GMR
The Original

SYNTHETIC BIOLOGY: *DE NOVO* DOMESTICATION, METABOLIC ENGINEERING OF CEREALS AND HORTICULTURAL CROPS

Namita Singh[1], Asha Kushwah[2]*, Meenakshi Sood[3], Dharmendra Singh Lagoriya[4], Zuby Gohar Ansari[5], Syed Kulsoom Fatima[6], Moinuddin[7] and Sheetanshu Gupta[8]

[1]Assistant Professor in Department of Genetics and Plant Breeding, College of Horticulture and Research Station Arjunda, Balod, Chhattisgarh 491225, Mahatma Gandhi Udyanikee Evam Vanikee Vishwavidyalaya, Sankra, Durg, Chhattisgarh
[2]Assistant Professor, B.M. College of Agriculture, Khandwa, RVSKVV Gwalior (M.P.)
[3]Associate Professor (Vegetable Science), University of Horticultural Sciences, Bagalkot, Karnataka
[4]DBT-AAU Centre, Assam Agricultural University, Jorhat-785013, Assam
[5]Assistant Professor (Plant Physiology), Faculty of Agriculture and Veterinary Sciences, Mewar University, Gangrar, Chittorgarh, Rajasthan 312901, India
[6]FS University, Shikohabad, U.P.,
[7]Professor, Department of Agronomy, Shri Guru Ram Rai University, Pathri Bagh. Dehradun
[8]Assistant Professor, Department of Food Safety and Quality Assurance, College of Food Technology
CAU, Imphal, Manipur 795004
***Corresponding author:** email id: ashakushwah.asha@gmail.com

ABSTRACT

A confluence of three disciplines, synthetic biology, *de novo* domestication and metabolic engineering, is quietly reshaping how we think about food security, nutrition and climate resilience in cereals, vegetables and other horticultural crops. Ten thousand years of conventional selection produced today's crops, but it also narrowed genetic diversity and, in many cases, uncoupled yield from stress tolerance. New molecular tools now let us design plants at the level of pathways, promoters and even whole genomes, and accelerate domestication of wild species from millennia into a handful of generations. This review synthesises the scientific foundations, landmark case studies and translational status of these three approaches, with a focus on tomato, rice, maize, groundcherry and other agriculturally important crops. Verified proof-of-concept studies include the CRISPR-Cas9-mediated *de novo* domestication of *Solanum pimpinellifolium*, *Physalis pruinosa*, and allotetraploid *Oryza alta*, together with the metabolic engineering of Golden Rice, multivitamin corn, and anthocyanin-enriched purple tomatoes. We conclude that these strategies, if coupled with responsible biosafety frameworks and pragmatic regulation, represent one of the most promising pathways to a nutritious, climate-smart food system for the twenty-first century.[1,2,3,4,5,6,7,8]

1. INTRODUCTION

Global agriculture is being asked to do things that would have seemed extraordinary only a few decades ago: feed close to ten billion people, do so on a shrinking land base, cope with more erratic weather, and simultaneously deliver micronutrient-rich food. Conventional plant breeding has served humanity remarkably well, but its pace and its reliance on the narrow gene pool of already-domesticated species are increasingly at odds with the speed of environmental change. Against this backdrop, a new toolbox has emerged that operates at the level of pathways, cis-regulatory elements and even whole genomes.

Three interrelated approaches now dominate the frontier of crop improvement. **Synthetic biology** applies engineering principles, standardised parts, rational design and iterative testing to biological systems in order to introduce, rewire or optimise pathways. ***De novo* domestication** uses genome editing to introduce a small set of well-characterised "domestication genes" into stress-tolerant crop wild relatives (CWRs), effectively re-running domestication in a controlled and highly compressed timeframe. **Metabolic engineering** targets specific biosynthetic pathways to enhance the accumulation of desired metabolites such as carotenoids, anthocyanins, vitamins or specialised amino acids. Together, these approaches allow crops to be redesigned rather than merely selected.[9,10,11] This paper examines each pillar with reference to verified, high-impact primary literature and considers their combined implications for cereal, vegetable and horticultural systems. Landmark studies including the CRISPR-Cas9 *de novo* domestication of the wild tomato *S. pimpinellifolium* by Zsögön and colleagues in 2018, the parallel work by Li and colleagues on stress-tolerant wild tomato accessions, the rapid improvement of the orphan Solanaceae *Physalis pruinosa* by Lemmon and coworkers, and the audacious *de novo* domestication of an allotetraploid wild rice (*Oryza alta*) by Yu and colleagues in 2021 anchor the discussion.[1,2,3,4]

