

Transgenic Approaches in Improving Physiological Processes for Crop Improvement

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Abstract

Modern agriculture faces the challenge of producing more food with fewer resources while adapting to climate change, emerging pests and diseases and the increasing scarcity of soil and water resources. Transgenic technology provides a route to introduce specific genes and modify physiological pathways that influence productivity, quality and stress adaptation. Early transgenic crops demonstrated insect resistance, herbicide tolerance and delayed fruit softening. Recent progress has expanded the field toward CRISPR/Cas genome editing, base editing, prime editing, multiplex editing, targeted insertion, de novo domestication and precision regulation of gene expression. These technologies are increasingly integrated with genomics, phenomics and speed breeding. This article highlights the principles, transformation procedures, major applications and recent developments of transgenic and genome-engineering approaches for sustainable crop improvement.

Introduction

Crop improvement traditionally depends on crossing, selection and recombination. However, many useful traits are absent from the crop gene pool, controlled by complex networks or difficult to transfer without undesirable linked characters. Genetic engineering can overcome some of these barriers by introducing a defined gene or construct into a plant. A transgene is genetic material introduced into an organism, while a transgenic plant expresses the introduced sequence and in stable transformation can transmit it to progeny. Modern biotechnology therefore complements conventional breeding rather than simply replacing it.

Transformation from gene to improved plant

A typical transgenic programme begins with identification of a candidate gene, validation of its function, construct design and selection of an appropriate promoter. The gene may be placed under a constitutive, tissue-specific, developmental or stress-inducible promoter. The construct is introduced into competent plant cells, transformed cells are selected, regenerated through tissue culture and molecularly characterized. PCR, sequencing, expression analysis and phenotyping are used to confirm the desired event.

Agrobacterium-mediated transformation remains one of the most important approaches. *Agrobacterium*

naturally transfers T-DNA into plant cells, researchers replace disease-associated sequences with the desired construct. Biolistic transformation uses DNA-coated microscopic particles accelerated into cells and is particularly useful for species or genotypes less amenable to *Agrobacterium*. Other approaches include PEG-mediated transformation of protoplasts and electroporation (Van vu *et al.*, 2025).

Major applications

Bt crops express insecticidal Cry proteins derived from *Bacillus thuringiensis* can protect crops such as cotton and maize against target insect pests. Virus-resistant plants have also been developed through pathogen-derived or RNA-mediated strategies. Engineering host immunity and susceptibility genes is now being explored with genome editing to create more durable disease resistance (Chen *et al.*, 2024).

Herbicide tolerance provides an effective weed management option, although repeated dependence on the same herbicide can select resistant weeds. Therefore, transgenic traits are most sustainable when combined with crop rotation, herbicide rotation and integrated weed management.

Improving nutritional quality is another important objective. Golden Rice was designed to accumulate provitamin A carotenoids in the endosperm. Transgenic approaches have also been used to modify oil composition, proteins, micronutrients and other metabolites. Fruit quality can be improved by altering enzymes involved in ripening and softening, the Flavr Savr tomato is an early example based on suppression of polygalacturonase expression.

Engineering physiological processes

Transgenic approaches can directly target physiological pathways rather than only visible resistance traits. Photosynthesis is a major target because improvements in light capture, carbon fixation, electron transport or photorespiration could increase biomass and yield. Chloroplast transformation provides an additional platform for manipulating photosynthetic functions and expressing multiple genes.

Abiotic stress tolerance is more challenging because drought, salinity, heat and cold involve interconnected signaling networks. Candidate genes include transcription factors, osmo protectant pathways, transporters, antioxidant

systems, chaperones and hormone-related regulators. For drought, researchers have investigated genes such as SNAC1 and pathways involving cytokinin metabolism. Such strategies seek to maintain cellular stability, stomatal regulation, water-use efficiency and delayed senescence under stress (Guo *et al.*, 2023).

Recent technologies: beyond classical transgenics

The major shift in the last decade has been from random or semi-random gene insertion toward precise genome engineering. CRISPR/Cas systems use guide RNAs to direct nucleases to selected genomic sequences. A targeted DNA break is repaired by cellular pathways, allowing gene knockout or with a suitable template, targeted sequence replacement. CRISPR has accelerated functional validation and trait development across crops. Recent reviews highlight its expanding applications in stress tolerance, yield, nutrition and quality improvement.

Base editing provides another level of precision. Engineered Cas proteins linked to deaminases can convert selected bases without creating conventional double-strand breaks. This is useful for generating specific alleles, including changes that mimic naturally occurring variants (Tuncel *et al.*, 2023).

Prime editing is an emerging “search-and-replace” technology. A Cas nickase is coupled with a reverse transcriptase and programmed by a prime-editing guide RNA. It can introduce defined substitutions, small insertions and deletions without requiring a conventional double-strand break or donor DNA template. Recent work has improved prime-editing efficiency in plants, including tomato, demonstrating its growing potential for crop improvement.

Multiplex CRISPR enables simultaneous modification of several genes or regulatory regions. This is particularly valuable for polygenic traits such as disease resistance, architecture, flowering and stress adaptation. Precise insertion technologies are also advancing, enabling targeted placement of genes or regulatory sequences and facilitating trait stacking (Lee *et al.*, 2025).

