple transgenes are used, these transgenes will likely integrate into different chromosomes and each will segregate independently, complicating downstream breeding. To address these potential shortcomings, researchers are investigating the use of SDNs to precisely introduce DNA elements into the plant genome at targeted DNA breakages via the plant's DNA homology directed repair pathway. Multiple introduced genes can be incorporated at a single genomic safe harbour, a defined chromosomal region with minimal positional effects to maximize efficacy of the transgene without perturbing necessary cell functions[144]. Ainley et al. developed a DNA 'landing pad' that when integrated into the genome allows sequential stacking of modular transgenes through defined sequence-specific nuclease cut sites. Its use in maize demonstrated co-segregation of inserted traits in the subsequent generation[145]. Despite these advances, site-specific integration of DNA via SDNs remains challenging in plants, as homology-directed repair is not always active during the plant cell life cycle, and it is difficult to deliver sufficient target DNA near the DNA breakage for high-efficiency insertion[146]. Efforts to address this include biasing the cell's repair machinery to favour homology-directed repair (for example, ref. [147]) and employing plant viral replication machinery to increase the amount of donor DNA, the latter of which has enhanced targeted insertion efficiency in tobacco[148], tomato[149] and wheat[150].

New methods to expand plant transformation technology

Generating transformed plants is often a major bottleneck for crop improvement[151]. Biological characteristics, such as recalcitrance to foreign DNA uptake and genomic integration or capacity for regeneration into whole plants after transformation, limit genetic engineering to amenable plant cultivars. The rigid plant cell wall presents a specific challenge to plant transformation protocols relative to those of mammalian cells, which lack such a barrier. Additionally, the transformation method employed can trigger differing governmental regulations and approval timelines for end products; for example, products produced in the United States using *Agrobacterium* fall under United States Department of Agriculture (USDA) oversight[152,153] and cannot be released until deregulated or non-regulated status is granted.

In plants, *Agrobacterium* transformation is restricted to susceptible host plant species. Bombardment can carry both DNA and/or other biomolecules such as gene-editing protein/RNA complexes[154], but the projectiles damage the impacted tissues. In both cases, production of suitable starting material and recovery of transformed plants can be time and labour intensive, typically extending several months, and unintended changes to the genome are likely[155,156]. A key research goal going forward is expanding the scope of transformable species and increasing transformation efficiency. Recent protocols couple transformation with expression of growth and developmental regulator genes *Baby Boom* and *Wuschel* to markedly improve transformation rates in the monocots maize, sorghum, sugarcane and rice[157], and refinement of this system shortened the timeframe for recovery of transformed plants to as little as four weeks[158]. The technique was successfully applied to vegetative leaf tissue for the first time in maize, reducing the time needed to grow adult plants to the flowering stage for source tissues. Similarly, developmental regulators were recently used to induce de novo transgenic shoots from vegetative tissues of tobacco, tomato, grape and potato[159].

Bypassing tissue culture methods for producing modified plants. The use of tissue culture to generate germplasm can yield unintended genomic changes affecting fitness or yield of the end product, necessitating additional screening of individual lines. This is a long-known phenomenon named somaclonal variation[160]. Whole genome sequencing revealed an increase in DNA deletions and variations in cultured tissues of rice[161], cotton[162] and potato[163]. Epigenetic marks widespread in the genome can be lost or retained, with these changes persisting for multiple generations after regeneration from tissue culture[164–166]. In some cases, these plants have altered gene expression profiles that resemble their source tissue[167] and display aberrant interactions with beneficial and pathogenic microorganisms. While sufficient screening and field testing can identify properly functioning individuals, the development of new methods bypassing or limiting tissue culture is desirable[151].