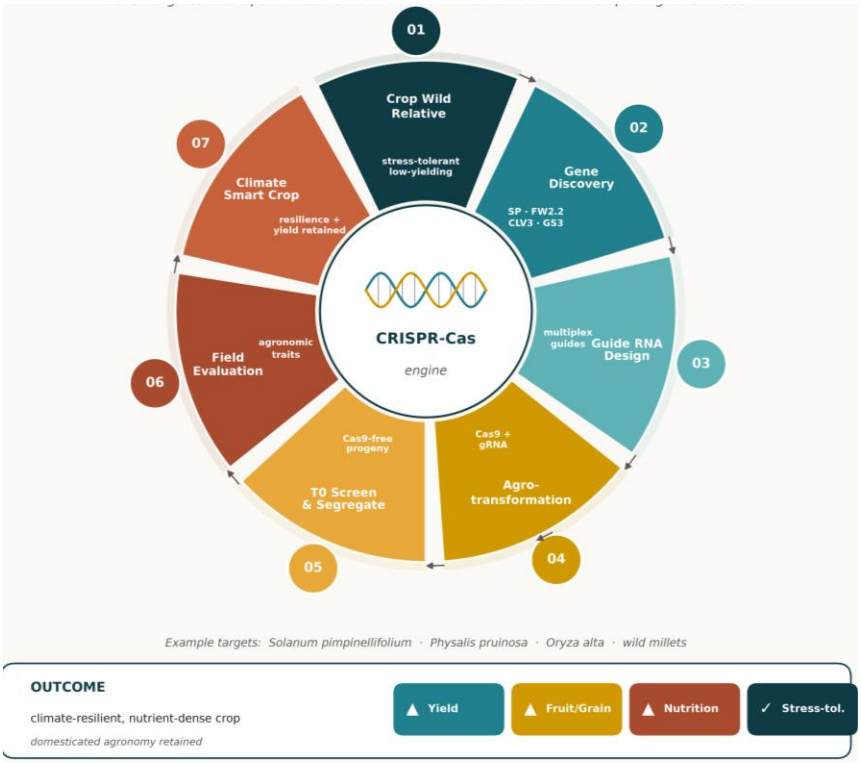

Figure 1. Conceptual workflow of de novo domestication of crop wild relatives using CRISPR-Cas genome editing. Adapted from Zsögön et al. (2018), Li et al. (2018) and Yu et al. (2021).

2. Synthetic Biology in Crop Improvement

2.1 Engineering principles applied to plants

Synthetic biology transfers the "design-build-test-learn" cycle from electrical engineering and industrial chemistry into biology. In plants, this means treating promoters, coding sequences, terminators, ribosome binding sites, transcription factors and even whole regulatory circuits as standardised, interchangeable parts. Rational design supported by modelling replaces trial-and-error transformation. Wurtzel and colleagues, in a widely cited 2019 perspective in *Nature Plants*, argued that synthetic biology can revolutionise agriculture by enabling engineered nitrogen fixation, drought resilience, photosynthetic efficiency and *de novo* metabolic pathways.[19] Several building blocks now underpin the field. Synthetic promoters allow tissue-specific and inducible expression; a canonical example is the endosperm-specific glutelin (Gt1) promoter used to drive β-carotene biosynthesis genes only in the rice grain endosperm without disturbing other tissues. Modular DNA assembly platforms such as Golden Gate, GoldenBraid and MoClo permit stacking of multiple transcriptional units in a single T-DNA. Chassis organisms, most notably yeast, are used to pre-test plant biosynthetic pathways before moving them into crops.[5,20]

2.2 From single genes to designer pathways

The clearest early demonstration of synthetic biology in cereals is Golden Rice, in which a heterologous carotenoid biosynthetic pathway was rebuilt inside rice endosperm using genes from daffodil and a soil bacterium. The prototype produced 1.6 µg of total carotenoids per gram of dry rice; a second-generation version substituted the maize phytoene synthase and boosted total carotenoids up to 37 µg/g, an approximately twenty-three-fold gain (Figure 2). Beyond this, multi-vitamin approaches such as the transgenic corn engineered by Naqvi and colleagues combined five constructs (Zm psy, Pa crtI, rice dhar, *E. coli* folE and a selectable marker) to raise β-carotene 169-fold, ascorbate sixfold and folate twofold in a single South African white-corn genotype, an outcome unattainable through conventional breeding alone.[5,6,7] Similar circuit-style engineering has been achieved in horticultural crops. In tomato, expression of the snapdragon (*Antirrhinum majus*) transcription factors Delila (bHLH) and Rosea1 (R2R3-MYB) under the tomato fruit-specific E8 promoter yielded fruit with anthocyanin levels comparable to blueberries and blackberries, the deep-purple "high-anthocyanin tomato" first reported by Butelli and coworkers, which extended the lifespan of cancer-susceptible *Trp53* knock-out mice fed a supplemented diet.[8]

