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Biotechnological Interventions in Horticulture Crops

(*Shivani Thakur, Dr. Shilpa Rana, Sonu Dhakar, Prashant Barnwal, Richa Sharma and Yukta Jaswal)
Sam Higginbottom University of Agriculture, Technology and Sciences, Prayagraj, India
*Corresponding Author's email: shthakur645@gmail.com

The rapid increase in human population in this changing climate scenario resulted in increased demand of plant-based food and energy sources. Fruits and vegetables are often considered protective foods rich in vitamins and minerals. The need of fruit and vegetables is growing consistently with the increasing population in developing countries. India ranks second in the world for horticultural produce after China. Biotechnology, in the present-day context, uses many methods of living things to create useful products. Tiny organisms have been used to help people since ancient times. This can be seen in making yogurt, wine, and bread as well as breeding animals and plants and producing important substances like antibiotics. Currently, India produces 283 million tonnes of horticultural products. This amount is more than the country's food grain production. Horticulture contributes about 30% to India's agriculture GDP. It uses only 17% of the land area. In the last decade, horticultural production has increased by 69%.

Introduction

Biotechnology is the application of scientific technology to modify and improve plants, animals, and microorganisms to enhance their value. The term biotechnology was coined by Karl Ereky, a Hungarian engineer in 1919. Biotechnology has changed horticulture by providing new ways to solve old problems and improve crop yield. It includes techniques like tissue culture, genetic transformation, market-assisted breeding, and genomics. These methods have greatly affected how we grow fruits, vegetables, and ornamental plants. Biotechnology in horticulture started in the mid-20th century with tissue culture techniques for growing plants in a controlled environment. Biotechnology has become a versatile tool in horticulture. It includes advanced research in genomics, proteomics, and precise gene editing methods. These innovation helps to improve crop quality, increase tolerance to environmental stresses, and enhance resistance to pests and diseases. Techniques like CRISPR-Cas9 have transformed gene editing, allowing for exact changes in the genetic makeup of horticulture crops. These advancements give horticulturists new ways to meet the growing demand for sustainable and nutritional crops. Biotech field crops are becoming popular worldwide. However, biotechnology has not succeeded in horticultural crops like fruit and vegetable crops.

The first biotech crop sold fresh was the 'Flavr saver' Tomato. This tomato has a longer shelf life. There was also a processing version of this tomato. It had higher viscosity, which made it more economically into puree and paste. Biotechnology has a lot of potential in horticulture. It can make important changes in crops like colour and aroma. Even small improvements can lead to big commercial benefits. Key areas in horticultural biotechnology include genetic transformation, micropropagation, and vitro conservation of germplasm.

Other important areas are synseed technology, virus cleaning, biofertilizers, biopesticides, and postharvest biotechnology.
The major areas of biotechnology which can be adopted for improvement of horticultural crops are:
1. Tissue culture
2. Genetic Engineering
3. Molecular markers
4. Development of Microbial Inoculums.
5. Chloroplast-mediated genetic transformation in horticultural crops.

1. Tissue Culture: Plant tissue culture and micropropagation are important techniques for growing and improving horticultural crops. These methods allow scientists to grow plant cells, tissues, or parts in a controlled environment. This helps produce many identical plants on a large scale.

Techniques of Plant Tissue Culture

- Initiation: Tissue culture begins with the selection of a suitable explant, like a piece of leaf, stem, root, and meristem. The explant is sterilized to eliminate contaminants and then placed on a nutrient medium containing vitamins, nutrients, and plant growth regulators.
- Multiplication: Under aseptic conditions, the explant forms a callus, which is a mass of undifferentiated cells. This callus can be subcultured to produce multiple plantlets. Another method of micropropagation allows multiple shoots or plantlets to grow directly from the explant without an intermediate callus phase.
- Rooting: Once enough shoots or plantlets are grown, they can be placed in a different medium to grow roots and become plants. This rooting medium usually has a lower amount of plant growth regulators to help the roots develop.
- Acclimatization: The regenerated plantlets are slowly adjusted to outside conditions. They move from the controlled lab environment to a greenhouse or open field. This process helps them adapt to their new surroundings.

