



## Biotechnological Approaches for the Improvement of Horticultural Crops

(Arpita A. Patel<sup>1</sup> and \*Rajesh D. Vekariya<sup>2</sup>)

<sup>1</sup>Anand Agricultural University, Anand, Gujarat, India-388 110

<sup>2</sup>Wheat Research Station, Navsari Agricultural University, Bardoli, Gujarat, India

\*Corresponding Author's email: [rajesh@nau.in](mailto:rajesh@nau.in)

With the country's growing population, there is a proportionate rise in the need for fruits and vegetables. How can we maintain horticulture output in line with the expanding population? There is an urgent need to include biotechnology to expedite agricultural development initiatives due to the rising demand for fruits and vegetables in emerging nations. One of the most important tools for the nation to implement its development strategies is biotechnology. These days, there are numerous research organizations around the nation working on horticulture biotechnology projects, which are both strategically important in the short and long terms., as follows: development of insect or disease-resistant crops and tolerant to abiotic stresses; improvement of the nutritional composition of edible crops; manipulation of secondary metabolite routes in plants; reduction of post-harvest storage losses.

**Key words:** Tissue culture; Genetic engineering; Transgene; molecular marker; genome editing; horticulture crops

### Introduction

Horticultural crops comprise of fruits, vegetables, medicinal, aromatic, plantation, and ornamental plants. They supply us with a range of nutrients like carbohydrates, proteins, organic acids, vitamins, and minerals which offers peptic and medicinal value and bring prosperity to a nation by providing economic gains. As a result, there is an increasing rise in demand for production of these crops. However, climate change has elevated production risks and can limit crop harvest by 70 % (Boyer, 1982). So far, improving these crops for better yield, quality, and tolerance to biotic-abiotic stresses has been addressed by conventional breeding approaches. But the constraints, like long time, low genetic variability, sexual incompatibility, low efficiency, and environmental dependence have limited their applications. With the advent of biotechnology, some of these bottlenecks can be overcome. Since its introduction about 20 years ago, plant biotechnology has achieved very important milestones in increasing global crop productivity to improve food, feed, and fiber security, and in reducing the environmental footprint of agriculture.

### Role of Biotechnology in Horticulture

**1. Plant Tissue Culture (PTC):** It is one of the widely known techniques used for the production of large numbers of genetically identical plants. Explants from the mother plant are utilized for *in vitro* propagation which rests on totipotency. It is practiced using three general steps: (i) preparation and sterilization of plant material, (ii) culture medium composition, and (iii) physical environmental conditions in the culture room and culture vessel. The benefits of micropropagation are rapid clonal propagation, decreasing diseases of

plantlets and the period of acclimatization *ex vitro*, and reducing the cost of micropropagation plantlets.

**2. Genetic Engineering (GE):** Genetically engineered crops contain modified genomes via special transformation techniques. Usually, the gene is inserted which is known as a transgene, and the entire process is called genetic transformation. The inserted gene might be from a different species of plant, bacteria, virus, or animal overcoming the prime limitation of sexual compatibility between species. Consequently, many transgenic crops have been commercialized and the area under transgenic cultivation has increased in many parts of the world.

**2.1 Herbicide Tolerance:** Weeds are a devastating factor in horticulture. Many herbicide-tolerant plants have been developed in tomato, tobacco, potato, soybean, cotton, corn, oilseed rape, petunia, etc.

**2.2 Pathogen/Pest/Virus Resistance:** Viruses and biotic stress are the major pests of crop plants that cause considerable yield losses. Many strategies have been applied to control virus infection using coat protein and satellite RNA. Potato virus-free seed is the best example in this regard. For providing protection against fungal and bacterial diseases, various genes like chitinase, glucanase, osmotin, defensin, and pathogenesis-related genes are being transferred to many horticultural crops the world over. A number of genes including natural and synthetic cry genes, protease inhibitors, trypsin inhibitors, and cystatin genes have been used to incorporate insect and nematode resistance.

