nature reviews bioengineering

https://doi.org/10.1038/s44222-023-00115-8

Review article

Check for updates

Genome-edited foods

Aytug Tuncel[1], Changtian Pan[2], Thorben Sprink[3], Ralf Wilhelm[3], Rodolphe Barrangou[4,5], Li Li[6,7], Patrick M. Shih[8,9,10,11], Rajeev K. Varshney[12], Leena Tripathi[13], Joyce Van Eck[7,14], Kranthi Mandadi[15,16,17] & Yiping Qi[1,18]

Abstract

Genome editing can transform agriculture and shape the future of food by improving crop yields and animal productivity, which in turn can help to achieve food security for the growing world population. CRISPR–Cas-based technologies are powerful gene editing tools that are applied to various food products. In this Review, we discuss the applications of CRISPR–Cas aimed at increasing the nutritional value of crops through macronutrient engineering and biofortification or the reduction of the amount of antinutrients. We examine the role of CRISPR–Cas in improving the flavour of crops and reducing post-harvest losses to increase consumer acceptance and decrease food waste. We also highlight the gene editing of animal food products and probiotics. We summarize the regulations for approval of gene-edited foods worldwide and the progressively evolving public view. Finally, we explore the strategies that can help to enhance the efficiency of genome editing techniques and the acceptance of genome-edited foods in the global market, and extend the technology to low-resource settings.

Sections

Introduction

CRISPR–Cas-based genome modifications in plants

Improving the nutritional value of crops

Genome-edited foods beyond crops

Regulations and evolving public view

Outlook

[1]Department of Plant Science and Landscape Architecture, University of Maryland, College Park, MD, USA. [2]Department of Horticulture, College of Agriculture and Biotechnology, Zhejiang University, Hangzhou, China. [3]Julius Kuehn Institute (JKI) – Federal Research Centre for Cultivated Plants, Institute for Biosafety in Plant Biotechnology, Quedlinburg, Germany. [4]Genomic Sciences Graduate Program, North Carolina State University, Raleigh, NC, USA. [5]Department of Food, Bioprocessing & Nutrition Sciences, North Carolina State University, Raleigh, NC, USA. [6]Robert W. Holley Center for Agriculture and Health, USDA-Agricultural Research Service, Cornell University, Ithaca, NY, USA. [7]Boyce Thompson Institute, Ithaca, NY, USA. [8]Department of Plant and Microbial Biology, University of California, Berkeley, Berkeley, CA, USA. [9]Environmental Genomics and Systems Biology Division, Lawrence Berkeley National Laboratory, Berkeley, CA, USA. [10]Feedstocks Division, Joint BioEnergy Institute, Emeryville, CA, USA. [11]Innovative Genomics Institute, University of California, Berkeley, Berkeley, CA, USA. [12]WA State Agricultural Biotechnology Centre, Centre for Crop and Food Innovation, Food Futures Institute, Murdoch University, Murdoch, WA, Australia. [13]International Institute of Tropical Agriculture (IITA), Nairobi, Kenya. [14]Plant Breeding and Genetics Section, School of Integrative Plant Science, Cornell University, Ithaca, NY, USA. [15]Texas A&M AgriLife Research and Extension Center, Texas A&M University, Weslaco, TX, USA. [16]Department of Plant Pathology and Microbiology, Texas A&M University, College Station, TX, USA. [17]Institute for Advancing Health Through Agriculture, Texas A&M AgriLife, College Station, TX, USA. [18]Institute for Bioscience and Biotechnology Research, University of Maryland, Rockville, MD, USA. e-mail: yiping@umd.edu

Key points

- CRISPR–Cas-mediated genome editing technologies enable the manipulation of the genome, epigenome and transcriptome.
- The macronutrient content of edible plant organs can be altered to create nutritionally healthier crops suitable for specific dietary needs.
- Crops can be engineered to biofortify vitamins, minerals and/or phytonutrients and reduce antinutrient content.
- Organoleptic food characteristics, such as colour, taste, flavour and texture, can be improved to optimize consumption and decrease post-harvest losses.
- Genome editing can be deployed in livestock, fish and bacteria to improve productivity.
- Contextual regulatory support and public acceptance are critical to enable the industrial deployment and commercialization of genome-edited foods.

Introduction

The global population is projected to expand from 8 billion people now to 10 billion by 2050 (ref. 1). The world food production (measured in crop calories) will consequently need to increase by about 50% compared with 2010 (refs. 2,3). Yet, today, around 765 million people face hunger[4]. More than 3 billion people cannot afford a healthy diet due to high costs, income inequality, persistent pandemic conditions and unceasing armed conflicts around the world[4]. Globally, the growth of one in five preschool children is stunted due to an inadequate intake of calories and/or micronutrients[5]. Whereas the undernourishment rate declines slowly, the rates of overweight and obesity soar[6]. The ratio of overweight to undernourished population is around 2.5, with more than 13% of the global population struggling with obesity[6].

Rising purchasing power and urbanization, and the mass production of food transform eating habits towards a calorie-rich diet. Therefore, the demand for fats, animal-based foods, added sugars, refined carbohydrates and processed food grows. Indeed, diet differences between different parts of the world have shrunk considerably over the past 50 years as a result of globalization[7]. Meanwhile, the prevalence of diet-related non-communicable diseases, including type 2 diabetes, cardiovascular complications, cancer and autoimmune disorders, has been increasing due to the high consumption of calorie-rich but nutritionally-poor foods. The global convergence of people's diets increases our dependence on a handful of crops with limited genetic diversity, posing a food security risk in a catastrophe, such as a drought or a pandemic.

The limited genetic diversity of elite cultivars constrains the improvements afforded by conventional breeding, which is complicated in vegetatively propagated crops (for example, potatoes and bananas) and time-consuming in perennial crops (for example, fruit trees). Crossbreeding with donor varieties, such as wild relatives, requires multiple generations of backcrossing to fix the desired trait[8]. Chemical or radiation-induced mutational breeding, first applied about a century ago, may introduce undesired random mutations that cannot be segregated out completely. Transgenic breeding, a technique first developed in the 1980s, which involves the transfer of genes between different species, provides a more straightforward approach to introducing novel traits. However, its application suffers from regulatory hurdles, prolonged commercial approval processes and a negative perception by the public opinion. By contrast, genome editing can be easily implemented as an alternative breeding technique to achieve the precise, fast and cheap production of transgene-free crops.

Genome editing tools, such as CRISPR–Cas, can be utilized to redesign our food. These tools enable the rapid development of nutritionally-rich, high-yield and stress-resilient crops, livestock and aquatic species. They can also be deployed in food-associated bacteria to increase food safety and quality, optimize fermentation and engineer novel probiotics with enhanced survival ability in the digestive tract that can produce more metabolites beneficial to gut health. In this Review, we describe food engineering strategies based on genome editing. We initially summarize how genome editing technologies are used to modify plant genomes; we then discuss the concepts and techniques utilized to increase the nutritional value of crops. Furthermore, we briefly discuss the genome editing of livestock, seafood and probiotics. We also review the status of regulatory policies around the world and the evolving public view of genome-edited foods. Finally, we present our vision on the prospects of plant genome editing and its role in the design of future foods.

CRISPR–Cas-based genome modifications in plants

Over the past three decades, plant genome editing has evolved rapidly from using meganucleases in the 1990s[9] to using zinc-finger nucleases in the 2000s[10] and transcription activator-like effector nucleases (TALENs) in the 2010s[11]. Technologies[12] and applications[13] based on the CRISPR–Cas system[14] have revolutionized genome editing over the past decade, as a result of its efficiency, versatility and ease of multiplexing and implementation[15]. The impact of the technology on plant engineering has been growing since its first demonstration in model plants (*Arabidopsis* and tobacco)[16,17] and crops (rice and wheat)[18].

Different CRISPR-based methods have been developed to introduce programmable changes in the genome, transcriptome and epigenome of plants[19–21] (Fig. 1). As an RNA-guided nuclease system, CRISPR–Cas is used for knocking out genes to create loss-of-function mutations that can be useful for plant metabolic engineering (for example, by disrupting competing pathways and shifting metabolism towards the accumulation of a certain compound) and boosting yield (for example, by decreasing the activity of genes that function as negative regulators in inflorescence development)[8,21]. Alternatively, gain-of-function mutations often require subtle nucleotide changes and can introduce novel traits into plants. However, homology-directed repair (HDR) is difficult to achieve with high efficacy in plants, so base editing is a viable alternative approach. For example, point mutations that hinder herbicide binding to acetolactate synthase (ALS), a key enzyme in the biosynthesis of branched-chain amino acids, are introduced in the *ALS* gene through base editing to confer herbicide resistance[8]. Nevertheless, base editing cannot achieve all possible base changes, and its implementation may result in undesirable bystander mutations (for example, the introduction of premature stop codons) in sites neighbouring the targeted base(s)[20]. Prime editors afford more diverse and precise genome editing outcomes (for example, all types of nucleotide changes without unintended mutations) than base editors, which are limited to a few types of nucleotide changes,

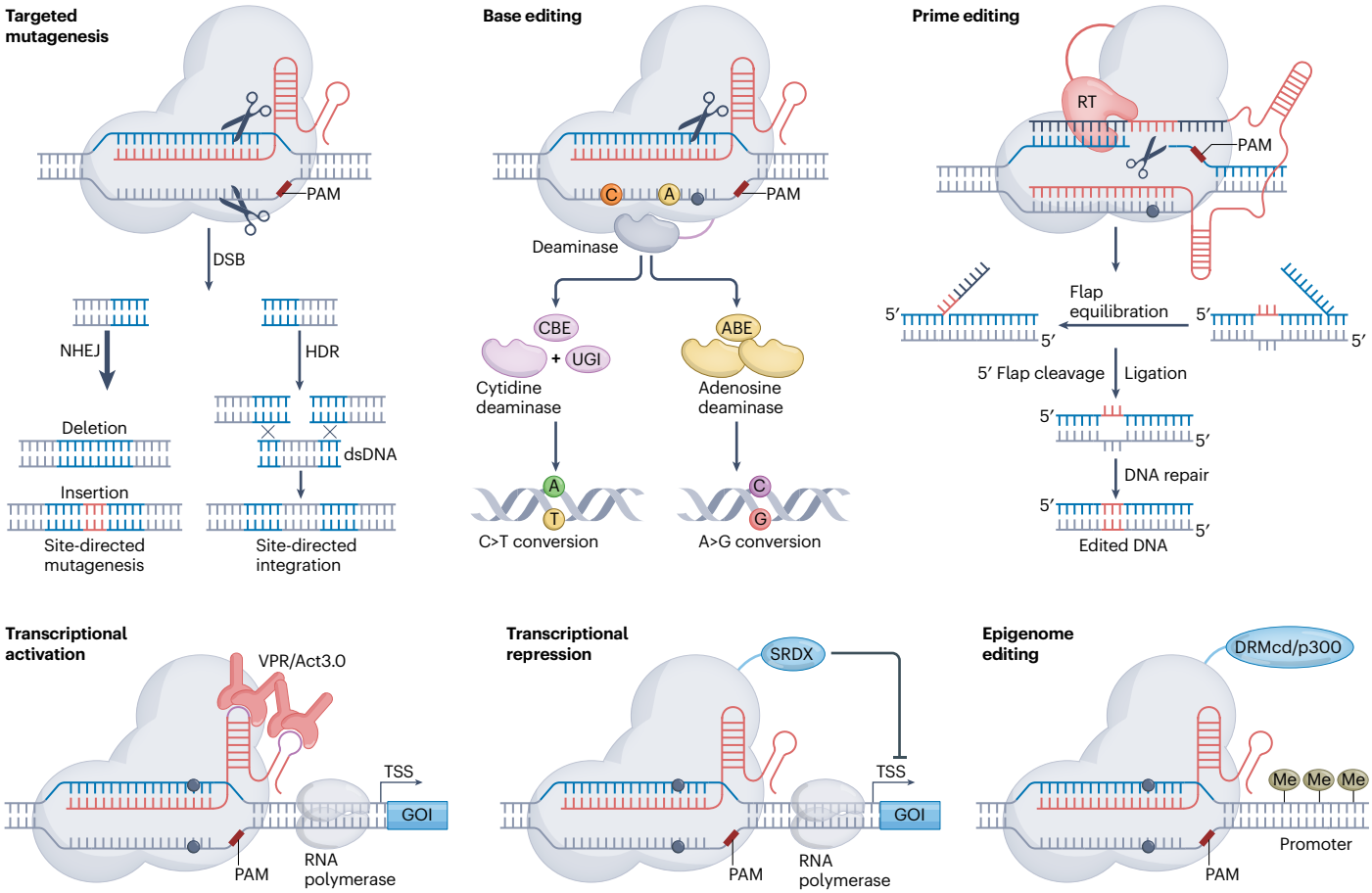

Fig. 1 | Methods of targeted gene editing by CRISPR–Cas. Targeted mutagenesis: the Cas protein introduces a double-strand break (DSB) at the target site, which can be repaired by non-homologous end joining (NHEJ) or, in the presence of a homologous template, by homology-directed repair (HDR) pathways. NHEJ often results in insertions and/or deletions that cause in-frame or out-of-frame mutations. Precise insertions (or deletions) can be introduced at the target site through HDR by supplying a template DNA harbouring the desired changes. Base editing: a catalytically impaired Cas protein (for example, a Cas9 nickase such as nCas9-D10A) introduces a nick in the target DNA sequence. nCas9 is fused with a cytidine or adenosine deaminase, which drive C>T or A>G base conversions, respectively. In the case of cytosine base editing (CBE), a uracil DNA glycosylase inhibitor (UGI) is also attached to nCas9 to inhibit excision of the uracil (formed as a result of deamination) by the repair enzyme uracil DNA glycosylase (UDG). Prime editing: nCas9-H840A is fused to an engineered reverse transcriptase (RT) and guided to the target site by a prime editing RNA composed by a modified single guide RNA (sgRNA) with RT template harbouring the desired mutation(s) and a complementary primer binding site. Nicking of the DNA followed by priming of reverse transcription results in the formation of edited 3′ and unedited 5′ flaps. The 5′ flap is cleaved by endonucleases or exonucleases in the cell, and the 3′ flap is ligated. The obtained DNA heteroduplex is then repaired by permanently installing the desired edits. Transcriptional activation: catalytically deactivated Cas (dCas) is guided to the target sequence by an sgRNA. In most activation cases, the target is a sequence in the promoter of the gene of interest (GOI). Various types of activators (for example, VP64, p65 and RTA (VPR) effectors or Act3.0)[22] attached to dCas help to recruit the transcriptional machinery and/or other activators, thus activating transcription. Transcriptional repression: transcriptional repressors fused to dCas recruit other repressors and/or directly inhibit assembly and/or binding of the transcriptional machinery at the TSS of the GOI. Depicted here is the notable EAR (ethylene-responsive element binding factor-associated amphiphilic repression) repression domain (SRDX). Epigenome editing: expression of a gene can be regulated by altering its epigenetic status through DNA or histone modifications. The GOI can be silenced by DNA methyltransferases (for example, domains rearranged methylase catalytic domain (DRMcd)) or activated by histone acetyltransferases (for example, p300) attached to dCas[19–21]. dsDNA, double-stranded DNA; Me, methyl group; PAM, protospacer adjacent motif; TSS, transcription start site.

such as C>T or A>G. However, the efficacy (for example, frequency of homozygous editing events) of prime editing should be improved for its applications in plants[20].

CRISPR–Cas can also be programmed to alter gene expression in plants (Fig. 1). Catalytically deactivated Cas (dCas) is fused to different types and/or numbers of effectors (activators or repressors) and targeted to the promoter of the gene of interest. Activation or repression strength can be adjusted by changing the number of guide RNAs (gRNAs) targeting different parts of the promoter[22]. Simultaneous gene editing and activation or inhibition can be achieved by gRNA-based orthogonal programming[23]. These changes in expression levels enable the non-disruptive fine-tuning of gene activity and are useful for functional genomics, boosting genome editing and accelerating the growth of plant tissue cultures[23]. Similarly, epigenome

editing (for example, by using DNA methyltransferase or histone acetyltransferase) allows to switch genes on or off without altering the genetic code. For example, the flowering time of the progeny can be altered through epigenome editing of the parental plant[19]. These rapidly evolving genome engineering tools[19–21] (Fig. 1), coupled with versatile transformation methods[24–26] (Fig. 2), offer new approaches to improving crops.