2.3 Chassis, sensors and beyond

A defining feature of contemporary plant synthetic biology is the use of orthogonal genetic elements, parts that behave predictably regardless of the host context. Curated libraries of synthetic promoters with dialled-in strengths, orthogonal transcription factors such as engineered dCas9-based activators and repressors, riboswitches, degrons and inducible split-protein systems now allow multiple pathways to be controlled independently within the same plant.

Chen and Nielsen have argued that yeast and other microbial chassis play an indispensable role as "biochemical breadboards" on which candidate plant enzymes and pathway variants can be pre-tested at throughput before the best combinations are moved into the target crop. This tightly coupled yeast–plant pipeline dramatically shortens the design-build-test-learn cycle and is already accelerating the rediscovery of native plant biosynthetic routes for high-value specialised metabolites such as taxanes, morphinans and terpenoid indole alkaloids.[20] Equally important is the maturation of plant biosensors and reporter circuits. Genetically encoded fluorescent sensors for auxin, sucrose, cytoplasmic pH and reactive oxygen species allow researchers to read out physiological states in living plants, and are now being redesigned as diagnostic tools that could be deployed in field settings. Taken together, these advances mean that synthetic biology in agriculture is moving decisively from "insert a single gene" toward the design of full multi-input, multi-output genetic circuits capable of sensing the environment, integrating cues and directing developmental or metabolic responses accordingly.[19,20]

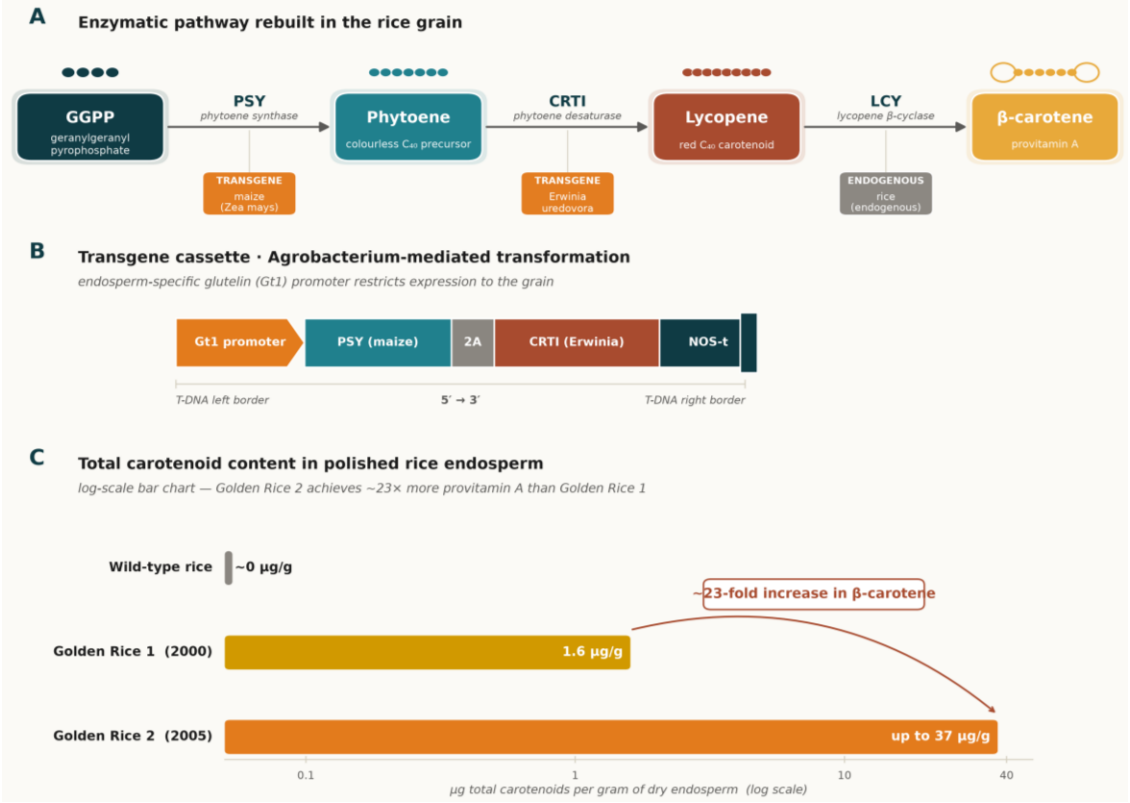

Figure 2. Metabolic engineering of the provitamin-A pathway in rice endosperm (the Golden Rice concept). Data from Ye et al. (2000) and Paine et al. (2005).