**2.3 Abiotic Stress Resistance:** Climate change has undoubtedly led to dynamic changes in weather conditions and increased the occurrence of various abiotic stresses such as drought, salinity, floods, heat, cold, nutrient deficiency, and metal toxicity and affects the growth and quality of crops. One of the greatest strategies to reduce losses by abiotic stresses is the development of climate-resilient crops. Various wild relatives and exotic germplasms are storehouses of robust abiotic stress-tolerant genes or QTLs that can be introgressed into susceptible cultivars to develop resistant crops. Resistance against chilling was introduced into tobacco plants by introducing a gene for glycerol-1-phosphate acyl-transferase enzyme from *Arabidopsis*. A number of genes encoding for biosynthesis of stress-protecting compounds including mannitol, glycine betaine, and heat shock proteins have been employed for abiotic stress tolerance besides various transcription factors like *DREB1*, *MAPK*, *WRKY*, etc. Antisense gene and *RNAi* technologies have revolutionized the pace of improvement of horticultural crops, particularly ornamentals for color modification, increasing shelf-life and reducing post-harvest losses.

**2.4 Quality improvement:** The application of genetic engineering to improve the quality in crops is brought through crop bio-fortification. Bio-fortified crops offer easy cost-effective, long-term, sustainable strategies to feed micronutrients to a large number of rural populations. Such crops can be developed by the introduction of novel genes, overexpression of genes present already, downregulation of definite genes, or interrupting the biochemical pathway of inhibitors. Additionally, genetic alterations can be made to redistribute micronutrients across tissues, increase the concentration of micronutrients in crops edible parts, boost the effectiveness of biochemical processes in edible tissues, or even recreate specific pathways. Crops such as apples, bananas, tomatoes, cassava, potato, sweet potato, cauliflower, lettuce, and carrot have been successfully bio-fortified for vitamins, minerals, secondary metabolites, antioxidants, etc., through transgenic approaches (Garg et al., 2018)

**3. Molecular Markers:** Now it has become possible to identify DNA segments and genes and to find their exact location by using molecular markers. The possibilities of using gene tags of molecular markers for selecting agronomic traits have made the job of breeders easier. It has been possible to score the plants for different traits or disease resistance at the

seedling stage itself. The use of RFLP (Restriction Fragment Length polymorphism), RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), and isozyme markers in plant breeding are numerous. RFLPs are advantageous over morphological and isozyme markers primarily because their number is limited only by genome size and they are not environmentally or developmentally influenced. Molecular maps now exist for a number of crop plants including corn, tomato, potato, rice, lettuce, wheat, brassica species, and barley. RFLPs have wide-ranging applications including cultivar fingerprinting, identification of quantitative trait loci, analysis of genome organization, germplasm introgression, and map-based cloning. AFLP is becoming the tool of choice for fingerprinting because of its reproducibility compared to RAPD. Microsatellite or simple sequence repeats (SSRs) markers have also become the choice for a wide range of applications in genotyping, genome mapping, and genome analysis.

**4. Genome Editing:** Precise DNA sequence-specific manipulation has now been possible with the advances in molecular biology. It involves the use of engineered nuclease that introduces targeted DNA Double Strand Breaks (DSB) which in turn stimulates cellular DNA repair processes. Zinc-Finger nucleases (ZNFs), transcription activator-like effector nucleases (TALENs), and RNA guided Clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR/associated protein 9 (Cas9) system are a few of the genome editing technologies that have developed in recent years. When one of the aforementioned methods is used to create DSBs, the plant's internal DNA repair systems have two options for repairing these DSBs: either through imprecise non-homologous end joining, which causes nucleotide insertions or deletions to cause gene knockouts, or through precise homologous recombination (HR), which results in gene replacements and insertions. In addition to generating indel mutations at target sequences, CRISPR/Cas systems have been adapted for precise base editing. These resources greatly increase the versatility of the tools that can be used for the precise manipulation of horticultural crops.