Applications of Plant Tissue Culture

- Mass clonal propagation: Plant tissue culture helps produce many uniform and healthy plants quickly. It is especially useful for growing valuable ornamental plants and fruit trees. This method ensures the plants are disease-free.
- Germplasm conservation: Tissue culture enables the preservation of rare, endangered, or otherwise difficult-to-maintain plant species. By maintaining plant collections in vitro genetic diversity is conserved for future generations.
- To obtain disease-free plants: Infected plant material can be treated through tissue culture to eliminate pathogens, resulting in the production of disease-free plants.
- Genetic Transformation: Plant tissue culture is a crucial step in genetic transformation techniques, allowing the introduction of desirable genes into plant genomes. This is essential for developing genetically modified (GM) horticultural crops.
- Varietal Improvement: Breeding programs utilize tissue culture to speed up the development of new plant varieties with improved traits, such as disease resistance, enhanced flavour, or longer shelf life.

2. Genetic Engineering: Genetic engineering has emerged as a powerful tool in the field of horticulture to enhance the traits of crops, leading to improved yield, quality, and resistance to biotic and abiotic stress. Genetic engineering, also known as genetic modification or gene editing, is a powerful and sophisticated set of biotechnological techniques used to manipulate the genetic material of organisms. This field of science allows researchers to selectively modify the DNA or RNA of an organism, enabling the introduction, removal, or alteration of specific genetic elements. The primary goal of genetic engineering is to bring about desired changes in the traits or characteristics of an organism, whether it be a plant, animal, or microbes. Techniques such as CRISPR-Cas9, RNA interference, and synthetic biology are commonly employed to precisely target and modify genes, facilitating the enhancement of desirable traits such as increased yield, improved nutritional content, resistance to diseases, and tolerance to environmental stresses. Genetic engineering has widespread application in agriculture, medicine, and industries, offering innovative solutions to address challenges in food security, health care, and producing valuable bioproducts. Genetic engineering involves three major steps:

1. Identification and isolation of suitable genes for transfer.
2. Delivery system to insert desired gene into recipient cells.
3. Expression of new genetic information in recipient cells.

Genetic engineering offers plant breeders access to an infinitely wide array of novel genes and traits, which can be inserted through a single event into high-yield elite cultivars of horticulture plants. The impact is focused on:

- Resistance to diseases, insects, and viruses.
- Tolerance to herbicides and abiotic stress factors.
- Increased effectiveness of biocontrol agents.
- Enhance nutritional value and postharvest quality.
- Altered photosynthetic activity, which in consequence has an increase in yield and other components like sugar and starch production.
- Knowledge of metabolic pathways and production of pharmaceuticals and vaccines.

3. Molecular markers: A molecular marker is a DNA sequence used for chromosome mapping, as it can be located at a specific site in a chromosome. A molecular marker is a heterozygosity for the same type of silent DNA variation, not associated with any measurable phenotypic variation. Molecular markers are identified as genetic markers.

- Molecular marker is a DNA or gene sequence within a recognized location on a
- chromosome which is used as an identification tool.
- In the pool of unknown DNA or a whole chromosome, these molecular markers help identify a particular sequence of DNA at a particular location.

Applications

- ➢ It plays a crucial role in gene mapping by identifying the position of linked genes in the chromosomes which inherited together.
- ➢ It also detects any alteration in a sequence of DNA or any genetic oddity. It ascertains genes involved in genetic disorders.
- ➢ It is used to determine different characters in a DNA sequence which is used to distinguish between individuals, populations, or species.
- ➢ Molecular markers possess unique genetic properties (i.e. they are heritable DNA sequences and phenotypically neutral) and are identified by techniques such as southern hybridization and PCR.

- **PCR-based genetic markers:** RAPD (Random Amplified Polymorphic DNA), AFLP (Amplified Fragment Length Polymorphism), SSR (Simple Sequence Repeat), STR (Single Tendem Repeats), VNTR (Variable Number Tendem Repeat), STS (Sequence

Tag Size), SNP (Single Nucleotide Polymorphism), EST (Expressed Sequence Tagged)

- **Hybridization-based genetic markers:** RFLP (Restriction Fragment Length Polymorphism).

Molecular markers also can be classified as

- 1st generation markers (at the time of 1980-1990): RFLP, RAPD
- 2nd generation markers (1990-2000): AFLP, SSR, STR, VNTR, STS
- 3rd generation markers (After 2000): SNP, EST

Quality for a good genetic marker:

- Molecular marker must possess the following desirable properties-
- It must be polymorphic so that diversity must be measured.
- It should be evenly distributed throughout the genome.
- It should be easily and fast detected.
- It must distinguish the homozygotes and heterozygotes.