3. *De novo* Domestication

3.1 Concept and rationale

De novo domestication (dnD) is a deceptively simple idea with far-reaching implications. Modern crops carry a small number of domestication mutations, most of them recessive loss-of-function alleles at loci controlling shattering, plant architecture, seed dormancy, day-length response and fruit or grain size. Rather than continuing to introgress climate-relevant traits from wild relatives into cultivated backgrounds (a slow process burdened by linkage drag), dnD reverses the flow: the small suite of domestication alleles is engineered directly into a stress-tolerant wild species. Zsögön and colleagues formalised this rationale in a landmark 2017 review in *Plant Science* before delivering experimental proof of concept the following year.[11,9]

3.2 Tomato, proof of principle in a vegetable crop

In October 2018, two independent teams published back-to-back papers in *Nature Biotechnology* that operationalised dnD in *Solanum pimpinellifolium*, the wild ancestor of the cultivated tomato. Zsögön and colleagues used multiplex CRISPR-Cas9 to edit six loci, SP, OVATE, FW2.2, MULT, CLV3 and CycB, controlling plant architecture, fruit shape, fruit weight, inflorescence branching, locule number and lycopene accumulation, respectively. The resulting plants produced fruit that was three times larger, ten times more numerous and contained roughly five times more lycopene than modern cultivated tomatoes, yet retained the disease resistance and stress tolerance of the wild parent.[1] Li and colleagues took a complementary approach, editing coding sequences, cis-regulatory regions and upstream open reading frames (uORFs) in four naturally stress-tolerant wild-tomato accessions. Their targets included SP, SP5G, CLV3, WUS and the uORF of SlGGP1, the latter of which controls vitamin C biosynthesis. Cas9-free progeny

of these edited lines had domesticated phenotypes yet retained bacterial-spot resistance and salinity tolerance, demonstrating that dnD does not necessarily create fitness penalties.[2]

3.3 Orphan crops, the groundcherry case

The Physalis Improvement Project, led by Zachary Lippman and Joyce Van Eck, extended dnD to orphan Solanaceae. In *Physalis pruinosa* (groundcherry, husk cherry, "strawberry tomato"), CRISPR-Cas9 editing of orthologs of the tomato genes SP, SP5G and CLV1 produced a substantially more compact plant, roughly fifty % more fruit per shoot and about twenty-four % heavier berries. The study, published in *Nature Plants* in 2018, is the clearest demonstration to date that knowledge from a model crop can be directly transferred to a distantly related orphan species in the same botanical family.[3]

3.4 Cereals, *de novo* domestication of an allotetraploid wild rice

Perhaps the most ambitious dnD effort so far is the 2021 *Cell* paper by Yu and colleagues, which engineered a route to a domesticated polyploid rice from the wild allotetraploid *Oryza alta* (CCDD genome). The authors first screened 28 wild polyploid rice lines and selected the *O. alta* accession "polyploid rice 1" (PPR1) on the basis of biomass, callus induction and regeneration properties, and stress tolerance. They then established an efficient tissue culture and CRISPR-Cas9 system for the species, built a high-quality reference genome resolved into its two subgenomes and demonstrated the rapid improvement of six agronomically important traits, shattering, awn length, hull colour, pericarp colour, panicle shape and grain width, by editing *O. alta* homologues of the corresponding diploid rice genes. This work compressed what took several thousand years for cultivated *Oryza sativa* into a handful of generations and pointed toward polyploid staples that combine buffered genomes with domesticated agronomy.[4]