Newly developed nanomaterials enable passive delivery of biomolecules such as DNA, RNA and proteins into cells for, among other applications, integration into, or gene editing of, host genomes. Physical and chemical properties of these materials such as size and electric charge can be tailored to allow uptake across plant cell walls and membranes of both plant and animal cells (see ref. [168] and references within), with application as simple as a foliar spray. Carbon nanomaterials have been demonstrated to passively traverse the plant cell wall to deliver DNA into leaf cells of arugula, wheat, cotton and the tobacco relative *Nicotiana benthamiana*[169], and selectively into arugula, spinach and tobacco leaf cell chloroplasts[170]. Expression of the delivered genes was short lived (less than 10 days) as the DNA did not integrate into the host genome[169]; such a system could be useful for CRISPR gene-editing in which integration is not necessary for function.

Gametic tissues in crop plants that propagate via sexual reproduction are being targeted to bypass tissue culture regeneration. Zhao et al. used a magnetic field to deliver transgene-carrying nanoparticles through apertures where the cell wall is thin and more permeable in cotton, pepper and pumpkin pollen, producing viable transgenic pollen[171]. Manual fertilization of flowers using this pollen produced seeds with stable, heritable transgene integration. Transgenic pollen can also serve as the delivery vector for gene-editing protein/RNA complexes. Using methods developed previously[172,173], a specific type of maize pollen carrying the

nuclease Cas9 protein fertilizes the egg cell, transferring gene-editing complexes that mutate the egg cell genome. Due to defects in the pollen, none of the genetic material it carries is permanently incorporated to the resulting haploid offspring, which are edited but transgene-free. The gene-editing complex could be passed from corn pollen to corn ovule, as well as corn pollen to wheat ovule. The mechanisms underpinning this haploid-inducing pollen appear conserved across monocot and dicot species[172], suggesting this method may have broad applicability, including genotypes recalcitrant to transformation themselves. However, as the development of the technologies described in this section remain active areas of research, further testing by the research community at large will be necessary to determine reproducibility, efficiency and ease of use for particular crop species.

Further considerations

The convergence of omics technology presents an unprecedented opportunity to identify genes controlling agronomically valuable traits and speed the development of genetically improved cultivars. Still, the lag time between fundamental research advances and commercialization is often lengthy[174]. For example, the process for commercialization of transgenic varieties is affected by a diverse array of political and socioeconomic concerns and can span decades[124], making it difficult to address urgent agricultural needs. Consequently, in many parts of the world, breeders and farmers do not have access to transgenic technologies. For example, while farmers in Bangladesh continue to adopt and cultivate Bt eggplant, the varieties remain prohibited in neighbouring India despite scientific support and similar farmer need[131]. Similarly, organic farmers do not have access to Bt and other transgenic varieties because methods that employ genetic engineering techniques are excluded from use in certified organic production[175], although other types of genetic alteration such as chemical and radiation mutagenesis are permitted[176].

The application of gene editing in agriculture has been met with mixed support among the general public[177], and regulation of transgene-free, gene-edited SDN1 products diverge across the globe (discussed in ref. [62]). For example, in the European Union, the products of gene editing are regulated as "genetically modified" according to a 2018 decision by the Court of Justice of the European Union[178], a designation that complicates European scientific field trials for SDN1/2 improved plants[179] and restricts farmer adoption[178]. By contrast, the USDA has no plans to regulate SDN1 crops as long as these crops are not plant pests or developed using plant pests[180]. This decision potentially saves years or even decades in bringing new varieties to US farmers[181]. The USDA National Organic Program (which develops the rules and regulations for the production, handling, labelling and enforcement of all USDA organic products) has not yet ruled on the use of SDN1 technologies in certified organic production and there is disagreement on how to proceed. For instance, whereas the National Organic Program Standards Board (a federal advisory board made up of 15 volunteers from the organic community) has recommended that diverse SDN technologies be disallowed; researchers in the US and Europe have called for the allowance of such technologies in organic farming systems[182–184].

These examples reflect the need for ongoing engagement of the scientific community with diverse stakeholders, including consumers and politicians, on the challenges faced by farmers and the use of plant biotechnologies to address these challenges. As described by communications scientists Scheufele and Krause, the increasingly polarized political environments and fundamental changes in how information is shared by media and audiences have given new urgency to the problem of the disconnect between public opinion in the United States and the scientific consensus on scientific topics[185].

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Competing interests

The authors declare no competing interests.

Additional information

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