Improving the nutritional value of crops

Half of the caloric need of the world through direct consumption is supplied by major cereals (wheat, rice and maize), with an even higher percentage supplied in the case of developing countries[27]. However, these cereals are not rich in nutrients, especially micronutrients, partly due to the selection of productivity traits over nutritional traits during the thousands of years of domestication and breeding. This challenge is amplified by the increase in greenhouse gas emissions, as plants grown in the presence of elevated CO_2 levels may lose nutritional quality (for example, they may contain decreased levels of protein, iron and zinc)[28]. The high dependence on these staple crops, particularly in low-income regions, manifests itself in the form of hidden hunger and associated diseases. In parallel, poor dietary choices and over-consumption of calories cause or worsen various health problems in the industrialized world. Adherence to dietary guidelines has been poor even in developed countries such as the USA[29].

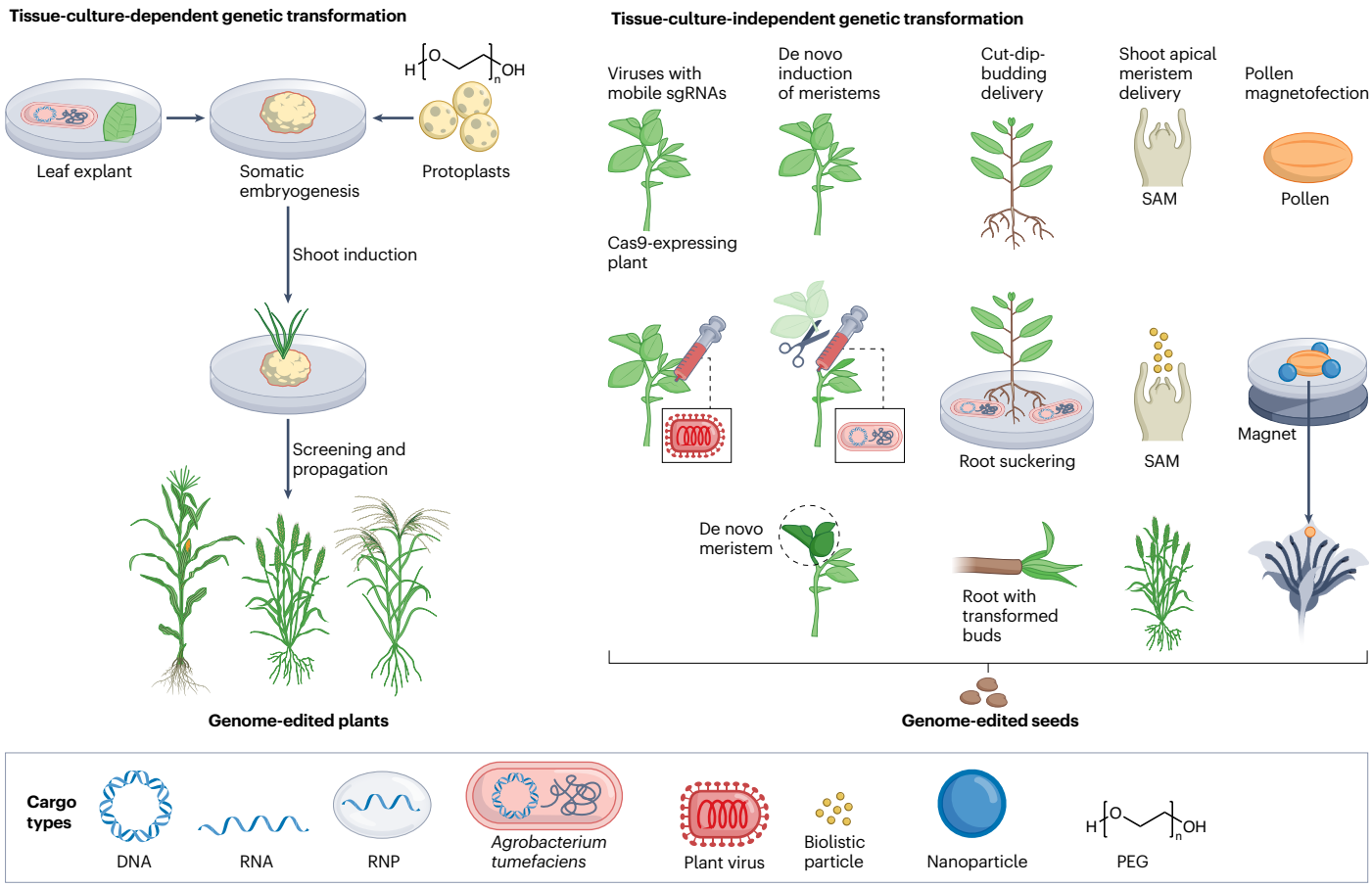

Fig. 2 | Transformation approaches of genome editing reagents into plant cells. Editing reagents can be delivered in the form of DNA, RNA or ribonucleoprotein (RNP) using various delivery strategies, including bacterial (for example, *Agrobacterium tumefaciens*), viral, biolistic, nanoparticle and chemical (for example, polyethylene glycol (PEG)) transformation. In tissue-culture-dependent genetic transformation methods, a plant explant (for example, a leaf or a protoplast) is transformed with a genome-editing cargo; somatic embryogenesis is then initiated on a selection medium often supplemented with antibiotics. Shoots are regenerated from the callus, and regenerants are screened for mutational events. In tissue-culture-independent transformation methods, lengthy and laborious steps (for example, callus induction and selection, or shoot regeneration) can be avoided. For example, Cas-expressing transgenic plants can be infiltrated with viruses expressing mobile single guide RNAs (sgRNAs) that can move to other parts of the plant, including flowers, from which new edited seeds can develop. Alternatively, de novo meristematic growth can be induced at cut shoot apices by inoculating the wound site with an *Agrobacterium* expressing the genome-editing elements and developmental regulators. The newly formed and edited meristem eventually gives rise to edited seeds. In the cut-dip-budding delivery, plants are transformed with *Agrobacterium rhizogenes* by suckering cut-roots from which hairy root formation is initiated. The gene-edited root segments are then used to induce the formation of shoots, which develop into whole plants producing edited progeny. Similar to de novo meristem induction, genome editing reagents can be delivered at the shoot apical meristem (SAM) by particle bombardment. As a result of this approach, the inflorescence structures developing from the transformed and edited SAM inherit the desired mutations. In pollen magnetofection, plasmid DNA is coated with magnetic nanoparticles and delivered into pollens using a magnetic field. Seeds developed from ovaries fertilized with the genome-edited pollens carry the desired mutations.

Table 1 | Genome-edited products available in the market or awaiting approval

Product	Target genes	Method	Phenotype	Market status	Company
Maize[35,189]	*GBSSI*	CRISPR–Cas	High-yield waxy	Pre-commercial	Corteva
Tomato[52]	*GAD3*	CRISPR–Cas	High GABA	Released, 2021	Sanatech Seed
Soybean[54,190]	*FAD2-1A*, *FAD2-1B*	TALEN	High oleic acid	Released, 2019	Calyxt
Red sea bream[157]	Myostatin	CRISPR–Cas	More muscle mass	Released, 2021	Regional Fish Institute
Tiger puffer fish[157]	Leptin receptor	CRISPR–Cas	Increased appetite	Released, 2021	Regional Fish Institute
Cattle[183]	Prolactin receptor	CRISPR–Cas	Heat-tolerant slick coat	FDA-approved	Recombinatics
Pennycress[191]	NA	CRISPR–Cas	Reduced erucic acid and fibre	Pre-commercial FDA-approved	CoverCress
Lettuce (GreenVenus)[192]	NA	CRISPR–Cas	Non-browning	Pre-commercial, expected release, 2023	Intrexon
Mustard greens[193]	Myrosinase	CRISPR–Cas	Reduced pungency	FDA-approved, expected release, 2023	Pairwise

FDA, US Food and Drug Administration; NA, not available; TALEN, transcription activator-like effector nuclease.

Today, 60% of adults in the USA struggle with one or more diet-related chronic disease[29]. Genome editing offers a compelling approach to achieving nutritional enrichment and/or diversification of crops to address these issues.

Macronutrient engineering

Starch. Starch is the main supplier of calories and the most important macronutrient worldwide, constituting about 70% of cereal grain weight. It consists of amylose and amylopectin molecules that are linear and branched chains of glucose, respectively. In starch, although the ratio of amylose to amylopectin varies between crop species and cultivars, it roughly equals 1:3 (ref. 30). This ratio determines the chemical and physical properties of starch, affecting its cooking and eating quality, its appearance, and its nutritional value[30]. For example, amylose-free waxy starch is used as a bulking, stabilizing or coating agent in the food industry[31].

The amylose-free waxy phenotype occurs due to a null mutation in granule-bound starch synthase I (*GBSSI*), which encodes the amylose synthesis enzyme. First identified in maize as a naturally occurring mutation[32], the waxy phenotype has been further generated in commercial crops, including rice[33], wheat[34], maize[35] and potato[36]. CRISPR–Cas9 editing of a waxy allele generates waxy corn, under pre-commercial launch, of higher yield performance than conventionally bred waxy corn. The genome-edited lines do not suffer from undesirable effects of linkage drag[35] (Table 1). In rice, the Wx^a allele expresses larger amounts of GBSSI than the Wx^b allele due to greater transcript stability caused by differential splicing[37]. Accordingly, indica cultivars, which mainly possess the Wx^a allele, produce drier, firmer and well-separated rice when cooked, whereas japonica cultivars with the Wx^b allele yield softer and stickier rice with higher moisture. CRISPR–Cas9-mediated or CRISPR–Cas12a-mediated promoter editing of *GBSSI* can generate a quantitative continuum of amylose[38–40]. These promoter-edited mutants with fine-tuned amylose contents (0–18%) diversify the rice germplasm for specific market breeding needs.

Refined and cooked starch is easily digested in the upper gut, leading to the rapid release of glucose, spiking of blood sugar level and insulin secretion. However, its frequent consumption, due to poor dietary choices and habits, can lead to the development of insulin resistance, which can progress to prediabetes and eventually to type 2 diabetes. About 39% of adults worldwide are overweight and 8% of global deaths are attributed to obesity[6,29]. The global economic cost of obesity is predicted to exceed 3 trillion US dollars by 2030 (ref. 41). Therefore, healthy carbohydrates are urgently needed to help to mitigate these problems. Engineering plants that contain resistant starch, such as amylose-rich starch, which is characterized by low caloric and glycaemic load and gut-microbiota-promoting abilities, presents a route to providing a healthy dietary option[42] (Fig. 3a).

The amylose-extender mutant of maize, which produces resistant starch with high amylose content, naturally occurs due to a null mutation in the endosperm main isoform of the starch branching enzyme (SBE)[43]. This mutant phenotype is reproduced in rice[44], wheat[45] and potato[46,47] by CRISPR–Cas9-mediated mutagenesis of *SBE* genes. In rice, upon the knocking out of *SBE2b*, amylose content in seeds increases up to 25%, from 15% in the wild type, with no loss in total starch content[44]. In wheat, the amylose content of total starch in fine flour increases from 31% to 65%, following the mutation of all six alleles of *SBE2a*[45]. However, this mutation decreases the total starch content and reduces cooking and sensory qualities. Increasing amylose content and reducing amylopectin branching is possible in potatoes when mutating leaf and tuber isoforms[45,47].

Proteins. Storage proteins in cereal grains are important energy suppliers and an alternative protein source, particularly for people following a plant-based diet. Certain types of seed storage proteins pose health risks to genetically predisposed individuals, for example, gluten poses such risks to people with coeliac disease, who have to avoid gluten-containing foods prepared from wheat, barley or rye (Fig. 3b).

In wheat, gluten comprises the prolamin-type proteins gliadin and glutenin, with α-gliadins being the immunodominant group associated with coeliac disease. Multiple α-gliadin genes (up to 35) are simultaneously edited using Cas9 and two gRNAs targeting the conserved regions, resulting in up to 85% reduction in wheat gluten immunoreactivity[48]. However, low-gluten wheat is still inconsumable for individuals with coeliac disease, and the flour prepared from such wheat can display undesirable baking properties. A similar approach is utilized in sorghum to reduce the level of α-kafirin, another poorly digestible prolamin-type storage protein[49]. Kernels from selected mutant lines have a reduced α-kafirin content and increased protein digestibility. Additionally, the total protein content of the kernels increases as other storage proteins offset the loss in α-kafirin.

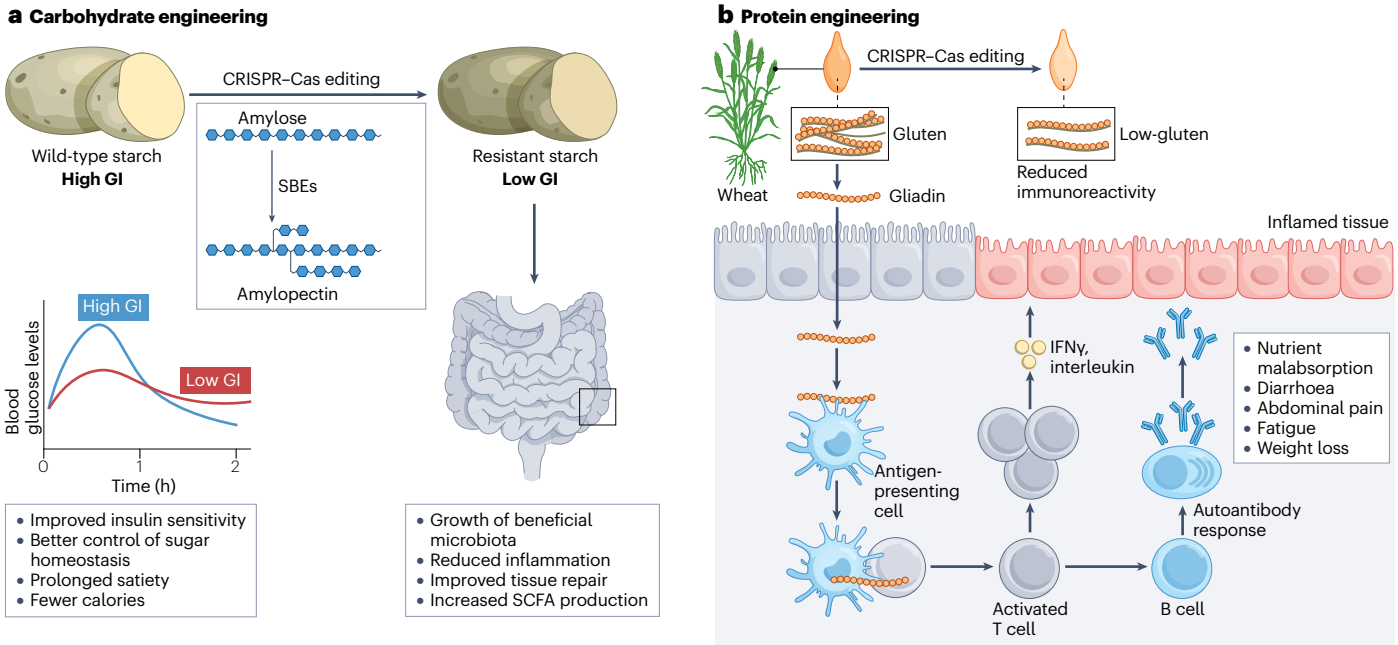
a Carbohydrate engineering
CRISPR–Cas editing
Wild-type starch
High GI
Amylose
SBEs
Amylopectin
Resistant starch
Low GI
High GI
Low GI
Blood glucose levels
Time (h)
• Improved insulin sensitivity
• Better control of sugar homeostasis
• Prolonged satiety
• Fewer calories
• Growth of beneficial microbiota
• Reduced inflammation
• Improved tissue repair
• Increased SCFA production
b Protein engineering
CRISPR–Cas editing
Wheat
Gluten
Gliadin
Low-gluten
Reduced immunoreactivity
Inflamed tissue
IFNγ, interleukin
Antigen-presenting cell
Activated T cell
B cell
Autoantibody response
• Nutrient malabsorption
• Diarrhoea
• Abdominal pain
• Fatigue
• Weight loss
c Lipid engineering
Soybean
Flax
OA
EA
Plastid
OA (C18:1)
LA (C18:2)
ALA (C18:3)
FAD2-1A
FAD2-1B
Desaturase
FAE1
Elongase
GA (C20:1)
EA (C22:1)
d Micronutrient biofortification
Wild-type rice
Increased β-carotene content
Golden rice
MEP pathway
GGPP
PSY
Cis-phytoene
PDS
Z-ISO
ZDS
CRTISO
Lycopene
CRTI
LCYε/LCYβ
α-Carotene
LCYβ
β-Carotene
Homology arms
CRTI-PSY
Glutelin
Precise knock-in
GSH
e Reducing post-harvest losses
Browning
Non-browning
Food wastage
Preservative use
Enzymatic browning reaction
PPO + O2
PPO + O2
Monophenol
Ortho-diphenol
Ortho-quinone
Self polymerization
Free amino acids
Proteins
Complex brown polymers
Melanin
f Prolonging shelf life
Nutritional value
Free radicals
Vitamins
Time
Shorter shelf life
15 days
15 days
CRISPR
Longer shelf life
RIN
NOR
CNR
CRISPR–Cas editing
PL
PG2a
TBG4
Ethylene
Ripening delay
Cell-wall remodelling