3.5 Orphan and semi-domesticated species

Conceptual and practical dnD demonstrations have now been reported for Solanaceae, cereals, legumes, feral crops and orphan species including teff, amaranth, cowpea, pigeon pea, quinoa and pennycress. Menon and colleagues, in a 2024 review in the *International Journal of Molecular Sciences*, argued that dnD is poised to become the fastest route to climate-smart staple crops, provided that transformation and regeneration bottlenecks are solved for a wider range of species. Fernie and Yan, writing in *Molecular Plant* in 2019, framed dnD as an "alternative route toward new crops for the future", noting that thousands of underused or semi-domesticated plant species contain traits, heat and drought tolerance, unusual protein profiles, distinctive flavour and colour compounds, that could be captured rapidly by editing a small number of domestication loci.[18,10] A particularly promising target is the family of C4 cereals adapted to marginal environments. Sorghum, pearl millet, finger millet and teff already tolerate drought and heat far better than rice or wheat, but they suffer from a mixture of low yields, shattering, poor threshability and, in teff, extremely small seeds that hamper mechanised harvest. Applying the dnD logic to these species, by editing well-characterised orthologs such as *SH1* (shattering), *GS3* (grain size), and *Ghd7* (heading date), would allow their inherent resilience to be combined with the agronomic properties needed for modern farming systems. Similar arguments apply to wild *Vigna* species that could serve as new legume crops for low-nutrient soils.[10,18]

4. Metabolic Engineering of Cereals and Horticultural Crops

4.1 Biofortification, the Golden Rice trajectory

Vitamin A deficiency remains a leading cause of preventable childhood blindness and mortality across South and Southeast Asia and sub-Saharan Africa. Because polished rice endosperm contains essentially no provitamin A carotenoids, no amount of conventional selection within cultivated *Oryza sativa* can solve the problem; the underlying biosynthetic capacity simply is not there. Ye and colleagues addressed this in a landmark 2000 paper in *Science* by introducing three transgenes into rice endosperm: phytoene synthase (psy) from daffodil, phytoene desaturase (crtI) from *Erwinia uredovora*, and lycopene β-cyclase from daffodil (later shown to be dispensable). The resulting "Golden Rice" produced ~1.6 μg of total carotenoids per gram of dry rice.[5] Five years later, Paine and colleagues improved on this in *Nature Biotechnology* by systematically testing plant psy sources; the maize enzyme substantially outperformed the daffodil version, driving flux through the carotenoid pathway and yielding "Golden Rice 2" with up to 37 μg of carotenoids per gram (~80% β-carotene). A more recent review by Kaul and coworkers in *Plant Science* summarised two decades of metabolic and regulatory challenges and highlighted the July 2021 approval of Golden Rice for commercial cultivation in the Philippines, the first country to do so. [6,12]

4.2 Multi-vitamin engineering in maize

Naqvi and colleagues took metabolic engineering one important step further. Rather than fortifying with a single micronutrient, they simultaneously engineered three separate biosynthetic pathways in the endosperm of an elite South African white-maize inbred by particle-bombardment delivery of five gene constructs. The resulting kernels accumulated 169 times the normal amount of β-carotene, six times the amount of ascorbate and roughly twice the amount of folate. Critically, these levels remained stable through at least the T3 homozygous generation, indicating a stable transgenic insertion.[7]

4.3 Anthocyanin-enriched horticultural crops

The 2008 purple tomato of Butelli, Martin and colleagues at the John Innes Centre remains the paradigm for transcription-factor–driven metabolic engineering in a fleshy fruit. Anthocyanin biosynthesis in wild-type tomato is largely inactive in the fruit because the necessary MYB and bHLH transcription factors are either poorly expressed or

non-functional there. By expressing Delila and Rosea1 from *Antirrhinum majus* under a fruit-specific E8 promoter, the researchers activated both early and late steps of the flavonoid pathway; the fruit accumulated ~2.83 mg anthocyanin per gram fresh weight, comparable to blueberries, and its hydrophilic antioxidant capacity was tripled. In *Trp53* knock-out mice supplemented with purple tomato powder, mean lifespan was extended by roughly 30% compared with a standard diet, providing the first isogenic demonstration of a dietary benefit from engineered plant polyphenols. The purple tomato received US regulatory approval in 2022 and reached the retail market in 2024.[8]

4.4 Editing endogenous regulatory space

A subtle but important extension of metabolic engineering is the deliberate editing of cis-regulatory regions to generate continuous quantitative variation. Rodríguez-Leal and colleagues, in a 2017 study in *Cell*, targeted the promoter regions of *CLV3*, *S* and *SP* in tomato, generating an allelic series that produced a graded spectrum of fruit sizes and inflorescence architectures. Rather than a binary knock-out, this approach delivered breeder-friendly quantitative variation, a powerful strategy for tuning yield-relevant traits without disturbing the underlying coding sequence.[15]