4. Development of Microbial Inoculums: Indiscriminate and injudicious use of chemical fertilizers and pesticides has caused environmental pollution. It has harmed soil, lead to resistance in many insects, and residue problems. Hence there is a great concern worldwide to use safer biofertilizers and biopesticides in the integrated nutrient management and pest management systems. Biofertilizers are microorganisms that help plants by fixing nitrogen from the air or making phosphorus in the soil available. Key organisms include nitrogen-fixing bacteria like Rhizobium, Azotobacter, and Azospirillum. Important phosphorus-solubilizing bacteria include Bacillus polymyxa, B. megaterium, and Pseudomonas striata, along with some fungi like Aspergillus and Penicillium.

The benefits of using micro-organisms as fertilizers are manyfold. They are less expensive, nontoxic to plants, do not pollute the groundwater nor render the soil acidic and unfit for growth of plants. Rhizobium forms nodules on the roots of leguminous plants and help in fixing nitrogen from the atmosphere to ammonium irons which get converted to amino acids in the plant system. Inoculation with this bacteria helps in reducing the addition of nitrogenous fertilizers to the soil. Azospirillum is also found colonizing intercellular spaces inside the root system. These bacteria also contribute substantially to the nitrogen requirement of the plants.

Phosphate-solubilizing bacteria are microorganisms that help break down insoluble phosphorus in the soil. This makes phosphorus available for crops to use.

Mycorrhiza is a helpful partnership between crop plant roots and non-pathogenic fungi. These fungi provide nutrients from deeper soil layers to the plants. When plants are inoculated with mycorrhiza, they grow better. Many fruit crops, like papaya, mango, banana, citrus, and pomegranate, depend on this association to get more phosphorus and other nutrients from the soil. Mycorrhiza also helps plants resist pathogens and improves soil quality.

Many researchers have studied how Arbuscular Mycorrhizal (AM) inoculation helps plants grow. This inoculation increases the yield of chilies, tomatoes, capsicum, and other vegetables. It also improves the growth of cashew seedlings. In Chrysanthemums and China asters, mycorrhizal inoculation leads to more flowers and longer vase life. It enhances rooting in cuttings of apple, cassava, and sweet potato. Mycorrhizal-inoculated avocado and cashew plants handle transplant shock better than those that are not inoculated.

Many researchers have noted that mycorrhizal inoculum helps develop crops with better traits. Improved growth and vigor have been seen in micro-propagated plantlets of grapevine, apple, avocado, pineapple, kiwifruit, strawberry, raspberry, asparagus, and banana when inoculated with mycorrhizal fungi. A ten-year-old mulberry plant and a one-and-a-half-year-old papaya tree also showed positive responses to mycorrhizal inoculation. Using controlled conditions in agriculture makes it easier to inoculate plants with mycorrhizal fungi using a small amount of inoculum. Proper use of these fungi in horticultural crops can lower cultivation costs and help protect the environment.

5. Chloroplast mediated genetic transformation in horticultural crops: In most cases of transgenic development, transgenes are added to the nuclear genome. Developing transgenics for the organelle genome, like chloroplasts, has some advantages. There is concern about pollen from transgenic plants mixing with nearby non-transgenic crops and wild relatives. Chloroplast transformation technology helps address this issue. In this process, DNA fragments with foreign genes are integrated into the chloroplast genome. Transgenic plants created this way show improved traits, such as herbicide resistance, insect resistance, better photosynthesis, enhanced nitrogen fixation, higher yields, and greater tolerance to drought, salinity, and bacterial and fungal pathogens.

The complete nucleotide sequence helped develop chloroplast transformation technology for horticultural crops like tomato, potato, and Brassica (mustard). Stable chloroplast transformation was successfully achieved in potatoes. Researchers used tobacco-specific chloroplast expression vectors to integrate the transgene into the potato's plastid genome. This method was then successfully applied to tomatoes as well. The genetic transformation of tomato plastids resulted in very high levels of transgene expression. A series of new plastid expression vectors were used for the transformation in tomatoes. Recently, this technology has also been developed for other crops, including carrots and Brassica.

Conclusion

Biotechnology interventions in horticultural crops offer significant benefits, including improved resistance to pests and diseases, enhanced nutritional content, and better adaptability to environmental stresses. Techniques like genetic modification, tissue culture, and chloroplast transformation enable the development of crops with desirable traits, leading to higher yields and more sustainable farming practices. These advancements can help reduce the reliance on chemical inputs and promote eco-friendly agriculture. Overall, biotechnology has the potential to transform horticulture, ensuring food security and supporting the needs of a growing population while protecting the environment.

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