Fig. 3 | Engineering the nutritional value of crops by genome editing. **a**, Carbohydrate engineering: resistant starch (RS), which is rich in amylose and long-chain amylopectin molecules, can be engineered by knocking out the activities of starch branching enzymes (SBEs). SBEs are glucosyltransferases that generate the α-1,6 glycosidic branches in starch by removing glucose chains linked through α-1,4 glycosidic bonds and reattaching them at the branching points. Therefore, losses in SBE activity cause the amylose content to increase and branching in amylopectin to decrease. The thus obtained starch with a low glycaemic index (GI) provides fewer calories, a more prolonged satiety and better control of blood sugar homeostasis than wild-type starch. In addition, the RS that is not digested in the upper gut passes to the colon and serves as an energy and substrate source for beneficial microbiota. Fermentation of RS by the lower-gut microbiota produces short-chain fatty acids (SCFAs) that are essential for several biological activities, including optimal functioning of pancreatic β-cells, which produce insulin, proliferation of epithelial cells, and maintenance of the gut barrier and gut–brain communication[42,194]. **b**, Protein engineering: coeliac disease is a chronic autoimmune disorder in which eating gluten (or related proteins) triggers inflammation and damaging of the intestinal lining, thus impairing proper nutrient absorption. When gluten is absorbed in the small intestine of individuals with coeliac disease, gliadin in gluten is recognized as an antigen. Presentation of the deaminated gliadins to T cells triggers their activation and the release of cytokines (for example, interferon-γ (IFNγ) and interleukins), thus promoting inflammation. Antibodies produced by B cells against gluten proteins can mistakenly attack intestinal cells and stimulate inflammation, thus damaging the intestinal lining (for example, flattening of villi) and causing nutrient malabsorption. Moreover, the damaged lining allows gluten proteins (and other leaked molecules) to enter the bloodstream and trigger immune responses in other body parts, such as skin and joints. As gluten proteins are encoded by multiple genes, it is virtually impossible to breed out the relevant trait from elite cultivars. Knocking out α-gliadin genes can produce low-gluten (or gluten-free) wheat with reduced immunoreactivity. **c**, Lipid engineering: manipulation of fatty acid (FA) composition in oilseeds. Reducing the activity of fatty acid desaturase (FAD) causes an increase in oleic acid (OA) content as a result of its conversion to linoleic acid (LA) being blocked. Similarly, knocking out fatty acid elongase 1 gene (*FAE1*) blocks the competing elongation pathway and inhibits the accumulation of erucic acid (EA); it also increases the contents of OA and downstream FAs. **d**, Micronutrient biofortification: CRISPR–Cas9-mediated targeted insertion of the carotenoid cassette into a rice genomic safe harbour (GSH). The maize phytoene synthase gene (*PSY*) and bacterial carotene desaturase gene (*CRTI*) are expressed under the rice endosperm-specific glutelin promoter. Precursors for the carotenoid pathway are supplied through the methylerythritol 4-phosphate (MEP) pathway in the endosperm. PSY catalyses the rate-limiting step of the carotenoid pathway. Bacterial CRTI can synthesize lycopene from phytoene, bypassing the intermediate steps. Lycopene serves as the branching point where knocking out *LCY-E* can reduce α-carotene synthesis and divert the pathway to the synthesis of β-carotene. Accumulation of β-carotene in the endosperm gives the characteristic golden colour to rice seeds. **e**, Reducing post-harvest losses: enzymatic browning is undesirable for some crops, such as apples, bananas, mushrooms and potatoes. This process is triggered when the plastid-localized polyphenol oxidases (PPOs) come into contact with the vacuole-localized phenolic substrates (for example, tyrosine and caffeic acid) due to mechanical damage of tissues and/or cells during poor post-harvest handling. A similar outcome is observed when precut, pureed or juiced fruits or vegetables are exposed to oxygen. The resulting quinones, in a series of reactions, self-polymerize or react with free amino acids to form brown-coloured pigments (for example, melanin). Various preservatives, such as sulfites, are added to prevent discolorations and prolong the shelf life of food products. Decreasing the activity of PPOs can help to slow or stop enzymatic browning, thus reducing food waste and the use of harmful preservatives. **f**, Prolonging shelf life: shelf life can be extended by targeting transcription factors and/or cell-wall-remodelling genes. Highly perishable plant products lose nutritional value as they age on the shelf. During spoilage, the concentration of free radicals increases, whereas that of vitamins decreases. In tomatoes, various ripening transcription factors (for example, ripening inhibitor (RIN), non ripening (NOR) and colourless ripening (CNR)) are known to affect ethylene production when mutated. Tomatoes with different levels of ripening can be obtained depending on the severity of the mutations. Alternatively, genes involved in cell-wall remodelling (for example, pectate lyase (PL), polygalacturonase 2a (PG2a) and β-galactanase (TBG4)) can be targeted to reduce softening. Shelf-life extension allows to improve the control of ripening time, thus reducing post-harvest losses. ALA, α-linolenic acid; CRTISO, carotenoid isomerase; GA, gondoic acid; GGPP, geranylgeranyl diphosphate; LCYβ, lycopene β-cyclase; LCYε, lycopene ε-cyclase; PDS, phytoene desaturase; ZDS, ζ-carotene desaturase; Z-ISO, ζ-carotene isomerase.

Moreover, protein-bound and free lysine contents in the seeds increase about twofold and tenfold, respectively.

A push strategy based on increasing the synthesis of a metabolite of interest is applied in tomatoes, fruits that are rich in GABA, to further increase the concentration of this acid. The non-proteinogenic amino acid GABA is linked to reduced stress, improved sleep quality and improved control of blood pressure[50]. Glutamate decarboxylase 3 (GAD3), the main isoform in tomatoes of the enzyme that catalyses GABA biosynthesis from glutamate, is edited by CRISPR–Cas9 at its auto-inhibitory C-terminal domain[51]. The edited tomatoes exhibit increased GAD3 activity and, consequently a sevenfold to 15-fold increase in GABA concentration[51]. Although the health benefits of the high GABA Sicilian Rouge tomatoes are not demonstrated in clinical trials, they have been sold in Japan since 2021 as the first CRISPR-edited plant product to enter the market[52] (Table 1).

Lipids. Oilseeds (for example, canola, sunflower, corn and soybean) are an essential part of a healthy diet. Replacing saturated animal fats with plant-based and fish-based mono-unsaturated and poly-unsaturated fatty acids (MUFAs and PUFAs) has several health benefits[29]. However, not all unsaturated fatty acids are equally stable. Due to the multiple carbon double bonds, PUFAs tend to undergo oxidation more quickly than MUFAs, a process that causes rancidity and shortens shelf life[53]. For example, oleic acid, as a MUFA, is more stable to oxidation and high temperatures than linoleic acid, the major dietary PUFA[53].

Given that many oilseeds contain high levels of PUFAs, increasing the MUFA content in storage organs of oil crops is essential to increase oxidative stability and shelf life. Improved soybean oil with high oleic acid to linoleic acid ratio is obtained by knocking out the fatty acid desaturase 2 gene (*FAD2*), which catalyses the desaturation of oleic acid to linoleic acid, using TALEN technology[54] (Table 1). Similarly, CRISPR–Cas9 is used for the gene editing of rapeseed[55], soybean[56], rice[57] and *Camelina*[58,59] (Fig. 3c). The increases in oleic acid content follow a gene dosage pattern; however, in some cases, knocking out all copies of *FAD2* causes developmental defects (for example, slow or stunted growth)[58,59]. Therefore, cytosine base editor is used to introduce base substitutions in *Arabidopsis FAD2* for subtle alterations in the enzyme activity, yet a threefold increase in oleic acid level is observed[60].

In rapeseed, fatty acid elongase 1 (FAE1) competes with FAD2 for oleic acid to sequentially synthesize gondoic acid and erucic acid in the so-called elongation pathway. High levels of erucic acid, commonly found in *Brassica* species such as *Brassica napus*, are associated with myocardial lipidosis; therefore, *FAE1* is an alternative genome editing

Box 1

The daily vitamin A requirement and golden rice

The two main sources of vitamin A in the human diet are animal-derived retinoids (pre-formed vitamin A), and plant-derived carotenoids (provitamin A), including α-carotene and β-carotene, as well as β-cryptoxanthin. Once ingested in the small intestine, β-carotene is converted to two molecules of retinal (retinaldehyde), which can be further converted into one of the two active forms of vitamin A: retinoic acid, a signaling molecule and hormone, and retinol, the transport and storage form of vitamin A[198]. The recommended dietary allowance (RDA) of vitamin A is measured in retinol activity equivalents (RAEs), in which 1 µg RAE is 1 µg of retinol or 2 to 24 µg of β-carotene, depending on the food matrix. Vitamin A requirements increase with age. Children aged 4–8 years (regardless of gender) require 400 µg RAE of vitamin A, whereas an adult requires twice that amount on average[199]. The absorption of provitamin A is inferior to that of the pre-formed vitamin A, making it even harder for individuals mainly relying on a plant-based diet to meet the RDA. GR2 accumulates up to 31 µg g^{-1} dry weight of β-carotene in the seeds. A simple calculation reveals that a 5-year-old preschool child relying solely on GR2 for vitamin A needs to consume about 52 g of GR2 to meet the recommended dietary allowance, assuming 4:1 RAE to β-carotene equivalency ratio (400 µg RAE x 4 / 31 µg/g=~52 g)[200]. Aiming for 25% RDA (a dose sufficient to prevent avitaminosis A) in a less conservative scenario, the same child would need to eat about 13 g of GR2 daily. However, β-carotene in GR2 degrades to a plateau of 3–5 µg g^{-1} after around 2 months of post-harvest storage due to oxidative decay[84]. Assuming a final β-carotene concentration of 4 µg g^{-1} after 2 months of storage, the calculated values of ~52 and ~13 g rise to about 400 and 100 g per day, respectively. Accordingly, eating fresh GR2 alone is sufficient and practical to meet the RDA of vitamin A for preschool children; however, meeting the RDA for vitamin A eating stale GR2 is impractical. Therefore, golden rice with further increased β-carotene accumulation and stability is needed, which may be achieved with genome editing.

target[61] (Fig. 3c). CRISPR–Cas9-mediated mutagenesis of *FAE1* in high erucic acid-containing *Brassica* cultivars yields seeds with reduced erucic acid and increased oleic acid contents[62]. Knocking out the two copies of *FAE1* eliminates erucic acid, whereas knocking out a single copy creates a gene dosage effect. Similarly, CRISPR–Cas9 mutating all three *FAE1* copies in *Camelia sativa* turns off the elongation pathway[63]. Pollen from the genome-edited homozygous *FAE1* mutant can pollinate transgenic *Camelina* plants producing polyunsaturated fish oils, eicosapentaenoic acid and docosahexaenoic acid[64]. Combining transgenic and genome editing approaches can be synergistic; for example, *FAE1*-edited transgenic *Camelina* seeds contain higher docosahexaenoic acid levels, 13%, than the parental transgenic seeds, 10%. Moreover, the total content of healthy long-chain fatty acids increases from 28% to 33%[64]. The modified seeds overexpressing multiple transgenes for docosahexaenoic acid and eicosapentaenoic acid biosynthesis, and edited for the FAE1, are the first of their kind to undergo a field trial[65].

Micronutrient biofortification

Iron, zinc, iodine, folate and vitamin A deficiencies affect over 2 billion people worldwide, and they are responsible for more than half of the mortality for children under the age of five globally[66,67]. Although supplementation programmes can help to alleviate these deficiencies, such programmes are hindered by issues related to supply management, availability of storage facilities, access to medical centers by rural populations and public education[68]. Alternatively, consumption of biofortified staple crops could be an effective and sustainable approach to addressing micronutrient deficiency. This approach could also help to alleviate micronutrient inadequacy, a condition prevalent in areas of high resources, which can lead to fatigue, poor cognition, cardiovascular diseases and cancer[69]. People with micronutrient inadequacies might excessively use dietary supplements to compensate for the a lack of a well-balanced diet. More than half of American adults reported using one or more dietary supplement within a month[70], with only less than a quarter of these supplements recommended by healthcare providers[71].

Dietary supplements are critical for tackling micronutrient deficiencies and in conditions in which the estimated average requirements are unlikely to be met. However, their risks and benefits in preventing mortality remain controversial[72,73]. Alternatively, the intake of vitamins and minerals (vitamins A and K, magnesium, copper and zinc) through foods, but not supplements, at or above the estimated average requirements, is associated with reduced all-cause or cardiovascular disease mortality[72]. Nutrients in foods can act synergistically and are more beneficial when consumed as a whole in food than separately. Therefore, biofortification of staple crops is necessary, and genome editing can help to accelerate the process.

Vitamin A. Around 250,000–500,000 cases of blindness are estimated to occur annually among preschool children in low-resource areas, with 50% mortality within 1 year due to vitamin A deficiency[74]. To reduce the prevalence of vitamin A deficiency, two golden rice varieties, GR1 and GR2, are developed to accumulate β-carotene, the most potent plant-derived provitamin A, in rice endosperm[75,76]. Both golden rice varieties are generated by 'pushing' the carotenoid biosynthesis pathway using transgenic techniques. GR2, characterized by the overexpression of the maize phytoene synthase gene (*PSY*) and bacterial carotene desaturase gene (*CRTI*) in the endosperm, accumulate 7–31 µg g^{-1} dry weight of β-carotene in the seeds[75]. Therefore, by consuming GR2 alone, the recommended dietary allowance of vitamin A can be reached[77] (Box 1). The Philippines was the first country to approve the cultivation of GR2 in 2021, overcoming two decades of regulatory hurdles[78]. GR2 is re-engineered with CRISPR–Cas9-mediated targeted insertion of the same carotenoid cassette, which was used to engineer GR2, into a genomic safe harbour[79] (Fig. 3d). An alternative blocking strategy, using CRISPR–Cas9, is utilized in bananas, and it involves the knocking out of lycopene ε-cyclase (*LCY-E*) to divert the carotenoid flux from α-carotene and lutein to β-carotene[80]. This approach moderately increases β-carotene level in the ripe fruit pulp up to sixfold (about 24 µg g^{-1} dry weight), due to the low abundance of lycopene in this tissue, suggesting yet another potential target to further increase β-carotene level in GR2.