5. Synthetic Biology Meets *De novo* Domestication and Metabolic Engineering

The three approaches described above are best understood as complementary rather than competing. Synthetic biology provides the parts, standards and modelling frameworks; *de novo* domestication delivers new starting germplasm suited to hostile environments; metabolic engineering equips those crops with the nutritional and secondary-metabolite profiles that consumers and public health authorities increasingly demand. In practice, the frontiers are converging. Chen and Nielsen argued in 2023 that plant biosynthetic pathway elucidation and engineering are being accelerated by yeast and plant chassis working in tandem, allowing enzyme candidates to be screened in a microbial host and then moved directly into an edited crop background.[20] The most obvious near-term application of this convergence is a stress-tolerant, nutritionally enhanced *de novo* domesticated cereal. For example, editing the shattering, day-length and grain-size loci in an allotetraploid rice such as *O. alta* would furnish domesticated agronomy; layering the endosperm-targeted carotenoid or folate cassettes on top would deliver micronutrients; and using cis-regulatory editing to fine-tune plant architecture would allow the same variety to be adapted rapidly to different latitudes. In horticulture, analogous stacked strategies applied to wild *Solanum* or *Physalis* species could yield climate-resilient, nutritionally superior vegetable and berry crops within a decade.[4,5,8]

6. Landmark Case Studies

S. No.	Crop / Species	Approach	Key edited genes / transgenes	Principal outcome
1	Wild tomato (*Solanum pimpinellifolium*)	*De novo* domestication (multiplex CRISPR-Cas9)	SP, OVATE, FW2.2, MULT, CLV3, CycB	Threefold larger fruit; tenfold more fruits; ~5× lycopene vs. cultivated tomato (Zsögön et al., 2018)
2	Wild tomato accessions (stress-tolerant lines)	Multiplex CRISPR-Cas9 (coding + cis-regulatory + uORF edits)	SP, SP5G, CLV3, WUS, SlGGP1	Compact architecture, day-neutral flowering, larger fruit, higher vitamin C; disease and salt tolerance retained (Li et al., 2018)
3	Groundcherry (*Physalis pruinosa*)	CRISPR-Cas9 in an orphan Solanaceae	SP, SP5G, CLV1 orthologues	More compact architecture; ~50% more fruit per shoot; ~24% heavier berries (Lemmon et al., 2018)
4	Wild allotetraploid rice (*Oryza alta*)	*De novo* domestication in a polyploid cereal	Homologues of shattering, awn, hull colour, grain-width, panicle, pericarp genes	Improved shattering, grain size and plant architecture; framework for a novel polyploid staple cereal (Yu et al., 2021)
5	Rice (*Oryza sativa*), Golden Rice 1	Metabolic pathway engineering	psy (Narcissus) + crtI (Erwinia) under endosperm-specific glutelin promoter	β-carotene accumulation in endosperm (~1.6 µg/g total carotenoids) (Ye et al., 2000)

S. No.	Crop / Species	Approach	Key edited genes / transgenes	Principal outcome
6	Rice (*Oryza sativa*), Golden Rice 2	Metabolic pathway optimisation	Maize psy + crtI	Up to ~37 µg/g total carotenoids; ~23-fold increase vs. Golden Rice 1 (Paine et al., 2005)
7	Maize (*Zea mays*), multivitamin corn	Multi-pathway transgene stacking	Zm psy + Pa crtI + rice dhar + E. coli folE	169× β-carotene, 6× ascorbate, 2× folate in endosperm (Naqvi et al., 2009)
8	Tomato (*S. lycopersicum* cv. Micro-Tom)	Transcription-factor–driven pathway activation	Delila (bHLH) + Rosea1 (R2R3-MYB) from Antirrhinum majus, E8 promoter	Anthocyanin content comparable to blackberries/blueberries; extended lifespan of cancer-prone mice on supplemented diet (Butelli et al., 2008)
9	Tomato, cis-regulatory quantitative variation	Promoter editing to create allelic series	CLV3, S (Compound inflorescence), SP promoter regions	Continuous quantitative variation in fruit size and inflorescence architecture usable for breeding (Rodríguez-Leal et al., 2017)
10	Tomato, day-neutral flowering	Natural allele identification and CRISPR-based dissection	SELF PRUNING 5G (SP5G)	Early yield and day-neutral flowering suitable for high-latitude cultivation (Soyk et al., 2017)

Table 1. Landmark studies in de novo domestication and metabolic engineering of cereals and horticultural crops.