Crop	Target gene	Transformation technique	Results	References
Apple	MdTFL1.1	Agrobacterium-mediated suspension cell transformation	Early flowering was observed	Charrier et al., 2019
Banana	eBSV	Agrobacterium-mediated suspension cell transformation	Increase the control on viral pathogens	Tripathi et al., 2019
Tomato	SINPR1	Agrobacterium-mediated transformation	Response to stress conditions	Li et al., 2019
Potato	SBE1 SBE2	Agrobacterium-mediated transformation and PEG-mediated transfection	Increase the initiation of starch phenotype	Tuncel et al., 2019
Cabbage	BoPDS BoSRK BoMS1	Agrobacterium-mediated transfection	Albino phenotypes were observed with self in compatibility and male sterility	Ma et al., 2019
Petunia	PhACO1 PhACO3 PhACO 4	PEG-mediated protoplast transfection	Functional in ethylene production and enhanced the longevity of flowers	Xu et al., 2019
	F3H	Protoplast transfection method	Reduction in anthocyanin level along with modifications in flower colour	Yu et al., 2021

## Conclusion

Over the coming decades, these populations will grow quickly. To recover the expenses of research and development, finance additional research to create even better genetically modified horticulture cultivars, and maintain competitiveness, reasonable profit margins are required. Furthermore, it is advised that a tier system for regulating genetically modified new crop varieties should put an emphasis on a plant's traits rather than how it was created. Bio-fortified crops have a very promising future since they could help millions of undernourished people, especially in developing nations, by removing micronutrient deficiency. Last but not least, new genetic engineering techniques have the potential to produce unique plant kinds that are difficult to tell apart genetically from plants bred using traditional methods or those that arise naturally.

## References

1. Boyer J. S. (1982). Plant productivity and environment. *Science*. Oct 29; 218:443-8.
2. Charrier, A., Vergne, E., Dousset, N.J.P., Richer, A., Petiteau, A., Chevreau, E., (2019). Efficient targeted mutagenesis in apple and first time edition of pear using the CRISPR-Cas9 system. *Front. Plant Sci.* 10, 40.
3. Garg, M., Sharma, N., Sharma, S., Kapoor, P., Kumar, A., Chunduri, V., & Arora, P. (2018). Biofortified crops generated by breeding, agronomy, and transgenic approaches are improving lives of millions of people around the world. *Frontiers in Nutrition*, 12.
4. Li, R., Liu, C., Zhao, R., Wang, L., Chen, L., Yu, W., Zhang, S., Sheng, J., Shen, L., (2019). CRISPR/CAS9-Mediated SINPR1 mutagenesis reduces tomato plant drought tolerance. *BMC Plant Biol.* 19, 38.
5. Ma, C., Liu, M., Li, Q., Si, J., Ren, X., Song, H., 2(019). Efficient BoPDS Gene Editing in Cabbage by the CRISPR/CAS9 System. *Hortic. Plant J.* 5, 164–169.
6. Tripathi, J.N., Ntui, V.O., Ron, M., Muiruri, S.K., Britt, A., Tripathi, L., (2019). CRISPR/CAS9 editing of endogenous banana streak virus in the B genome of *Musa* spp. overcomes a major challenge in banana breeding. *Commun. Biol.* 2, 46.
7. Tuncel, A., Corbin, K.R., Ahn-Jarvis, J., Harris, S., Hawkins, E., Smedley, M.A., Harwood, W., Warren, F.J., Patron, N.J., Smith, A.M., (2019). CAS9-mediated mutagenesis of potato starch-branching enzymes generates a range of tuber starch phenotypes. *Plant Biotechnol. J.* 17, 2259–2271.
8. Xu, J., Kang, B.C., Naing, A.H., Bae, S.J., Kim, J.S., Kim, H., Kim, C.K., (2020). CRISPR/ CAS9-mediated editing of 1-aminocyclopropane-1-carboxylate oxidase1 (ACO 1) enhances *Petunia* flower longevity. *Plant Biotechnol. J.* 18(1):287-297.
9. Yu, Q. H., Wang, B., Li, N., Tang, Y., Yang, S., Yang, T. and Asmutola, P. (2017). CRISPR/Cas9-induced targeted mutagenesis and gene replacement to generate long-shelf life tomato lines. *Scientific reports*, 7(1), 1-9.