Preventing β-carotene degradation is equally important to increasing its yield, because this compound is highly susceptible to oxidative

degradation. For example, β-carotene undergoes specific enzymatic oxidative breakdown catalysed by carotenoid cleavage dioxygenases (CCDs)[81]. RNAi-based silencing of *CCD1* and *CCD4a* increases rice seed carotenoid content 1.4-fold and 1.6-fold, respectively[82]. β-Carotene is also degraded by non-specific enzymes, such as lipoxygenases and peroxidases[81]. Accordingly, RNAi silencing of the *LOX1* locus in another golden rice variety reduces lipoxygenase activity and improves β-carotene retention during storage[83]. CRISPR–Cas-mediated genome editing can reduce the activities of CCDs and lipoxygenases to improve β-carotene storage. However, non-enzymatic oxidative degradation also accounts for substantial post-harvest β-carotene loss in GR2 (ref. 84) and sorghum[85]. Therefore, alternative approaches are needed to prevent the non-enzymatic oxidative decay of β-carotene[86]. Further improvement of β-carotene may be achieved by combining push (increasing levels of upstream metabolites), pull (improving its storage capacity) and block (preventing its degradation or conversion to other metabolites) approaches[87].

Vitamin C. Vitamin C (also known as ascorbic acid) is an antioxidant that is essential to plant stress response and the human immune system. Overexpression of GDP-l-galactose phosphorylase (GGP), the enzyme that catalyses the rate-limiting step of the ascorbate biosynthetic pathway in plants, is a promising transgenic approach to boosting ascorbate levels in the edible parts of plants[88]. Alternatively, controlling post-transcriptional regulation can increase protein abundance, thus improving enzyme activity in the cell. Such a strategy is utilized by editing upstream open reading frames (uORFs) of lettuce GGP isoforms (*GGP1* and *GGP2*) to boost foliar ascorbic acid level[89]. Whereas small deletions (1–14 bp) in or near the uORF initiation codon yield moderate increases (35 to 98%, depending on the isoform and mutation), a large deletion (92 bp) in *GGP2* increase ascorbic acid levels by more than 150%[89]. Although this approach relies on Cas9, promoter editing through Cas12a could be a more effective strategy for generating large deletions[40,90]. Notably, the uORF editing strategy is also implemented to downregulate protein levels by introducing de novo start codons or by extending the length of naturally occurring uORFs through base or prime editing[91].

Vitamin D. Plants are poor dietary sources of vitamin D compared with fish and dairy. This increases the likelihood of vitamin D deficiency, which affects about 1 billion people worldwide, for individuals adopting plant-based diets[92]. Humans partially meet their vitamin D requirements (15 µg for children and adults) through the cutaneous conversion of 7-dehydrocholesterol to pre-vitamin D_3 upon exposure to ultraviolet-B light. However, the efficacy of this conversion is limited by the duration of exposure, and it decreases as the darkness of the skin, the age of the individual and the distance from the equator increase. As conventional diets may fail to provide the recommended dietary allowance of vitamin D, biofortification of food crops is highly desirable.

The gene encoding 7-dehydrocholesterol reductase isoform (SI7-DR2), which catalyses the conversion of 7-dehydrocholesterol to cholesterol that is used in steroidal glycoalkaloid synthesis, is knocked out in tomato by CRISPR–Cas9 (ref. 93). The mature fruits accumulate 7-dehydrocholesterol, resulting in up to 2 µg of vitamin D per fruit (equivalent to the contents of two medium-sized eggs or 170 g of tuna). Seven to eight medium-sized tomatoes (each with 8–10 g of dry weight) are sufficient to provide the recommended dietary allowance of vitamin D. Moreover, the loss of SI7-DR2 activity does not come with any yield penalty because of a duplicated pathway that can also supply cholesterol for steroidal glycoalkaloid synthesis[94]. By contrast, null mutants of the sterol Δ^7-reductase in *Arabidopsis* inhibit brassinosteroid biosynthesis, resulting in severe dwarfism due to the lack of the duplicated pathway[95].

Minerals. Boosting the mineral content of staple crops is vital to combat micronutrient deficiencies. Iron and zinc are poorly present in primary cereals, so biofortification is essential, especially considering their deficiencies are the most prevalent worldwide, with severe consequences. For example, iron deficiency and associated anaemia affect more people (1.5–2 billion) than any other deficiency, and it causes 20% of maternal deaths globally[96,97]. Similarly, about 1.2 billion people are estimated to have inadequate zinc intakes[68].

Iron and zinc biofortification in primary cereals mostly relies on conventional breeding or transgenic methods through overexpression of genes involved in the uptake and translocation of these elements[98]. The application of genome editing for mineral biofortification is limited. In rice, CRISPR–Cas9-mediated mutagenesis of *CYP735A3* and *CYP735A4*, which are genes involved in the synthesis of trans-zeatin type cytokinins that control zinc uptake and transport, results in seed zinc concentrations exceeding 20 µg g^{-1} dry weight, a more than 10% increase[99]. To meet 30% of the estimated average requirement of iron and zinc, HarvestPlus breeding programmes aim for 13 and 28 µg g^{-1} dry weight in rice, respectively. However, these levels are the minimum to achieve biologically meaningful nutritional impacts[100]. Genome editing could help to increase the mineral content in target crops by CRISPR–Cas-mediated uORF editing, promoter swapping (with a stronger one) or transcriptional activation of genes involved in the uptake and translocation of iron and zinc[19,22]. Multiplexed genome editing may help to increase the nutritional value of staple crops by introducing cooperative traits, such as iron and vitamin C, that are absorbed and function synergistically.

Anthocyanins. Anthocyanins are pigments that can be powerful antioxidants. As anthocyanins belong to the flavonoid group of compounds, their biosynthesis in plants involves the interaction of several genes and transcription factors. The ternary complex MBW, which is formed by transcription factors myeloblastosis protein (MYB), basic helix–loop–helix protein (bHLH) and WD-repeat protein (WDR), plays a key role in activating multiple genes in the anthocyanin pathway[101]. Unlike the purple-coloured members of the Solanaceae plants, such as the eggplant, most tomato cultivars do not produce anthocyanins due to incomplete activation of the pathway and suboptimal flavonoid levels[102]. Accordingly, tomatoes overexpressing the snapdragon genes encoding the bHLH and MYB transcription factors accumulate anthocyanins and turn deep purple[103]. When used to supplement diets, the resulting anthocyanin-rich transgenic tomatoes extend the lifespan of cancer model mice (tumorigenesis-prone *Trp53*$^{-/-}$ or *p53* knockout), presumably due to their high antioxidant capacity[103]. The United States Department of Agriculture (USDA) authorized the regulatory approval of these purple tomatoes[104].

Expression of the MYB-encoding anthocyanin mutant 1 gene (*ANT1*) is ectopically boosted by HDR-mediated promoter swap[105,106]. However, subtle edits at single nucleotide level can increase anthocyanin accumulation in tomatoes. For example, the point mutations in genes *SlAN2-like* (encoding the MYB transcription factor) and *SlMYBATV* (encoding an anthocyanin repressor), which account for the red and purple colours of Ailsa Craig and Indigo Rose cultivars, respectively, are identified[107]. In the Ailsa Craig cultivar, the mutation

in *SlAN2-like* renders the activator non-functional, whereas the mutation in *SlMYBATV* makes the repressor functional. Collectively, these mutations suppress anthocyanin accumulation in this tomato cultivar. Accordingly, fruit-specific overexpression of the functional *SlAN2-like* generates anthocyanin-rich Ailsa Craig tomatoes exhibiting a purple colour[107]. Therefore, comparable results can be obtained using base editing to substitute the relevant nucleotides. Indeed, CRISPR–Cas9 genome editing is applied in three elite rice cultivars to revert a non-functional *bHLH* transcription factor (due to a premature stop codon arising from a 14 base-pair frameshift deletion) to the functional form, which confers proanthocyanidin-rich red pericarp in the wild relative *Oryza rufipogon*[108]. Single-nucleotide polymorphisms (SNPs), which suppress anthocyanin biosynthesis, are also present in other species. For example, SNPs affecting anthocyanin accumulation are identified in the dihydroflavonol-4-reductase gene (*DFR*) from different eggplant species[109]. By providing high quality sequence data from different cultivars or species, comparative genomics can help to identify anthocyanin-suppressing SNPs that can be targeted by base or prime editing to boost anthocyanin levels.

Other carotenoids. In addition to β-carotene, plants synthesize other types of carotenoids, including lycopene, α-carotene and xanthophylls[81]. Although not a provitamin A, lycopene has strong antioxidant properties[81]. Moreover, it serves as the precursor of α-carotene, β-carotene and xanthophylls. Therefore, increasing lycopene content is desirable. Tomato, being rich in lycopene, is an excellent model species for lycopene content manipulation. Simultaneous targeting of lycopene β-cyclase isoforms (*LCY-B1* and *LCY-B2*) and the lycopene ε-cyclase gene (*LCY-E*), which encode enzymes that collectively convert lycopene to downstream metabolites, together with stay-green 1 (*SGR1*), which inhibits the activity of PSY, produces a range of tomato mutants with increased levels of lycopene and β-carotene[110]. Among these mutants, knocking out *SGR1* alone resulted in the highest level of lycopene in comparison with any other mutant combination. Additionally, knocking out *LCY-B* in wild tomato (*Solanum pimpinellifolium*) results in a fivefold increase in lycopene content compared with Micro-Tom, a model tomato cultivar that is similar in size to its wild relative, without negatively affecting the accumulation of β-carotene or lutein[111]. Comparable increases in lycopene, β-carotene and lutein contents are achieved when the UV-damaged DNA-binding protein 1 gene (*DDB1*) and deetiolated 1 gene (*DET1*) are mutagenized (in addition to *LCY-B*) by cytosine base editing[112].

Reducing antinutrients

Phytic acid is the most noteworthy antinutrient causing micronutrient deficiency. Phytic acid chelates divalent cations (for example, Fe^{2+} and Zn^{2+}) in the small intestine, hindering their absorption. Genome editing in wheat and rapeseed targets the enzymes catalysing the last and preceding steps of phytic acid biosynthesis, respectively[113,114]. Knocking out three functional paralogues of the inositol tetrakisphosphate kinase gene (*ITPK*) in rapeseed reduces phytic acid content by up to 35%[114]. Similarly, knocking out the seed dominant homologue of inositol pentakisphosphate 2-kinase 1 gene (*IPK1*) in wheat reduces phytic acid content and increases the apparent Fe^{2+} and Zn^{2+} contents about twofold[113]. Leguminous crops, which are naturally rich in phytic acid, could be genome-edited. As phytic acid is essential for plant germination, subtle changes in gene expression by promoter editing, rather than complete disruption, might be necessary to avoid developmental and yield penalties.

Although most antinutrients are naturally found in crops, acrylamide forms during high-temperature food processing (baking, toasting and frying, but not boiling). This carcinogen accumulates when reducing sugars (for example, glucose and fructose) react with free amino acids, such as asparagine, through the nonenzymatic Maillard reaction[115]. Therefore, potato-based and wheat-based products, such as French fries, chips and toasted bread, raise safety concerns. Although acrylamide presence in foods is not regulated in the USA, benchmark levels are set for different types of food in Europe for consumer protection (for example, 50 and 750 μg kg^{-1} for wheat bread and potato chips, respectively)[116]. One strategy to reduce acrylamide formation in potatoes is to reduce the activity of vacuolar invertase (VINV), the main enzyme responsible for the degradation of sucrose to glucose and fructose during post-harvest cold storage. Transgenic potatoes with RNAi-silenced *VINV* have been available in the US market. Moreover, tubers from full *VINV*-knockout non-transgenic lines, generated by TALEN-mediated gene editing, accumulate undetectable levels of reducing sugars resulting in up to 73% less acrylamide in chips[117].

Reduced acrylamide levels are also achieved by CRISPR–Cas9 editing of *VINV* and/or the asparagine synthase 1 gene (*ASN1*) in potato[118,119]. Wheat acrylamide formation is mainly controlled by free asparagine concentration in the seeds[120]. Accordingly, the seed-specific isoform *ASN2* is edited using CRISPR–Cas9 (ref. 121). Free asparagine concentration is reduced by more than 90% in second generation T2 seeds of a total *ASN2* knockout. Partially edited plants display a gradient reduction in free asparagine concentration, indicating that the trait is fine-tuneable. The flour prepared from the seeds of a full *ASN2*-knockout mutant has about 50% less acrylamide after being heated[122]. This trait represents the first CRISPR–Cas-edited wheat being evaluated in field trials in Europe[122].

Evaluation of the potential of genome editing in reducing the antinutrients is in its early stages. Examples include decreasing cyanogenic glycosides in cassava[123], lowering steroidal glycoalkaloids in nightshades[124] and reducing the uptake of heavy metals[125] or even radioactive isotopes[126] in rice. These examples can be expanded to genome-edited crops producing decreased levels of allergenic proteins, such as albumin[127] and α-amylase/trypsin inhibitor[128].

Improving flavour

Although improving flavour may not be recognized to be as important as improving the nutritional content, it can positively influence the public view of genome-edited crops and encourage people to diversify their diets. Various consumers agree that heirloom varieties are richer in flavour than modern cultivars due to breeding focus on producer traits (for example, yield and shelf life) over those of consumers. Nevertheless, engineering flavour (predominantly sensed by taste and smell) is challenging due to the complex nature of genetic control over the trait, subjectivity of the stimuli to people, and the vast number of metabolites (for example, sugars and acids) and volatile compounds that contribute to flavour. This challenge is magnified by environmental variations (for example, seasonal changes and soil type)[129].

Tomato flavour is improved by matching consumer responses with metabolite profiles from several different cultivars and mapping these profiles to relevant genes and transcription factors[130,131]. For example, promoter variations in tomato lipoxygenase (*TomLoxC*), encoding the protein responsible for the production of flavour-associated lipid-derived and carotenoid-derived volatiles, are identified between the heirlooms and modern cultivars[132]. Genome editing can be

leveraged to introduce changes in promoters and genes as more data on flavour become available. Such a strategy is also utilized to fine-tune sugar content in strawberry fruit[133]. Editing of the uORF within the sucrose-responsive promoter region of basic leucine zipper protein (FvebZIP1.1) by cytosine base editing creates a range of mutations resulting in a 34–84% increase in the sugar content, without severe yield penalties[133].

In rice, 2-acetyl-1-pyrroline (among 200 volatile compounds) is responsible for the popcorn-like aroma in fragrance-rich cultivars, such as Jasmine and Basmati[134]. These cultivars accumulate 2-acetyl-1-pyrroline due to naturally occurring mutations in the betaine aldehyde dehydrogenase 2 gene (*BADH2*)[134]. TALEN is used to mimic these natural mutations in the non-fragrant Nipponbare cultivar[135]. Mutant kernels homozygous for *BADH2* accumulate as much 2-acetyl-1-pyrroline as kernels from a fragrance-rich control group[135]. Similar results are obtained with other rice cultivars using CRISPR–Cas9 mutagenesis[136–138]. Moreover, metabolite analysis reveals increases in the levels of several other volatile compounds in the edited rice seeds, such as pyridine, isophytol and benzaldehyde, indicating a broad impact of the mutations[136,138]. Unlike rice, maize lacks natural mutants of *BADH2*, necessitating the use of genome editing to introduce such variations. Simultaneous (but not separate) editing of the two homologous genes *BADH2a* and *BADH2b* results in 2-acetyl-1-pyrroline accumulation in mutant kernels, generating aromatic maize[139].

Reducing post-harvest losses

About one-third of the food produced globally goes unconsumed due to unintentional post-harvest losses or intentional wasting[140,141]. For low-resource areas, losses occur mostly during post-harvest stages (handling, storage and distribution), whereas in high-resource areas losses occur through wasting at the end of the supply chain because of the high cosmetic and flavour expectations of consumers[141]. In general, extending shelf life requires cold and dry storage and the application of various chemicals to prevent or delay ripening, especially in the case of roots, tubers, fruits and vegetables, as they are more perishable than cereals, pulses and oilseeds[141]. These practices can be costly, hazardous and often reduce the nutritional quality and/or flavour, increasing the waste[140].