7. Challenges and Regulatory Landscape

Despite the striking advances discussed above, several bottlenecks remain. First, plant transformation and regeneration are still inefficient in many wild species; the *O. alta* effort by Yu and colleagues succeeded partly because the authors screened dozens of accessions before finding one amenable to callus induction. Second, epistasis, pleiotropy and structural variation revealed by pan-genome studies mean that single-gene edits may not always yield the expected phenotype, particularly for traits under quantitative control. Third, off-target effects and unintended metabolic consequences must be rigorously screened, although Gao and colleagues showed in a 2021 review in *Cell* that modern CRISPR variants (base editors, prime editors) can dramatically reduce collateral edits.[4,14]

Regulation continues to evolve. Several jurisdictions, including Argentina, Brazil, Australia, Japan, the United States and, more recently, India, have adopted policies that exempt certain SDN-1 and SDN-2 genome-edited crops (edits producing no foreign DNA) from the strict transgenic biosafety pathway. This distinction is scientifically important: a *de novo* domesticated wild tomato whose Cas9-free progeny carry only small indels is genetically closer to a conventional mutation-bred variety than to a classical GMO. Public and consumer acceptance nevertheless remains uneven and requires transparent communication, particularly around the difference between transgenic biofortified products such as Golden Rice or purple tomato and edit-only products that carry no exogenous DNA.[13,22]

A further set of practical challenges concerns intellectual property, seed systems and farmer access. Many of the enabling patents on CRISPR-Cas9, base editors and prime editors are held by a small number of institutions and companies, and the resulting licensing landscape can slow deployment in low-income countries. The humanitarian licensing model pioneered by the Golden Rice inventors, in which the technology was donated for use by resource-poor farmers with incomes below a defined threshold, offers one workable template, and its recent 2021 regulatory approval in the Philippines shows that patient, coalition-based deployment can eventually succeed. Ku and Ha, reviewing genome-editing-based nutritional improvement in *Frontiers in Plant Science*, emphasised that seed-system integration and farmer training will ultimately determine whether these technologies reach the households that need them most.[21,22]

8. CONCLUSIONS

Synthetic biology, *de novo* domestication and metabolic engineering together constitute the most consequential shift in crop improvement since the Green Revolution. Where the twentieth century sought to optimise a narrow set of already-domesticated species, the twenty-first is beginning to expand the pool of crops by molecular design. The

proof-of-concept work in tomato, groundcherry and allotetraploid rice, along with the maturation of Golden Rice, multi-vitamin maize and anthocyanin-enriched purple tomato, demonstrate that this shift is no longer speculative.
Three priorities will define the next decade. First, delivery: robust, tissue-culture-independent transformation systems (for example, nano-carriers and viral vectors carrying CRISPR reagents) will need to be scaled across a much wider range of crops and wild relatives. Second, integration: dnD-edited backgrounds should be combined with rationally engineered metabolic modules and with genomic-selection breeding pipelines to accelerate delivery to farmers. Third, equity: the greatest need for nutritionally enhanced, climate-resilient crops is in low- and middle-income countries, and the corresponding regulatory, intellectual-property and extension systems must be designed accordingly. If these priorities are addressed together, the coming decade could deliver a genuinely nutritious, climate-smart and biodiverse food system built on both ancient wild germplasm and cutting-edge molecular design.[18,19]

REFERENCES

1. Zsögön, A., Čermák, T., Naves, E. R., et al. (2018). *De novo* domestication of wild tomato using genome editing. Nature Biotechnology, 36, 1211–1216. https://doi.org/10.1038/nbt.4272

2. Li, T., Yang, X., Yu, Y., Si, X., Zhai, X., Zhang, H., Dong, W., Gao, C., & Xu, C. (2018). Domestication of wild tomato is accelerated by genome editing. Nature Biotechnology, 36, 1160–1163. https://doi.org/10.1038/nbt.4273

3. Lemmon, Z. H., Reem, N. T., Dalrymple, J., Soyk, S., Swartwood, K. E., Rodriguez-Leal, D., Van Eck, J., & Lippman, Z. B. (2018). Rapid improvement of domestication traits in an orphan crop by genome editing. Nature Plants, 4, 766–770. https://doi.org/10.1038/s41477-018-0259-x

4. Yu, H., Lin, T., Meng, X., Du, H., Zhang, J., Liu, G., et al. (2021). A route to *de novo* domestication of wild allotetraploid rice. Cell, 184(5), 1156–1170.e14. https://doi.org/10.1016/j.cell.2021.01.013

5. Ye, X., Al-Babili, S., Klöti, A., Zhang, J., Lucca, P., Beyer, P., & Potrykus, I. (2000). Engineering the provitamin A (β-carotene) biosynthetic pathway into (carotenoid-free) rice endosperm. Science, 287(5451), 303–305. https://doi.org/10.1126/science.287.5451.303