Procedure for trait development and validation

For practical breeding, the workflow should begin with a clearly defined target phenotype and measurable physiological indicator. Candidate genes can be identified through QTL mapping, GWAS, transcriptome analysis, mutant screening or comparative genomics. Functional evidence is then obtained through overexpression, knock down or targeted editing. The selected construct or editing reagents are delivered into suitable explants or protoplasts. Regenerated plants are screened for the intended molecular change and evaluated for stability across generations.

Physiological characterization is essential. For drought tolerance, measurements may include relative water content, stomatal conductance, chlorophyll fluorescence, canopy temperature, root traits and yield under stress. For nutrient-use efficiency, researchers can examine nutrient uptake, transporter expression, biomass partitioning and grain nutrient concentration. For photosynthetic improvement, gas exchange, chlorophyll fluorescence, leaf area, biomass accumulation and harvest index provide complementary evidence. Multi-location testing is needed because performance observed under controlled conditions may not translate directly to farmers’ fields.

A useful principle is to distinguish tolerance from yield protection. A plant may survive severe stress yet produce little grain. Therefore, successful crop improvement should demonstrate agronomic value under realistic stress intensity and identify whether the trait carries a yield penalty under favourable conditions. Tissue-specific or inducible promoters can help address such trade-offs by activating a gene only where or when it is required.

Examples of physiological engineering

Manipulation of transcription factors illustrates how a single regulatory gene can influence multiple downstream processes. NAC, DREB, bZIP and related regulators have been investigated for drought, salinity, heat and oxidative stress responses. However, constitutive expression may produce undesirable effects such as growth retardation. Recent strategies therefore emphasize stress-inducible promoters, precise allele editing and modification of regulatory sequences rather than simple overexpression.

Genome editing can modify metabolic enzymes or transport processes to increase beneficial compounds while reducing anti-nutritional factors. In vegetables and fruits, editing can target ripening regulators, browning pathways and disease-susceptibility genes, potentially reducing post-harvest losses. Such approaches connect molecular breeding with consumer-oriented traits and value addition (Chen *et al.*, 2019).

Some genome-editing products contain only small targeted changes and may not retain an introduced transgene. Nevertheless, both approaches belong to the broader toolbox of modern crop biotechnology and should be evaluated according to their traits, risks and intended uses. FAO describes genome editing as a group of techniques capable of introducing targeted genetic changes for agricultural purposes, including traits related to food production, quality and climate resilience.

For developing countries, technology transfer, affordable transformation platforms, public-sector breeding capacity and transparent regulatory systems will be as

important as laboratory advances. Public breeding programmes can use these technologies to address region-specific problems such as drought-prone cereals, salinity, emerging diseases and micronutrient deficiencies. Farmer participation and field-based evaluation can ensure that technically successful traits also provide practical value.

Research priorities for agriculture

Future programmes should give greater attention to traits that are difficult to improve through single-gene approaches. Root system architecture, nutrient-use efficiency, heat tolerance and reproductive-stage stress tolerance often involve many genes and strong environmental interactions. Combining genomic prediction with targeted editing may allow breeders to identify high-value alleles and assemble favourable combinations more efficiently.

Another promising direction is precision gene regulation. Instead of permanently increasing or eliminating gene activity, researchers can modify promoters, enhancers or upstream regulatory sequences to produce intermediate levels of expression.

Digital phenotyping and artificial intelligence can strengthen this process by rapidly measuring canopy temperature, disease symptoms, plant architecture, chlorophyll status and growth dynamics. When these datasets are integrated with genomic information, breeders can identify plants that combine desirable molecular changes with stable field performance. The convergence of gene editing, high-throughput phenotyping and data-driven

breeding is therefore likely to define the next phase of crop biotechnology.

Literature Cited

- Tuncel, A; Pan, C; Sprink, T; Wilhelm, R; Barrangou, R.; Li, L; Shih, P.M; Varshney, R.K; Tripathi, L; Van Eck, J. 2023. Genome-edited foods. *Nat. Rev. Bioeng.* 799-816.
- Chen, K; Wang, Y; Zhang, R; Zhang, H; Gao, C. CRISPR/Cas Genome Editing and Precision Plant Breeding in Agriculture. 2019. *Annu. Rev. Plant Biol.* 70: 667-697.
- Chen, F., Chen, L., Yan, Z., Xu, J., Feng, L., He, N., Guo, M., Zhao, J., Chen, Z., Chen, H., Yao, G. and Liu, C. 2024. Recent advances of CRISPR-based genome editing for enhancing staple crops. *Frontiers in Plant Science.* 15: 1478398.
- Guo, Y.; Zhao, G.; Gao, X.; Zhang, L.; Zhang, Y.; Cai, X.; Yuan, X.; Guo, X. 2023. CRISPR/Cas9 gene editing technology: A precise and efficient tool for crop quality improvement. *Planta.* 258, 36.
- Lee, J., Kweon, J. and Kim, Y. 2025. Emerging trends in prime editing for precision genome editing. *Experimental & Molecular Medicine.* 57: 1381–1391.
- Van Vu, T., Nguyen, N.T., Kim, J., Sung, Y.W., Chung, W.S. and Kim, J.Y. 2025. The evolving landscape of precise DNA insertion in plants. *Nature Communications.* 16: 10428.