Fruits and vegetables undergo enzymatic browning, which reduces their nutritional value and consumer appeal (Fig. 3e). CRISPR–Cas genome editing of the polyphenol oxidase genes (*PPO*) is applied to potato and eggplant[142,143]. Potatoes with tetra-allelic mutations in the *PPO2* isoform are obtained by ribonucleoprotein delivery of Cas9 and gRNAs into protoplasts followed by plant regeneration. The tubers of the plants exhibit up to 69% reduction in total PPO activity, which translates to a 73% decrease in browning compared with the wild type[143]. Browning can be further reduced by targeting the other tuber isoforms (*PPO1*, *PPO3* and *PPO4*). Such multiplex strategy is applied to eggplant by targeting a region conserved among the three *PPO* isoforms (*PPO4*, *PPO5* and *PPO6*), showing the highest expressions upon cutting of the fruit[142]. The PPO activity in the genome-edited fruits decreases by about 50% compared with the wild type, a change accompanied by visible reductions in browning. The non-browning mushroom is the first CRISPR-edited crop exempted from USDA regulation[144].

The shelf life of perishable climacteric fruits depends on their softening and ripening properties. Although harvesting earlier than full ripeness extends shelf life, it also reduces nutritional quality and flavour. By contrast, harvesting at full ripeness ensures nutrient and flavour richness but shortens shelf life[140]. The timing-sensitive ripening process is controlled by ethylene status, making the genes involved in ethylene biosynthesis ideal genome editing targets to extend shelf life[140]. CRISPR–Cas9 mutagenesis of aminocyclopropane-1-carboxylate oxidase 1 (*ACO1*), which encodes the main enzyme isoform in banana catalysing the final step of ethylene biosynthesis, delays fruit ripening by about 2 months[145]. Moreover, the thus obtained bananas ripen around the same time as the wild type, when ripening is induced by ethephon, though their organoleptic properties are uncertain.

Ripening is regulated by different transcription factors acting as master regulators[146]. For example, editing of the ripening inhibitor gene (*RIN*) delays ripening in tomatoes, which display a wide range of pigmentation, depending on the level of editing[147,148]. Similarly, CRISPR–Cas9 mutants of the non ripening gene (*NOR*) express truncated forms of the transcription factor and, consequently, the plants produce tomatoes with the partial non-ripening phenotype (reduced red colouring) as opposed to the naturally occurring *NOR* mutant, the fruits of which remain unripened and green[149]. By contrast, truncations in the colourless ripening gene (*CNR*) create tomatoes with the wild-type colour, delaying ripening by a few days[149]. Introducing the T137A point mutation into *NOR* (corresponding to a valine to aspartic acid mutation in the NOR protein) through CRISPR–Cas9-mediated HDR extends the shelf life of tomatoes with no other visible effect[150], indicating that the ripening time can be fine-tuned by subtle changes in the protein structures of these transcription factors or their expression (Fig. 3f).

Excessive delays in ripening are often accompanied by a low nutritional value and a poor organoleptic quality[140]. These setbacks can be avoided by delaying over-ripening, rather than slowing it, by targeting the softening process, which occurs through changes in cuticle characteristics and the actions of several cell-wall-remodelling enzymes. For example, an isoform of the pectin-degrading enzyme pectate lyase is identified as being highly expressed during tomato ripening[151]. RNAi silencing and CRISPR–Cas9 mutagenesis of pectate lyase improve pericarp texture and water retention and substantially delay softening without changing the levels of metabolites related to colour and flavour in the genome-edited fruits[151,152]. Accordingly, combining pectate lyase mutation with one of the minor transcription factor mutations could further extend the shelf life of tomatoes without compromising other properties (Fig. 3f).

Genome-edited foods beyond crops

Two-thirds of the global agricultural land is used as pasture fields and one-third of the cereal production is fed to animals. Moreover, the demand for animal-based foods will increase by 70% by mid-century[3]. Therefore, improving livestock and aquaculture should be considered[3,153], in which genome editing is utilized to increase productivity, control diseases and improve the nutritional value of animal food products[154–156].

Among the genes promoting growth rate in animals, myostatin (*MSTN*) stands out owing to its dramatic effect on muscle development. Naturally occurring *MSTN* mutations result in pronounced muscle development in cattle and sheep breeds[156]. Consequently, knocking out the gene in different species, including cattle, sheep, goat, pig and fish, produces animals with more muscle mass, leaner meat and higher feed efficiency than wild types[155,156]. In 2021, Japan approved the sale of *MSTN* CRISPR-edited sea bream, which grows 1.2 times larger on the same amount of feed[157] (Table 1). Porcine reproductive and respiratory syndrome virus (PRRSV), which causes massive productivity losses in the pork industry[158], presents a notable example of engineering disease resistance in livestock. CRISPR–Cas9 mutagenesis of the cluster of differentiation 163 (*CD163*), encoding the receptor responsible

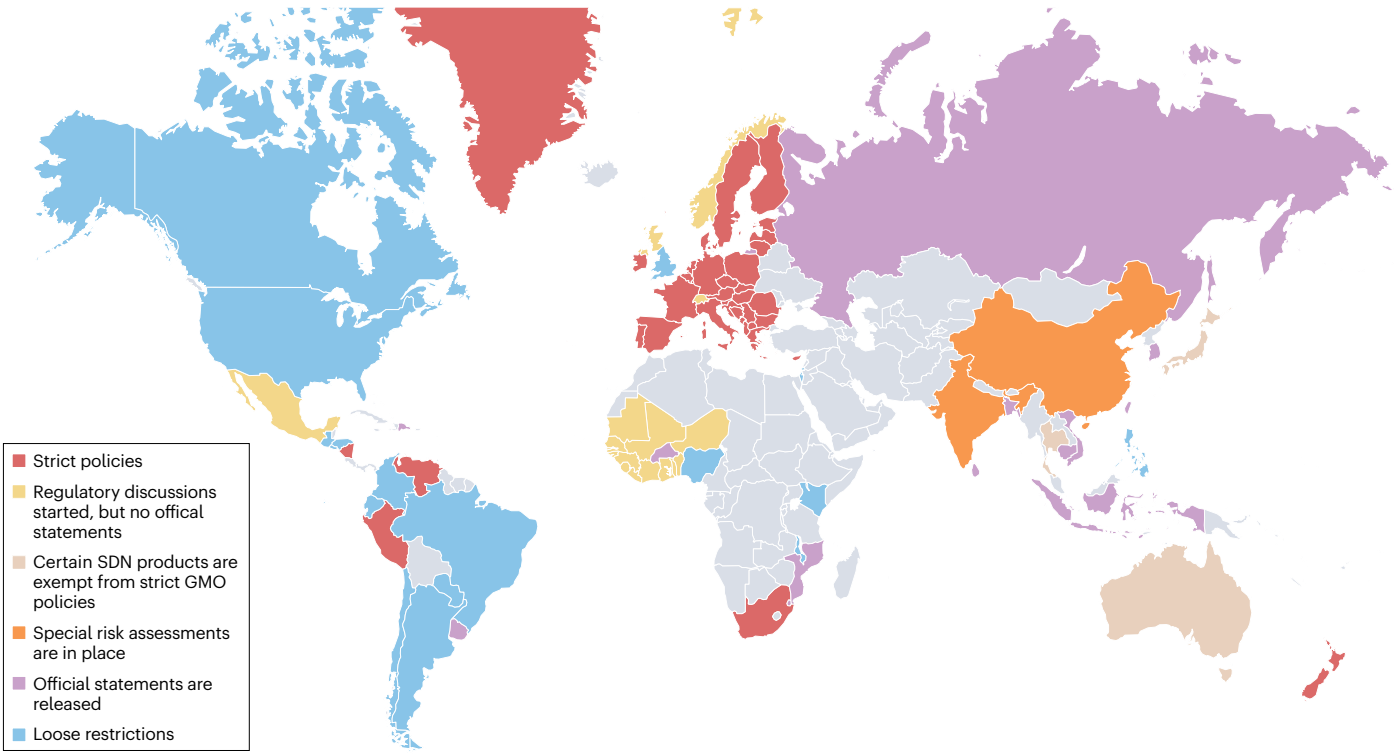

Fig. 4 | Current regulations of genome-edited crops around the world. The map is coloured to indicate the various types of regulations. In red: strict policies that regulate genome-edited crops as genetically modified organisms (GMOs) are in place (the European Union (EU) and other non-member states in the region, New Zealand, South Africa, Venezuela, Peru and Nicaragua). The discussions in some of these countries (New Zealand and in Europe) are ongoing, with the first encouraging decisions in EU released in 2023 (ref. 195). Moreover, the UK is conducting field trials with genome-edited products for possible exemption from the laborious risk assessment. Further laws in the UK allow certain genome-edited products, which could be produced by conventional breeding, to be exempt from GMO regulations. For example, cisgenic insertions are defined as "precision bred" and as not containing "foreign DNA" in the new legislation. In yellow: regulatory discussions have started, but official statements are yet to be released. The discussions are most advanced in Norway and Switzerland, with possible flexibility on site-directed nuclease 1 (SDN-1). In light brown: SDN products without foreign DNA integration are exempt from strict GMO policies (Australia, Japan and Thailand). Australia and Japan released guidelines in 2019. Australia exempts SDN-1, provided DNA templates are not used. The Japanese guidelines refer to the Cartagena protocol and exempt SDN-1, SDN-2 and oligonucleotide-directed mutagenesis, provided that the absence of foreign DNA integration is proven in the cases of SDN-2 and oligonucleotide-directed mutagenesis. In orange: special risk assessments are in place (China and India). In China, guidelines provide four requirement categories based on the risk profile of the target trait and focus on the potential for increasing crop and/or environmental safety risks. These regulations only cover SDN-1 and SDN-2, with SDN-3 products being regulated as GMOs. In 2022, India exempted SDN-1 and SDN-2 genome-edited products to accelerate the development of novel crop varieties with improved disease and drought resistance. However, SDN-1 edits will be assessed for the absence of any biologically relevant off-target genomic changes. For SDN-2, the assessment is extended to phenotypic equivalence and trait efficacy under contained field trials. By contrast, SDN-3 products are assessed strictly as conventional GMOs. In light purple: discussions are ongoing, and official statements about the potential future regulations have been released, or the country supports the International Statement on Agricultural Application of Precision Biotechnology submitted to the World Trade Organization. In light blue: loose restrictions are in place, allowing the commercialization of certain types of genome-edited crops. In the USA, SDN-1 genome-edited crops without transgenes are regarded as conventionally bred crops. SDN-2 with one base change is also deregulated if the edited crop carries no transgene. SDN-2 with more than one base change and SDN-3 are regulated and require case-by-case evaluation according to the SECURE rule[196]. Canada follows product-based regulations focusing on the genome-edited crop, rather than the process that creates it. In 2015, Argentina became one of the first countries to release guidelines for the handling of genome-edited crops[197]. The Argentine regulations established a template for other South American countries to introduce similar guidelines[170]. In Africa, Nigeria became the first country to release the relevant guidelines in 2020, exempting the genome-edited products, provided they do not have a novel gene combination or integrated recombinant DNA. In 2022, Kenya released comparable guidelines to those of Nigeria[169]. Philippines deregulates genome-edited products that do not possess a novel combination of genetic material that cannot be achieved with conventional breeding. In 2023, decisions on genome editing regulations are expected to be published in South Korea, Taiwan, Uruguay, the UK and the EU. Several African countries (Malawi, Ethiopia and Ghana) also discuss such regulations. However, it is unclear when the policies will be implemented.

for the entry of PRRSV into cells, confers PRRSV resistance to pigs[159]. Analogous to seed glutens, cow and goat milk contains caseins and β-lactoglobulin (BLG), which can be allergenic to some people. Accordingly, *BLG*-knockout goats produce milk with undetectable levels of the allergenic protein[160].

CRISPR deployment in bacteria has various food applications, particularly in the fermentation processes. Yoghurt was the first naturally CRISPR-enhanced food, in which the fermenting bacteria express CRISPR–Cas systems to acquire immunity against invading viruses[161]. In fact, Cas9 genome editing effector is first identified and characterized

in dairy starter cultures[161]. Historically, the first commercial application of CRISPR is improving phage resistance in yoghurt and cheese cultures[162]. Beneficial bacteria are also widely formulated in feed, foods and dietary supplements as probiotics, defined as live microorganisms that confer benefits to the host when administered in sufficient amounts[163]. The gut microbiota composition is impacted by lifestyle and dietary habits[164], and it influences the body's immune response and outcomes of autoimmune diseases[165]. Therefore, manipulation of gut microbiota and correcting dysbiosis has great potential for preventative and therapeutic purposes. The most commonly used probiotic organisms include *Escherichia coli* Nissle 1917 and strains from certain species of *Lactobacillus*, *Bifidobacterium* and *Saccharomyces*[166]. Engineered variants (predominantly transgene-expressing mutants) of these species are widely used in the treatment of animal models of autoimmune diseases, cancer, infections, inflammation and metabolic disorders, with only a limited number of them being tested in early-phase human clinical trials[166]. For example, engineered *E. coli* Nissle 1917, metabolizing phenylalanine, and *Lactobacillus lactis*, delivering human proinsulin and IL-10, are trailed for the treatment of phenylketonuria and type 1 diabetes, respectively[166]. Furthermore, genome editing is applied to bacterial probiotics, such as *Lactobacillus*[167] and *Bifidobacterium*[168], to improve the host gut adaptability and colonization properties of these species[167].

Regulations and evolving public view

Genome editing mutations are classified as site-directed nuclease 1 (SDN-1), SDN-2 and SDN-3 at the regulatory level[169]. Sequence-specific nucleases induce double-strand breaks in DNA. Template-independent repair of double-strand breaks through the non-homologous end joining pathway can lead to small indels (targeted mutagenesis) (Fig. 1) that are comparable to those achieved by conventional breeding or classical mutagenesis techniques and are defined as SDN-1. Alternatively, SDN-2 mutations refer to specific point mutations and small indels generated using a repair template. By contrast, SDN-3 mutations refer to template-based insertions of large DNA sequences (for example, promoter or an entire gene) in the genome. In general, the SDN-1 category of mutations (and in certain SDN-2 cases, such as in the absence of foreign DNA in the final crop product) are deregulated in countries with flexible regulations, whereas SDN-3-type mutations are more strictly regulated[169]. However, the demarcations between SDN-1, SDN-2 and SDN-3 are not always clear-cut and may require evaluation on a case-by-case basis of the genome-edited organism. The distinction between the categories is further complicated by the recently developed base and prime editors, as the relevant techniques utilize Cas proteins with modified nuclease activity (for example, a Cas9 nickase such as nCas9-D10A, which introduces single-strand breaks), so they do not fit into the conventional SDN definition[20].

Supportive governmental regulations and consumer acceptance are required to deploy genome editing technologies commercially. The rapid emergence of CRISPR–Cas systems challenges the regulations worldwide, as most agencies and existing frameworks need to cope with these novel modalities and their applications. Some genome-edited products have already entered the markets in the Americas and Asia, with more product approvals on the horizon (Table 1). Regulatory agencies should determine whether and how these edited products should be (de)regulated with harmonized regulatory schemes to be defined internationally. Some countries issue new guidelines, whereas others amend active regulations to ensure compliance with their existing biotechnology policies, leading to a globally diverse, misaligned mosaic of regulatory policies[169,170] (Fig. 4).

Regardless of the de-regulations passed by governmental bodies, the market viability of genome-edited foods ultimately depends on public acceptance and interest. In contrast to genetically modified organisms, which contain transgenes, genome-edited food is

Box 2

Genome editing in low-resource settings

Deployment of genome editing technologies presents unique opportunities for food and nutrition security in low-resource settings. Yields of locally grown orphan crops can be improved, in addition to post-harvest characteristics and resistance to environmental stresses, such as drought and pests. These improvements can impact the food supply in regions where crop failure and yield losses are prevalent due to limited resources and unfavourable environmental conditions. Moreover, genome editing can improve the nutritional quality of crops grown and consumed locally within communities in which diets disproportionately rely on a few types of crops. However, challenges are associated with transferring genome editing technologies to low-resource regions. These challenges include limited infrastructure (for example, temperature-controlled environments), inadequate laboratory facilities, lack of equipment (for example, advanced sequencing and analysis instruments) and skilled personnel, and scarcity of funding. In addition, low-resource regions lack well-defined regulatory frameworks and consumers and/or producers may have concerns regarding the safety of genome-edited crops. Furthermore, intellectual property rights and access to genetic resources can be challenging. Overcoming these challenges requires a multitude of approaches. Targeted capacity-improvement programmes supported by global funding and international collaboration networks among public and private research institutions to provide training opportunities and promote knowledge sharing are essential to empowering local scientists. Developing low-cost, open-source tools (for example, plasmid sharing) and resource-efficient simplified protocols, and streamlining the genome editing workflow to minimize the consumption of expensive or rare reagents can improve accessibility. Moreover, access to genetic diversity (for example, germplasm resources) and plant material should also be easily granted to researchers. Providing regulatory oversight and informing local communities about the benefits of genome-edited crops can help to accelerate the adoption of the technology. Implementing these strategies will be essential to ensuring sustainable agriculture in low-resource areas, as they are expected to have the highest population increases and to be affected by climate change.