6. Paine, J. A., Shipton, C. A., Chaggar, S., Howells, R. M., Kennedy, M. J., Vernon, G., et al. (2005). Improving the nutritional value of Golden Rice through increased pro-vitamin A content. Nature Biotechnology, 23, 482–487. https://doi.org/10.1038/nbt1082

7. Naqvi, S., Zhu, C., Farre, G., Ramessar, K., Bassie, L., Breitenbach, J., et al. (2009). Transgenic multivitamin corn through biofortification of endosperm with three vitamins representing three distinct metabolic pathways. Proceedings of the National Academy of Sciences, 106(19), 7762–7767. https://doi.org/10.1073/pnas.0901412106

8. Butelli, E., Titta, L., Giorgio, M., Mock, H.-P., Matros, A., Peterek, S., et al. (2008). Enrichment of tomato fruit with health-promoting anthocyanins by expression of select transcription factors. Nature Biotechnology, 26, 1301–1308. https://doi.org/10.1038/nbt.1506

9. Gasparini, K., dos Reis Moreira, J., Peres, L. E. P., & Zsögön, A. (2021). *De novo* domestication of wild species to create crops with increased resilience and nutritional value. Current Opinion in Plant Biology, 60, 102006. https://doi.org/10.1016/j.pbi.2021.102006

10. Fernie, A. R., & Yan, J. (2019). *De novo* domestication: an alternative route toward new crops for the future. Molecular Plant, 12(5), 615–631. https://doi.org/10.1016/j.molp.2019.03.016

11. Zsögön, A., Čermák, T., Voytas, D., & Peres, L. E. P. (2017). Genome editing as a tool to achieve the crop ideotype and *de novo* domestication of wild relatives: case study in tomato. Plant Science, 256, 120–130. https://doi.org/10.1016/j.plantsci.2016.12.012

12. Kaul, T., Sony, S. K., Verma, R., Motelb, K. F. A., Prakash, A. T., Eswaran, M., et al. (2022). Revisiting the two decades of Golden Rice—Lessons learned for inspiring future metabolic engineering. Plant Science, 316, 111220. https://doi.org/10.1016/j.plantsci.2021.111220

13. Chen, K., Wang, Y., Zhang, R., Zhang, H., & Gao, C. (2019). CRISPR/Cas genome editing and precision plant breeding in agriculture. Annual Review of Plant Biology, 70, 667–697. https://doi.org/10.1146/annurev-arplant-050718-100049

14. Gao, C. (2021). Genome engineering for crop improvement and future agriculture. Cell, 184(6), 1621–1635. https://doi.org/10.1016/j.cell.2021.01.005

15. Rodríguez-Leal, D., Lemmon, Z. H., Man, J., Bartlett, M. E., & Lippman, Z. B. (2017). Engineering quantitative trait variation for crop improvement by genome editing. Cell, 171(2), 470–480.e8. https://doi.org/10.1016/j.cell.2017.08.030

16. Soyk, S., Müller, N. A., Park, S. J., Schmalenbach, I., Jiang, K., Hayama, R., et al. (2017). Variation in the flowering gene SELF PRUNING 5G promotes day-neutrality and early yield in tomato. Nature Genetics, 49(1), 162–168. https://doi.org/10.1038/ng.3733

17. Tuncel, A., & Qi, Y. (2022). CRISPR/Cas mediated genome editing in potato: past achievements and future directions. Plant Science, 317, 111199. https://doi.org/10.1016/j.plantsci.2021.111199

18. Menon, U., et al. (2024). Future-proofing agriculture: *de novo* domestication for sustainable and resilient crops. International Journal of Molecular Sciences, 25(4), 2374. https://doi.org/10.3390/ijms25042374

19. Wurtzel, E. T., et al. (2019). Revolutionizing agriculture with synthetic biology. Nature Plants, 5(12), 1207–1210. https://doi.org/10.1038/s41477-019-0539-0
20. Chen, Y., & Nielsen, J. (2023). Leveraging synthetic biology and metabolic engineering to overcome obstacles in plant pathway elucidation. Current Opinion in Plant Biology, 71, 102330. https://doi.org/10.1016/j.pbi.2022.102330
21. ISAAA (2023). Global Status of Commercialized Biotech/GM Crops and Golden Rice, Philippines becomes the first country to approve commercial cultivation. https://www.isaaa.org
22. Ku, H.-K., & Ha, S.-H. (2020). Improving nutritional and functional quality by genome editing of crops: status and perspectives. Frontiers in Plant Science, 11, 577313. https://doi.org/10.3389/fpls.2020.577313