Box 3

Development process of genome-edited crops

Developing genome-edited crops involves multiple steps that require a comprehensive understanding of the relevant trait(s) at genetic, biochemical and physiological levels. The first step is to carefully determine the target(s) in the genome (avoiding any potential off-target effects) and the desired modification(s) (such as small indels or base substitutions). It is highly recommended to test these targets (and guide RNAs, if CRISPR is used) with small-scale experiments (for example, cell-based transient assays) to ensure reasonable editing efficacy. The appropriate plant tissue is then transformed, stably or transiently, with the editing reagents and the edited lines regenerated. Once the desired change or changes in the genome is or are confirmed (by DNA and/or RNA sequencing), plants are further propagated to obtain homozygous lines and (unless editing reagents are delivered transiently) to breed out the transgenes, such as the Cas gene and the selection marker. Multiple genome-edited lines grown in controlled environment conditions (for example, in greenhouse) are then analysed. Phenotypically, edited plants with no yield penalty (or acceptable yield loss) are desired. Biochemically, the nutritional content (for example, micronutrient concentration) has to be improved compared with the wild type. Genetically, the absence of off-target effects and any foreign DNA is proven by whole genome sequencing. After passing these analyses, the selected lines go through field trials to test whether the improved trait(s) can be reproduced in the natural environment. It is preferable to conduct field trials multiple times during various growing seasons in different locations to reach conclusive results. After the successful completion of the field trials, a permit is filed to the relevant agency of that country to grow and market the product, which can take years to obtain. After its approval, the market success of the product depends on the public view and on the interest of the consumers and producers. Finally, marketing campaigns supported by convincing scientific evidence are required, such as animal and human trials that show improved health conditions due to consumption of the genome-edited nutrient-fortified crop (for example, vitamin-biofortified crops). Similarly, farmers could willingly grow genome-edited crops provided that related yield loss, if any, is economically outscored by the consumer benefit and interest. In this regard, producer interest can be raised by concurrently improving yield traits, such as disease and/or drought tolerance.

more accepted due to its perceived naturalness and novelty of the technology[171,172]. Genome editing is mostly used for creating knockouts, which aligns with the observation that gene deletions are perceived to be more natural than insertions[173]. Additionally, genome-edited plants are more favoured than their livestock counterparts[174]. Nevertheless, surveys around the world reveal that public knowledge and awareness are low, with the misconception that parties other than consumers will benefit more from genome-edited foods[171,174]. Therefore, consumers may trust environmental organizations, for example, more than the government and biotech industry[171]. Considering the societal impact, younger generations and people with higher education levels and incomes are found to be more accepting of genome-edited foods as studied in the USA[175].

Outlook

Genome editing has the potential of shaping the future of agriculture. CRISPR-based technologies allow to alter the genomes of diverse crops with unparalleled precision and ease. Research involves harnessing and/or engineering highly specific and efficient Cas variants and developing new genome editing tools using additional effectors. Achieving precise HDR events with high efficacy in plants is still challenging. Otherwise, HDR alone can generate all types of SDN edits. The potential of chromosome engineering in generating alternative cisgenic events for crop improvement or even creating novel species is only being realized[176]. For example, CRISPR–Cas-mediated chromosomal translocations are demonstrated in *Arabidopsis*[177], allowing to break linkage-drags or to link desired traits in crops[176]. Similarly, the inversion of chromosomal regions devoid of natural crossovers allows to unlock new recombination events, adding more genetic variations in crop breeding[178,179].

Implementing genome editing in crops requires intensive tissue culture, one of the major bottlenecks in plant transformation[25]. The expression of morphogenic factor genes may help to alleviate this challenge[26]. Improvements in plant tissue culture and genome editing can be simultaneously achieved by combining the activation of morphogenic genes with the editing of target genes using the CRISPR-Combo system[23]. Although well-established transformation protocols exist for primary crops, relevant research should expand to orphan crops and wild species. Therefore, innovative and universal approaches are needed to streamline the delivery of genome editing reagents into plant cells. Viral delivery, grafting and nanomaterials hold great potential for this purpose[180–182]. Challenges extend to a lack of in-depth understanding of the metabolic pathways at genetic and biochemical levels and implementation of genome editing technologies in low-resource settings (Box 2).

Although primary cereals and horticultural crops are the focus of plant genome editing, considerable interest is expected to shift towards orphan crops such as yam, cassava and millet. These endeavours will help to maintain food and nutrition security for small communities dependent on local farming. So far, genome editing is mostly used to improve one trait at a time: yield or nutritional quality. Upcoming research may be multi-trait-oriented, which is important in perennial trees (for example, citrus and apple) that benefit only to a small extent from traditional breeding due to the long generational times required to grow mature plants capable of producing offspring. Multi-trait improvement will also be critical for satisfying the consumers and producers and for ensuring the commercial success of engineered crops. On the livestock and aquaculture fronts, genome-edited animals have started to be introduced in some countries (for example, the genome-edited fish in Japan)[157,183] (Table 1). However, research

focuses mainly on increasing productivity rather than improving nutritional quality[154,156]. Other traits to improve include reducing allergens in meat, altering fat and lean meat composition, and enhancing flavour. Possibilities extend to generating environmentally friendly animals that require less feed and produce less waste. Alternatively, plant-based and cultured meat represent healthier, more sustainable and humane alternatives. For lab-grown meat, genome editing can be utilized to program stem cells to proliferate faster or for altering the composition of muscle and fat cells in tissue culture. However, economic feasibility and consumer acceptance of these cell-based products are uncertain[184].

Progress in regulatory policies and the general public view of genome-edited food is still slow and insufficient. Countries such as the USA, the UK, Japan and Argentina grant some flexibility to use genome editing techniques for making relatively small genomic changes. More countries are expected to follow with expanding public acceptance. Large cisgenic edits of SDN-3 (for example, promoter replacement or insertion of multiple gene copies) are next to pass regulatory restrictions, because cisgenic changes are perceived as more natural, and thus favoured by the public, than transgenic ones[185]. However, SDN-3, though expanding trait engineering opportunities, is often comparable to classical genetic modification at the regulatory level. Research organizations should provide transparency on genome editing research and participate in outreach activities informing the public about the development and benefits of genome-edited foods at the consumer and producer levels (Box 3).

Although CRISPR-related genome editing is mainly directed to medical applications, given the therapeutic and financial implications, deploying the technology in agriculture will impact more people and benefit the environment. Genome editing can provide sustainable agriculture and increase the nutritional value of diet that subside the onset of food-related diseases at over-consumption and under-consumption levels, thereby indirectly lowering the burden on healthcare systems. Diet also plays a major role in our development, disease susceptibility, response to environmental exposures and aging by altering the expression of genes through epigenetic marks[186]. Growing nutrigenomics evidence indicates that we are what we eat at the epigenetic level, and so are our children[187]. The epigenetic marks in our genomes can be transgenerational, impacting the health and well-being of offspring[187,188]. Early childhood genetic screening to determine susceptibility to non-communicable diseases and assessment of epigenetic status later in life (for example, during diagnosis) will help to craft personalized diets for individuals. The role of biofortified nutritious food will be important in preventative medicine. Eating nutrient-biofortified food, in combination with healthy lifestyle choices, will improve the quality of life and extend life expectancy. In this regard, genome editing can drive the diversification and improvement of the food we eat and help to develop personalized diets.

Published online: 04 October 2023

References

1. United Nations Department of Economic and Social Affairs, Population Division. World population prospects 2022: summary of results (UN DESA, 2022).
2. van Dijk, M., Morley, T., Rau, M. L. & Saghai, Y. A meta-analysis of projected global food demand and population at risk of hunger for the period 2010–2050. *Nat. Food* **2**, 494–501 (2021).
3. Searchinger, T., Waite, R., Hanson, C. & Ranganathan, J. Creating a sustainable food future: a menu of solutions to feed nearly 10 billion people by 2050 (World Resources Institute, 2019).
4. Food and Agriculture Organization of the United Nations, International Fund for Agricultural Development, United Nations Children's Fund, World Food Programme & World Health Organization. The state of food security and nutrition in the world 2022: repurposing food and agricultural policies to make healthy diets more affordable (FAO, 2022).
5. United Nations Children's Fund, World Health Organization & World Bank. Levels and trends in child malnutrition: UNICEF/WHO/The World Bank Group joint child malnutrition estimates: key findings of the 2021 edition (UNICEF, 2021).
6. Ritchie, H. & Roser, M. Obesity. *Our World in Data* https://ourworldindata.org/obesity (2017).
7. Fischetti, M. One-world menu. *Sci. Am.* **315**, 76 (2016).
8. Chen, K., Wang, Y., Zhang, R., Zhang, H. & Gao, C. CRISPR/Cas genome editing and precision plant breeding in agriculture. *Annu. Rev. Plant Biol.* **70**, 667–697 (2019).
9. Puchta, H., Dujon, B. & Hohn, B. Homologous recombination in plant cells is enhanced by in vivo induction of double strand breaks into DNA by a site-specific endonuclease. *Nucleic Acids Res.* **21**, 5034–5040 (1993).
10. Lloyd, A., Plaisier, C. L., Carroll, D. & Drews, G. N. Targeted mutagenesis using zinc-finger nucleases in *Arabidopsis*. *Proc. Natl Acad. Sci. USA* **102**, 2232–2237 (2005).
11. Christian, M. et al. Targeting DNA double-strand breaks with TAL effector nucleases. *Genetics* **186**, 757–761 (2010).
12. Anzalone, A. V., Koblan, L. W. & Liu, D. R. Genome editing with CRISPR-Cas nucleases, base editors, transposases and prime editors. *Nat. Biotechnol.* **38**, 824–844 (2020).
13. Wang, J. Y. & Doudna, J. A. CRISPR technology: a decade of genome editing is only the beginning. *Science* **379**, eadd8643 (2023).
14. Jinek, M. et al. A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. *Science* **337**, 816–821 (2012).
15. Malzahn, A., Lowder, L. & Qi, Y. Plant genome editing with TALEN and CRISPR. *Cell Biosci.* **7**, 21 (2017).
16. Nekrasov, V., Staskawicz, B., Weigel, D., Jones, J. D. & Kamoun, S. Targeted mutagenesis in the model plant *Nicotiana benthamiana* using Cas9 RNA-guided endonuclease. *Nat. Biotechnol.* **31**, 691–693 (2013).
17. Li, J. F. et al. Multiplex and homologous recombination-mediated genome editing in *Arabidopsis* and *Nicotiana benthamiana* using guide RNA and Cas9. *Nat. Biotechnol.* **31**, 688–691 (2013).
18. Shan, Q. et al. Targeted genome modification of crop plants using a CRISPR-Cas system. *Nat. Biotechnol.* **31**, 686–688 (2013).
19. Pan, C., Sretenovic, S. & Qi, Y. CRISPR/dCas-mediated transcriptional and epigenetic regulation in plants. *Curr. Opin. Plant Biol.* **60**, 101980 (2021).
20. Molla, K. A., Sretenovic, S., Bansal, K. C. & Qi, Y. Precise plant genome editing using base editors and prime editors. *Nat. Plants* **7**, 1166–1187 (2021).
21. Zhang, Y., Malzahn, A. A., Sretenovic, S. & Qi, Y. The emerging and uncultivated potential of CRISPR technology in plant science. *Nat. Plants* **5**, 778–794 (2019).
22. Pan, C. et al. CRISPR-Act3.0 for highly efficient multiplexed gene activation in plants. *Nat. Plants* **7**, 942–953 (2021).
23. Pan, C. et al. Boosting plant genome editing with a versatile CRISPR-Combo system. *Nat. Plants* **8**, 513–525 (2022).
24. Ghogare, R., Ludwig, Y., Bueno, G. M., Slamet-Loedin, I. H. & Dhingra, A. Genome editing reagent delivery in plants. *Transgenic Res.* **30**, 321–335 (2021).
25. Altpeter, F. et al. Advancing crop transformation in the era of genome editing. *Plant Cell* **28**, 1510–1520 (2016).
26. Chen, Z., Debernardi, J. M., Dubcovsky, J. & Gallavotti, A. Recent advances in crop transformation technologies. *Nat. Plants* **8**, 1343–1351 (2022).
27. D'Odorico, P., Carr, J. A., Laio, F., Ridolfi, L. & Vandoni, S. Feeding humanity through global food trade. *Earths Future* **2**, 458–469 (2014).
28. Smith, M. R. & Myers, S. S. Impact of anthropogenic CO_2 emissions on global human nutrition. *Nat. Clim. Change* **8**, 834–839 (2018).
29. U.S. department of agriculture and U.S. department of health and human services *Dietary Guidelines for Americans, 2020–2025* 9th edn (2020).
30. Zeeman, S. C., Kossmann, J. & Smith, A. M. Starch: its metabolism, evolution, and biotechnological modification in plants. *Annu. Rev. Plant Biol.* **61**, 209–234 (2010).
31. Slattery, C. J., Kavakli, I. H. & Okita, T. W. Engineering starch for increased quantity and quality. *Trends Plant Sci.* **5**, 291–298 (2000).
32. Shure, M., Wessler, S. & Fedoroff, N. Molecular identification and isolation of the *Waxy* locus in maize. *Cell* **35**, 225–233 (1983).
33. & Ma, X. et al. A robust CRISPR/Cas9 system for convenient, high-efficiency multiplex genome editing in monocot and dicot plants. *Mol. Plant* **8**, 1274–1284 (2015).
34. Zhang, S. et al. CRISPR/Cas9-mediated genome editing for wheat grain quality improvement. *Plant Biotechnol. J.* **19**, 1684–1686 (2021).
35. Gao, H. et al. Superior field performance of waxy corn engineered using CRISPR-Cas9. *Nat. Biotechnol.* **38**, 579–581 (2020).
36. Andersson, M. et al. Efficient targeted multiallelic mutagenesis in tetraploid potato (*Solanum tuberosum*) by transient CRISPR-Cas9 expression in protoplasts. *Plant Cell Rep.* **36**, 117–128 (2017).
37. Isshiki, M. et al. A naturally occurring functional allele of the rice *waxy* locus has a GT to TT mutation at the 5′ splice site of the first intron. *Plant J.* **15**, 133–138 (1998).
38. Zeng, D. et al. Quantitative regulation of *Waxy* expression by CRISPR/Cas9-based promoter and 5′UTR-intron editing improves grain quality in rice. *Plant Biotechnol. J.* **18**, 2385–2387 (2020).
39. Huang, L. et al. Creating novel *Wx* alleles with fine-tuned amylose levels and improved grain quality in rice by promoter editing using CRISPR/Cas9 system. *Plant Biotechnol. J.* **18**, 2164–2166 (2020).
40. Zhou, J. et al. An efficient CRISPR-Cas12a promoter editing system for crop improvement. *Nat. Plants* **9**, 588–604 (2023).

41. World Obesity Federation. The economic impact of overweight & obesity in 2020 and 2060: 2nd edition with estimates for 161 countries (World Obesity Federation, 2022).
42. DeMartino, P. & Cockburn, D. W. Resistant starch: impact on the gut microbiome and health. *Curr. Opin. Biotechnol.* **61**, 66–71 (2020).
43. Li, L., Jiang, H. X., Campbell, M., Blanco, M. & Jane, J. L. Characterization of maize amylose-extender (*ae*) mutant starches. Part I: relationship between resistant starch contents and molecular structures. *Carbohydr. Polym.* **74**, 396–404 (2008).
44. Sun, Y. et al. Generation of high-amylose rice through CRISPR/Cas9-mediated targeted mutagenesis of starch branching enzymes. *Front. Plant Sci.* **8**, 298 (2017).
45. Li, J. et al. Modification of starch composition, structure and properties through editing of *TaSBEIIa* in both winter and spring wheat varieties by CRISPR/Cas9. *Plant Biotechnol. J.* **19**, 937–951 (2021).
46. Zhao, X. et al. Amylose starch with no detectable branching developed through DNA-free CRISPR-Cas9 mediated mutagenesis of two starch branching enzymes in potato. *Sci. Rep.* **11**, 4311 (2021).
47. Tuncel, A. et al. Cas9-mediated mutagenesis of potato starch-branching enzymes generates a range of tuber starch phenotypes. *Plant Biotechnol. J.* **17**, 2259–2271 (2019).
48. Sanchez-Leon, S. et al. Low-gluten, nontransgenic wheat engineered with CRISPR/Cas9. *Plant Biotechnol. J.* **16**, 902–910 (2018).
49. Li, A. et al. Editing of an alpha-kafirin gene family increases, digestibility and protein quality in sorghum. *Plant Physiol.* **177**, 1425–1438 (2018).
50. Hepsomali, P., Groeger, J. A., Nishihira, J. & Scholey, A. Effects of oral gamma-aminobutyric acid (GABA) administration on stress and sleep in humans: a systematic review. *Front. Neurosci.* **14**, 923 (2020).
51. Nonaka, S., Arai, C., Takayama, M., Matsukura, C. & Ezura, H. Efficient increase of ɣ-aminobutyric acid (GABA) content in tomato fruits by targeted mutagenesis. *Sci. Rep.* **7**, 7057 (2017).
52. Waltz, E. GABA-enriched tomato is first CRISPR-edited food to enter market. *Nat. Biotechnol.* **40**, 9–11 (2022).
53. Liu, H. R. & White, P. J. Oxidative stability of soybean oils with altered fatty-acid compositions. *J. Am. Oil Chem. Soc.* **69**, 528–532 (1992).
54. Haun, W. et al. Improved soybean oil quality by targeted mutagenesis of the fatty acid desaturase 2 gene family. *Plant Biotechnol. J.* **12**, 934–940 (2014).
55. Okuzaki, A. et al. CRISPR/Cas9-mediated genome editing of the *fatty acid desaturase 2* gene in *Brassica napus*. *Plant Physiol. Biochem.* **131**, 63–69 (2018).
56. Do, P. T. et al. Demonstration of highly efficient dual gRNA CRISPR/Cas9 editing of the homeologous *GmFAD2-1A* and *GmFAD2-1B* genes to yield a high oleic, low linoleic and alpha-linolenic acid phenotype in soybean. *BMC Plant Biol.* **19**, 311 (2019).
57. Abe, K., Araki, E., Suzuki, Y., Toki, S. & Saika, H. Production of high oleic/low linoleic rice by genome editing. *Plant Physiol. Biochem.* **131**, 58–62 (2018).
58. Lee, K. R. et al. Increasing monounsaturated fatty acid contents in hexaploid *Camelina sativa* seed oil by *FAD2* gene knockout using CRISPR-Cas9. *Front. Plant Sci.* **12**, 702930 (2021).
59. Morineau, C. et al. Selective gene dosage by CRISPR-Cas9 genome editing hexaploid *Camelina sativa*. *Plant Biotechnol. J.* **15**, 729–739 (2017).
60. Park, M. E., Yun, J. Y. & Kim, H. U. C-to-G base editing enhances oleic acid production by generating novel alleles of *FATTY ACID DESATURASE 2* in plants. *Front. Plant Sci.* **12**, 748529 (2021).
61. Knutsen, H. K. et al. Erucic acid in feed and food. *EFSA J.* **14**, e04593 (2016).
62. Liu, Y. et al. CRISPR/Cas9-targeted mutagenesis of *BnaFAE1* genes confers low-erucic acid in *Brassica napus*. *Front. Plant Sci.* **13**, 848723 (2022).
63. Ozseyhan, M. E., Kang, J., Mu, X. & Lu, C. Mutagenesis of the *FAE1* genes significantly changes fatty acid composition in seeds of *Camelina sativa*. *Plant Physiol. Biochem.* **123**, 1–7 (2018).
64. Han, L., Haslam, R. P., Silvestre, S., Lu, C. & Napier, J. A. Enhancing the accumulation of eicosapentaenoic acid and docosahexaenoic acid in transgenic *Camelina* through the CRISPR-Cas9 inactivation of the competing FAE1 pathway. *Plant Biotechnol. J.* **20**, 1444–1446 (2022).
65. Napier, J. A. & Betancor, M. B. Engineering plant-based feedstocks for sustainable aquaculture. *Curr. Opin. Plant Biol.* **71**, 102323 (2023).
66. Ritchie, H. & Roser, M. Micronutrient deficiency. *Our World in Data* https://ourworldindata.org/micronutrient-deficiency (2017).
67. Ahmed, T., Hossain, M. & Sanin, K. I. Global burden of maternal and child undernutrition and micronutrient deficiencies. *Ann. Nutr. Metab.* **61**, 8–17 (2012).
68. Bailey, R. L., West, K. P. Jr. & Black, R. E. The epidemiology of global micronutrient deficiencies. *Ann. Nutr. Metab.* **66**, 22–33 (2015).
69. Fairfield, K. M. & Fletcher, R. H. Vitamins for chronic disease prevention in adults: scientific review. *J. Am. Med. Assoc.* **287**, 3116–3126 (2002).
70. Mishra, S., Stierman, B., Gahche, J. & Potischman, N. Dietary supplement use among adults: United States, 2017–2018. *NCHS* https://www.cdc.gov/nchs/products/databriefs/db399.htm (2021).
71. Bailey, R. L., Gahche, J. J., Miller, P. E., Thomas, P. R. & Dwyer, J. T. Why US adults use dietary supplements. *J. Am. Med. Assoc. Intern. Med.* **173**, 355–361 (2013).
72. Chen, F. et al. Association among dietary supplement use, nutrient intake, and mortality among U.S. adults. *Ann. Intern. Med.* **170**, 604–613 (2019).
73. Jenkins, D. J. A. et al. Supplemental vitamins and minerals for CVD prevention and treatment. *J. Am. Coll. Cardiol.* **71**, 2570–2584 (2018).
74. World Health Organization. Vitamin A deficiency. *WHO* https://www.who.int/data/nutrition/nlis/info/vitamin-a-deficiency (2009).
75. Paine, J. A. et al. Improving the nutritional value of Golden Rice through increased pro-vitamin A content. *Nat. Biotechnol.* **23**, 482–487 (2005).
76. Ye, X. et al. Engineering the provitamin A (beta-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. *Science* **287**, 303–305 (2000).
77. Swamy, B. P. M. et al. Compositional analysis of genetically engineered GR2E "Golden Rice" in comparison to that of conventional rice. *J. Agric. Food Chem.* **67**, 7986–7994 (2019).
78. International Rice Research Institute. Philippines becomes first country to approve nutrient-enriched "Golden Rice" for planting. *IRRI* https://www.irri.org/news-and-events/news/philippines-becomes-first-country-approve-nutrient-enriched-golden-rice (2021).
79. Dong, O. X. et al. Marker-free carotenoid-enriched rice generated through targeted gene insertion using CRISPR-Cas9. *Nat. Commun.* **11**, 1178 (2020).
80. Kaur, N. et al. CRISPR/Cas9 directed editing of *lycopene epsilon-cyclase* modulates metabolic flux for beta-carotene biosynthesis in banana fruit. *Metab. Eng.* **59**, 76–86 (2020).
81. Sun, T., Rao, S., Zhou, X. & Li, L. Plant carotenoids: recent advances and future perspectives. *Mol. Hortic.* **2**, 3 (2022).
82. Ko, M. R. et al. RNAi-mediated suppression of three carotenoid-cleavage dioxygenase genes, *OsCCD1, 4a*, and *4b*, increases carotenoid content in rice. *J. Exp. Bot.* **69**, 5105–5116 (2018).
83. Gayen, D., Ali, N., Sarkar, S. N., Datta, S. K. & Datta, K. Down-regulation of *lipoxygenase* gene reduces degradation of carotenoids of golden rice during storage. *Planta* **242**, 353–363 (2015).
84. Schaub, P. et al. Nonenzymatic beta-carotene degradation in provitamin A-biofortified crop plants. *J. Agric. Food Chem.* **65**, 6588–6598 (2017).
85. Che, P. et al. Elevated vitamin E content improves all-trans beta-carotene accumulation and stability in biofortified sorghum. *Proc. Natl Acad. Sci. USA* **113**, 11040–11045 (2016).
86. Watkins, J. L. & Pogson, B. J. Prospects for carotenoid biofortification targeting retention and catabolism. *Trends Plant Sci.* **25**, 501–512 (2020).
87. Morelli, L. & Rodriguez-Concepcion, M. Open avenues for carotenoid biofortification of plant tissues. *Plant Commun.* **4**, 100466 (2023).
88. Bulley, S. et al. Enhancing ascorbate in fruits and tubers through over-expression of the L-galactose pathway gene GDP-L-galactose phosphorylase. *Plant Biotechnol. J.* **10**, 390–397 (2012).
89. Zhang, H. et al. Genome editing of upstream open reading frames enables translational control in plants. *Nat. Biotechnol.* **36**, 894–898 (2018).
90. Zhang, Y. et al. Expanding the scope of plant genome engineering with Cas12a orthologs and highly multiplexable editing systems. *Nat. Commun.* **12**, 1944 (2021).
91. Xue, C. et al. Tuning plant phenotypes by precise, graded downregulation of gene expression. *Nat. Biotechnol.* https://doi.org/10.1038/s41587-023-01707-w (2023).
92. van Schoor, N. & Lips, P. in *Vitamin D. Volume 2: Health, Disease and Therapeutics* (ed. Feldman, D.) 15–40 (Academic, 2018).
93. Li, J. et al. Biofortified tomatoes provide a new route to vitamin D sufficiency. *Nat. Plants* **8**, 611–616 (2022).
94. Sonawane, P. D. et al. Plant cholesterol biosynthetic pathway overlaps with phytosterol metabolism. *Nat. Plants* **3**, 16205 (2016).
95. Choe, S. et al. Lesions in the sterol delta reductase gene of *Arabidopsis* cause dwarfism due to a block in brassinosteroid biosynthesis. *Plant J.* **21**, 431–443 (2000).
96. GBD 2015 Disease and Injury Incidence and Prevalence Collaborators. Global, regional, and national incidence, prevalence, and years lived with disability for 310 diseases and injuries, 1990-2015: a systematic analysis for the Global Burden of Disease Study 2015. *Lancet* **388**, 1545–1602 (2016).
97. World Health Organization. Worldwide prevalence of anaemia 1993–2005 (WHO, 2008).
98. Van Der Straeten, D. et al. Multiplying the efficiency and impact of biofortification through metabolic engineering. *Nat. Commun.* **11**, 5203 (2020).
99. Gao, S. et al. Cytokinin-dependent regulatory module underlies the maintenance of zinc nutrition in rice. *New Phytol.* **224**, 202–215 (2019).
100. Bouis, H. E., Hotz, C., McClafferty, B., Meenakshi, J. V. & Pfeiffer, W. H. Biofortification: a new tool to reduce micronutrient malnutrition. *Food Nutr. Bull.* **32**, S31–S40 (2011).
101. Li, S. H., He, Y. J., Li, L. Z., Li, D. L. & Chen, H. Y. New insights on the regulation of anthocyanin biosynthesis in purple *Solanaceous* fruit vegetables. *Sci. Hortic.* **297**, 110917 (2022).
102. Colanero, S., Perata, P. & Gonzali, S. What's behind purple tomatoes? Insight into the mechanisms of anthocyanin synthesis in tomato fruits. *Plant Physiol.* **182**, 1841–1853 (2020).
103. Butelli, E. et al. Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. *Nat. Biotechnol.* **26**, 1301–1308 (2008).
104. Coker, R. APHIS issues first regulatory status review response: Norfolk Plant Sciences' purple tomato. *APHIS* https://www.aphis.usda.gov/aphis/newsroom/stakeholder-info/sa_by_date/sa-2022/purple-tomato (2022).
105. Vu, T. V. et al. Highly efficient homology-directed repair using CRISPR/Cpf1-geminiviral replicon in tomato. *Plant Biotechnol. J.* **18**, 2133–2143 (2020).
106. Čermák, T., Baltes, N. J., Čegan, R., Zhang, Y. & Voytas, D. F. High-frequency, precise modification of the tomato genome. *Genome Biol.* **16**, 232 (2015).
107. Sun, C. et al. A transcriptional network promotes anthocyanin biosynthesis in tomato flesh. *Mol. Plant* **13**, 42–58 (2020).

108. Zhu, Y. et al. CRISPR/Cas9-mediated functional recovery of the recessive *rc* allele to develop red rice. *Plant Biotechnol. J.* **17**, 2096–2105 (2019).
109. Wang, X. et al. Discovery of a DFR gene that controls anthocyanin accumulation in the spiny Solanum group: roles of a natural promoter variant and alternative splicing. *Plant J.* **111**, 1096–1109 (2022).
110. Li, X. et al. Lycopene is enriched in tomato fruit by CRISPR/Cas9-mediated multiplex genome editing. *Front. Plant Sci.* **9**, 559 (2018).
111. Zsögön, A. et al. *De novo* domestication of wild tomato using genome editing. *Nat. Biotechnol.* **36**, 1211–1216 (2018).
112. Hunziker, J. et al. Multiple gene substitution by Target-AID base-editing technology in tomato. *Sci. Rep.* **10**, 20471 (2020).
113. Ibrahim, S. et al. CRISPR/Cas9 mediated disruption of *Inositol Pentakisphosphate 2-Kinase 1* (*TaIPK1*) reduces phytic acid and improves iron and zinc accumulation in wheat grains. *J. Adv. Res.* **37**, 33–41 (2022).
114. Sashidhar, N., Harloff, H. J., Potgieter, L. & Jung, C. Gene editing of three *BnITPK* genes in tetraploid oilseed rape leads to significant reduction of phytic acid in seeds. *Plant Biotechnol. J.* **18**, 2241–2250 (2020).
115. Mottram, D. S., Wedzicha, B. L. & Dodson, A. T. Acrylamide is formed in the Maillard reaction. *Nature* **419**, 448–449 (2002).
116. European Commission. Establishing mitigation measures and benchmark levels for the reduction of the presence of acrylamide in food. *EUR-Lex* https://eur-lex.europa.eu/eli/reg/2017/2158 (2017).
117. Clasen, B. M. et al. Improving cold storage and processing traits in potato through targeted gene knockout. *Plant Biotechnol. J.* **14**, 169–176 (2016).
118. Ly, D. N. P., Iqbal, S., Fosu-Nyarko, J., Milroy, S. & Jones, M. G. K. Multiplex CRISPR-Cas9 gene-editing can deliver potato cultivars with reduced browning and acrylamide. *Plants* **12**, 379 (2023).
119. Yasmeen, A. et al. CRISPR/Cas-mediated knockdown of vacuolar invertase gene expression lowers the cold-induced sweetening in potatoes. *Planta* **256**, 107 (2022).
120. Halford, N. G., Raffan, S. & Oddy, J. Progress towards the production of potatoes and cereals with low acrylamide-forming potential. *Curr. Opin. Food Sci.* **47**, 100887 (2022).
121. Raffan, S. et al. Wheat with greatly reduced accumulation of free asparagine in the grain, produced by CRISPR/Cas9 editing of asparagine synthetase gene *TaASN2*. *Plant Biotechnol. J.* **19**, 1602–1613 (2021).
122. Raffan, S. et al. Field assessment of genome-edited, low asparagine wheat: Europe's first CRISPR wheat field trial. *Plant Biotechnol. J.* **21**, 1097–1099 (2023).
123. Juma, B. S., Mukami, A., Mweu, C., Ngugi, M. P. & Mbinda, W. Targeted mutagenesis of the *CYP79D1* gene via CRISPR/Cas9-mediated genome editing results in lower levels of cyanide in cassava. *Front. Plant Sci.* **13**, 1009860 (2022).
124. Zheng, Z. Z. et al. Editing sterol side chain reductase 2 gene (*StSSR2*) via CRISPR/Cas9 reduces the total steroidal glycoalkaloids in potato. *All Life* **14**, 401–413 (2021).
125. Tang, L. et al. Knockout of *OsNramp5* using the CRISPR/Cas9 system produces low Cd-accumulating indica rice without compromising yield. *Sci. Rep.* **7**, 14438 (2017).
126. Nieves-Cordones, M. et al. Production of low-Cs^+ rice plants by inactivation of the K^+ transporter OsHAK1 with the CRISPR-Cas system. *Plant J.* **92**, 43–56 (2017).
127. Assou, J. et al. Removing the major allergen Bra j I from brown mustard (*Brassica juncea*) by CRISPR/Cas9. *Plant J.* **109**, 649–663 (2022).
128. Camerlengo, F. et al. CRISPR-Cas9 multiplex editing of the α-amylase/trypsin inhibitor genes to reduce allergen proteins in durum wheat. *Front. Sustain. Food Syst.* **4**, 104 (2020).
129. Goff, S. A. & Klee, H. J. Plant volatile compounds: sensory cues for health and nutritional value? *Science* **311**, 815–819 (2006).
130. Colantonio, V. et al. Metabolomic selection for enhanced fruit flavor. *Proc. Natl Acad. Sci. USA* **119**, e2115865119 (2022).
131. Tieman, D. et al. A chemical genetic roadmap to improved tomato flavor. *Science* **355**, 391–394 (2017).
132. Gao, L. et al. The tomato pan-genome uncovers new genes and a rare allele regulating fruit flavor. *Nat. Genet.* **51**, 1044–1051 (2019).
133. Xing, S. et al. Fine-tuning sugar content in strawberry. *Genome Biol.* **21**, 230 (2020).
134. Hashemi, F. S. G. et al. Biochemical, genetic and molecular advances of fragrance characteristics in rice. *CRC Crit. Rev. Plant Sci.* **32**, 445–457 (2013).
135. Shan, Q., Zhang, Y., Chen, K., Zhang, K. & Gao, C. Creation of fragrant rice by targeted knockout of the *OsBADH2* gene using TALEN technology. *Plant Biotechnol. J.* **13**, 791–800 (2015).
136. Hui, S. et al. Production of aromatic three-line hybrid rice using novel alleles of *BADH2*. *Plant Biotechnol. J.* **20**, 59–74 (2022).
137. Tang, Y. et al. CRISPR/Cas9 induces exon skipping that facilitates development of fragrant rice. *Plant Biotechnol. J.* **19**, 642–644 (2021).
138. Ashokkumar, S. et al. Creation of novel alleles of fragrance gene *OsBADH2* in rice through CRISPR/Cas9 mediated gene editing. *PLoS ONE* **15**, e0237018 (2020).
139. Wang, Y. et al. Creation of aromatic maize by CRISPR/Cas. *J. Integr. Plant Biol.* **63**, 1664–1670 (2021).
140. Shipman, E. N., Yu, J., Zhou, J., Albornoz, K. & Beckles, D. M. Can gene editing reduce postharvest waste and loss of fruit, vegetables, and ornamentals? *Hortic. Res.* **8**, 1 (2021).
141. Gustavsson, J., Cederberg, C., Sonesson, U., van Otterdijk, R. & Meybeck, A. Global food losses and food waste : extent, causes and prevention (FAO, 2011).
142. Maioli, A. et al. Simultaneous CRISPR/Cas9 editing of three PPO genes reduces fruit flesh browning in *Solanum melongena* L. *Front. Plant Sci.* **11**, 607161 (2020).
143. Gonzalez, M. N. et al. Reduced enzymatic browning in potato tubers by specific editing of a polyphenol oxidase gene via ribonucleoprotein complexes delivery of the CRISPR/Cas9 system. *Front. Plant Sci.* **10**, 1649 (2019).
144. Waltz, E. Gene-edited CRISPR mushroom escapes US regulation. *Nature* **532**, 293 (2016).
145. Hu, C. et al. CRISPR/Cas9-mediated genome editing of *MaACO1* (aminocyclopropane-1-carboxylate oxidase 1) promotes the shelf life of banana fruit. *Plant Biotechnol. J.* **19**, 654–656 (2021).
146. Wang, R., Angenent, G. C., Seymour, G. & de Maagd, R. A. Revisiting the role of master regulators in tomato ripening. *Trends Plant Sci.* **25**, 291–301 (2020).
147. Ito, Y. et al. Re-evaluation of the *rin* mutation and the role of *RIN* in the induction of tomato ripening. *Nat. Plants* **3**, 866–874 (2017).
148. Ito, Y., Nishizawa-Yokoi, A., Endo, M., Mikami, M. & Toki, S. CRISPR/Cas9-mediated mutagenesis of the *RIN* locus that regulates tomato fruit ripening. *Biochem. Biophys. Res. Commun.* **467**, 76–82 (2015).
149. Gao, Y. et al. Diversity and redundancy of the ripening regulatory networks revealed by the fruitENCODE and the new CRISPR/Cas9 *CNR* and *NOR* mutants. *Hortic. Res.* **6**, 39 (2019).
150. Yu, Q. H. et al. CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. *Sci. Rep.* **7**, 11874 (2017).
151. Uluisik, S. et al. Genetic improvement of tomato by targeted control of fruit softening. *Nat. Biotechnol.* **34**, 950–952 (2016).
152. Wang, D. et al. Characterization of CRISPR mutants targeting genes modulating pectin degradation in ripening tomato. *Plant Physiol.* **179**, 544–557 (2019).
153. Godfray, H. C. et al. Food security: the challenge of feeding 9 billion people. *Science* **327**, 812–818 (2010).
154. Wani, A. K. et al. Genome centric engineering using ZFNs, TALENs and CRISPR-Cas9 systems for trait improvement and disease control in animals. *Vet. Res. Commun.* **47**, 1–16 (2023).
155. Wang, S. et al. Application of gene editing technology in resistance breeding of livestock. *Life* **12**, 1070 (2022).
156. Tait-Burkard, C. et al. Livestock 2.0 - genome editing for fitter, healthier, and more productive farmed animals. *Genome Biol.* **19**, 204 (2018).
157. No authors listed. Japan embraces CRISPR-edited fish. *Nat. Biotechnol.* **40**, 10 (2022).
158. Valdes-Donoso, P., Alvarez, J., Jarvis, L. S., Morrison, R. B. & Perez, A. M. Production losses from an endemic animal disease: porcine reproductive and respiratory syndrome (PRRS) in selected midwest US sow farms. *Front. Vet. Sci.* **5**, 102 (2018).
159. Whitworth, K. M. et al. Gene-edited pigs are protected from porcine reproductive and respiratory syndrome virus. *Nat. Biotechnol.* **34**, 20–22 (2016).
160. Zhou, W. et al. Generation of beta-lactoglobulin knock-out goats using CRISPR/Cas9. *PLoS ONE* **12**, e0186056 (2017).
161. Barrangou, R. et al. CRISPR provides acquired resistance against viruses in prokaryotes. *Science* **315**, 1709–1712 (2007).
162. Barrangou, R. et al. Genomic impact of CRISPR immunization against bacteriophages. *Biochem. Soc. Trans.* **41**, 1383–1391 (2013).
163. Hill, C. et al. Expert consensus document. The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. *Nat. Rev. Gastroenterol. Hepatol.* **11**, 506–514 (2014).
164. Fan, Y. & Pedersen, O. Gut microbiota in human metabolic health and disease. *Nat. Rev. Microbiol.* **19**, 55–71 (2021).
165. Miyauchi, E., Shimokawa, C., Steimle, A., Desai, M. S. & Ohno, H. The impact of the gut microbiome on extra-intestinal autoimmune diseases. *Nat. Rev. Immunol.* **23**, 9–23 (2023).
166. Aggarwal, N., Breedon, A. M. E., Davis, C. M., Hwang, I. Y. & Chang, M. W. Engineering probiotics for therapeutic applications: recent examples and translational outlook. *Curr. Opin. Biotechnol.* **65**, 171–179 (2020).
167. Goh, Y. J. & Barrangou, R. Harnessing CRISPR-Cas systems for precision engineering of designer probiotic lactobacilli. *Curr. Opin. Biotechnol.* **56**, 163–171 (2019).
168. Pan, M. et al. Genomic and epigenetic landscapes drive CRISPR-based genome editing in *Bifidobacterium*. *Proc. Natl Acad. Sci. USA* **119**, e2205068119 (2022).
169. Sprink, T., Wilhelm, R. & Hartung, F. Genome editing around the globe: an update on policies and perceptions. *Plant Physiol.* **190**, 1579–1587 (2022).
170. Menz, J., Modrzejewski, D., Hartung, F., Wilhelm, R. & Sprink, T. Genome edited crops touch the market: a view on the global development and regulatory environment. *Front. Plant Sci.* **11**, 586027 (2020).
171. Strobbe, S., Wesana, J., Van Der Straeten, D. & De Steur, H. Public acceptance and stakeholder views of gene edited foods: a global overview. *Trends Biotechnol.* **41**, 736–740 (2023).
172. Baum, C. M., Kamrath, C., Bröring, S. & De Steur, H. Show me the benefits! Determinants of behavioral intentions towards CRISPR in the United States. *Food Qual. Prefer.* **107**, 104842 (2023).
173. Siegrist, M. & Hartmann, C. Consumer acceptance of novel food technologies. *Nat. Food* **1**, 343–350 (2020).
174. Kato-Nitta, N., Inagaki, Y., Maeda, T. & Tachikawa, M. Effects of information on consumer attitudes towards gene-edited foods: a comparison between livestock and vegetables. *CABI Agric. Biosci.* **2**, 14 (2021).
175. Cummings, C. & Peters, D. Who trusts in gene-edited foods? Analysis of a representative survey study predicting willingness to eat- and purposeful avoidance of gene edited foods in the United States. *Front. Food Sci. Technol.* **2**, 858277 (2022).

176. Ronspies, M., Dorn, A., Schindele, P. & Puchta, H. CRISPR-Cas-mediated chromosome engineering for crop improvement and synthetic biology. *Nat. Plants* **7**, 566–573 (2021).
177. Beying, N., Schmidt, C., Pacher, M., Houben, A. & Puchta, H. CRISPR-Cas9-mediated induction of heritable chromosomal translocations in Arabidopsis. *Nat. Plants* **6**, 638–645 (2020).
178. Schwartz, C. et al. CRISPR-Cas9-mediated 75.5-Mb inversion in maize. *Nat. Plants* **6**, 1427–1431 (2020).
179. Schmidt, C. et al. Changing local recombination patterns in Arabidopsis by CRISPR/Cas mediated chromosome engineering. *Nat. Commun.* **11**, 4418 (2020).
180. Zhang, C., Liu, S., Li, X., Zhang, R. & Li, J. Virus-induced gene editing and its applications in plants. *Int. J. Mol. Sci.* **23**, 10202 (2022).
181. Yang, L., Machin, F., Wang, S., Saplaoura, E. & Kragler, F. Heritable transgene-free genome editing in plants by grafting of wild-type shoots to transgenic donor rootstocks. *Nat. Biotechnol.* **41**, 958–967 (2023).
182. Demirer, G. S. et al. Nanotechnology to advance CRISPR-Cas genetic engineering of plants. *Nat. Nanotechnol.* **16**, 243–250 (2021).
183. [No authors listed] CRISPR beef cattle get FDA green light. *Nat. Biotechnol.* **40**, 448 (2022).
184. Post, M. J. et al. Scientific, sustainability and regulatory challenges of cultured meat. *Nat. Food* **1**, 403–415 (2020).
185. Gaskell, G. et al. The 2010 Eurobarometer on the life sciences. *Nat. Biotechnol.* **29**, 113–114 (2011).
186. Zhang, Y. & Kutateladze, T. G. Diet and the epigenome. *Nat. Commun.* **9**, 3375 (2018).
187. Guo, T. Y., Luo, F. J. & Lin, Q. L. You are affected by what your parents eat: diet, epigenetics, transgeneration and intergeneration. *Trends Food Sci. Technol.* **100**, 248–261 (2020).
188. Miska, E. A. & Ferguson-Smith, A. C. Transgenerational inheritance: models and mechanisms of non-DNA sequence-based inheritance. *Science* **354**, 59–63 (2016).
189. Canadian Biotechnology Action Network. GM waxy corn (CBAN, 2021).
190. US Food and Drug Administration. Biotechnology notification file No. 000164: high oleic acid soybean, FAD2KO (FDA, 2019).
191. Robin, M. Researchers attempt to turn weed into a crop. *The Western Producer* https://www.producer.com/news/researchers-attempt-to-turn-weed-into-a-crop (2022).
192. International Service for the Acquisition of Agri-biotech Applications. Non-browning GreenVenus romaine lettuce advances to commercial trials in the US. *ISSA* https://www.isaaa.org/kc/cropbiotechupdate/article/default.asp?ID=17569 (2019).
193. Karlson, D. et al. Targeted mutagenesis of the multicopy *myrosinase* gene family in allotetraploid *Brassica juncea* reduces pungency in fresh leaves across environments. *Plants* **11**, 2494 (2022).
194. Silva, Y. P., Bernardi, A. & Frozza, R. L. The role of short-chain fatty acids from gut microbiota in gut-brain communication. *Front. Endocrinol.* **11**, 25 (2020).
195. Stokstad, E. European Commission proposes loosening rules for gene-edited plants. *Science* https://www.science.org/content/article/european-commission-proposes-loosening-rules-gene-edited-plants (2023).
196. Hoffman, N. E. Revisions to USDA biotechnology regulations: the SECURE rule. *Proc. Natl Acad. Sci. USA* **118**, e2004841118 (2021).
197. Goberna, M. F., Whelan, A. I., Godoy, P. & Lewi, D. M. Genomic editing: the evolution in regulatory management accompanying scientific progress. *Front. Bioeng. Biotechnol.* **10**, 835378 (2022).
198. Blaner, W. S. in *Present Knowledge in Nutrition* 11th edn (eds Marriott, B. P., Birt, D. F., Stallings, V. A., & Yates, A. A.) 73–91 (Academic, 2020).
199. National Institutes of Health Office of Dietary Supplements. Vitamin A and carotenoids. *NIH ODS* https://ods.od.nih.gov/factsheets/VitaminA-HealthProfessional (2022).
200. Tang, G., Qin, J., Dolnikowski, G. G., Russell, R. M. & Grusak, M. A. Golden rice is an effective source of vitamin A. *Am. J. Clin. Nutr.* **89**, 1776–1783 (2009).

Acknowledgements
This work was supported by the National Science Foundation (IOS-1758745, IOS-2029889, IOS-2132693 and IOS-2224203), the USDA National Institute of Food and Agriculture (2018-33522-28789, 2020-33522-32274, 2020-70029-33161, 2021-67013-34554 and 2021-70029-36056), the Foundation for Food & Agriculture Research (21010111), the United States Agency for International Development (22010332) and the Food Futures Institute of Murdoch University.

Author contribution
Y.Q. proposed the project and planned the content with A.T. A.T. drafted the outline and the manuscript. T.S. drafted the section 'Regulations and evolving public view'. C.P., T.S. and A.T. prepared the figures and the table. A.T., C.P., T.S. and Y.Q. researched the data for the article and contributed to discussing, writing, reviewing and editing the manuscript before submission. R.W., R.B., L.L., P.M.S., R.K.V., L.T., J.V.E. and K.M. contributed to reviewing and editing of manuscript before submission.

Competing interests
The authors declare no competing interests.

Additional information
Peer review information *Nature Reviews Bioengineering* thanks Kan Wang, Cathie Martin and the other, anonymous, reviewer for their contribution to the peer review of this work.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Related links
HarvestPlus: https://www.harvestplus.org/