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Biotechnology: Approaches and methods in horticultural crops

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Abstract
The review was conducted at Teerthankkar Mahaveer University, Moradabad, in the month of April 2024, with an aim of gathering information regarding recent advances in the biotechnology in horticultural crops. Horticultural crops play a crucial role in ensuring food and nutritional security. Additionally, they are a vital component of the agricultural economy, possessing significant economic potential. These crops are a valuable source of nutrients essential for human health. Global production of horticultural crops has seen substantial growth over recent decades. Nevertheless, significant losses due to both biotic and abiotic stresses remain significant challenges in their effective utilization. Biotechnology is often regarded as a solution to various horticultural production challenges. Plant biotechnology encompasses three primary areas: *in vitro* propagation and tissue culture for producing disease-free plants, the use of molecular markers for enhanced selection in plant breeding, and genetic engineering. The review highlights role of biotechnology in improvement of horticultural crops. It explores micropropagation's contributions to elite plant varieties, reviews genetic transformation methods and GM crops in horticulture. The literature includes Agrobacterium-mediated and biolistics transformation techniques for crop enhancement. Marker-assisted breeding strategies are discussed in the context of enhancing crop resilience, productivity, and nutritional quality as well as latest technology like CRISPR Cas9. Overall, this review synthesizes current knowledge and identifies future directions in biotechnological approaches aimed at advancing horticultural crop improvement and sustainability.

Keywords: Horticultural biotechnology, GM, marker assisted breeding, CRISPR Cas9

1. Introduction
Horticulture crops include vegetables, fruits, flower, plantations, aromatics and medicinal crops. These crops are of high value to human health and play an important role in food security. Horticultural crops, mainly fruits and vegetables, are rich in carbohydrates, vitamins, proteins, minerals and organic acids. According to the new guidelines of World Health Organization (WHO), the daily recommended intake of fruits and vegetables should be more than 400 g day^{-1} (Anonymous, 2023) [7]. Although horticultural crops play a crucial role in ensuring nutrition and food security, advancements in these crops have not kept pace with those seen in most staple food crops. With the increasing population, the demand for horticulture produce is increasing day by day, from the requirement of quality planting material to the higher production of the crops which cannot be fulfilled by the conventional practices. Apart from this, with changing environmental conditions like irregular rainfall patterns, rising temperature, drought, chilling or frost and with changing environmental conditions introductions of new pest and diseases are creating new challenges for quality production of horticultural crops. Therefore, there is pressing priority to adopt biotechnological practices which will aid in the upliftment of horticulture sector. With the implementation of biotechnology, it is possible to target specific genes and its expression can be regulation.
Biotechnology is a broad term, this include employment of biological operations, organisms or system for the betterment of human life. The term “biotechnology” was first coined by a Hungarian engineer Karl Ereky (Ereky, 1919) [28]. The early historical accounts of biotechnological processes include practices like fermentation and breeding, which were developed by the ancient Egyptians (Bigey *et al*., 2021) [10]. Biotechnological tools are poised to have significant effects on agriculture, especially in cases where even subtle

alterations, such as improvements in color, fragrance, and post-harvest behavior, can result in substantial commercial benefits. Genetic modification of plants has been implemented for over thirty years. Genetic modification, micropropagation, germplasm preservation through *in vitro* techniques, synchronized technology, biofertilizers, biopesticides and post-harvest biotechnology all represent crucial facets of biotechnology's role in the improvement of horticultural crops. Contemporary biotechnological methodologies enable the manipulation of genes sourced from diverse origins and their integration into plants to confer beneficial characteristics aimed at enhancing crop quality (Irfan *et al*., 2023) [43]. Despite the growing global acceptance of biotech field crops, biotechnology has seen limited commercial success so far in horticultural crops, such as fruits, vegetables, flowers, and landscape plants. (Numerous traits desirable to both growers and consumers of horticultural crops, such as innovative genetic techniques for disease and insect resistance, weed management, extended flower longevity, and slower-growing grass, have been developed and tested but have not yet reached commercialization (Choudhary *et al*., 2014) [21]. To create a novel high-yielding variety, it is crucial to produce functional recombinants and formulate a suitable approach for their selection and subsequent progress. Apart from this, insufficient understanding of genetic variability type, extent, and relationships among key traits would hinder the design of an effective breeding strategy for genetic enhancement.

Biotechnology has significantly advanced horticultural crops, such as genetically engineered papaya resistant to the Papaya Ringspot Virus (PRSV) and virus-resistant sweet potatoes and cucumbers. These developments have improved crop resilience and safeguarded vital crops from losses. The Flavr Savr tomato, developed with the polygalacturonase (PG) gene, was the first commercialized horticultural crop (Sheehy *et al*., 1988). This review discusses biotechnology tools, techniques, applications, and achievements in horticultural crops.

2. Materials and Methods

A comprehensive literature search was conducted using databases such as PubMed, ScienceDirect, Web of Science, and Google Scholar to gather information on biotechnological applications in horticultural crops. Keywords such as “biotechnology”, “horticultural crops”, “genetic engineering”, “tissue culture”, “marker-assisted selection”, and “genome editing” were used. Only peer-reviewed articles, reviews, and book chapters in English were included, while articles not directly related to horticultural crops or lacking methodological detail were excluded. Information was systematically extracted on various biotechnological methods, including genetic engineering (e.g., CRISPR/Cas9), tissue culture (e.g., micropropagation) and marker-assisted selection, emphasizing success rates, challenges, and future prospects. Methodologies were critically evaluated for reproducibility, scalability, and impact. Findings were synthesized to provide an overview of current trends, advancements, and gaps, highlighting future research areas and the implications of biotechnology on sustainable horticulture.

3. Biotechnological Tools and Techniques for Improvement of Horticultural Crops

Various biotechnological tools and techniques have been employed in to enhance the production and quality of horticultural crops. Using techniques like genetic engineering, RNA antisense technology, gene silencing etc improvement of the targeted character can be possible. In manufacturing of true to type and disease free planting material tissue culture technique is quite successful. Plant biotechnology allows for enhancements that cannot be achieved solely through traditional crossbreeding of closely related species. Such approaches have been concisely discussed below:

3.1 Tissue culture

Plant tissue culture is the *in vitro* cultivation of explants on nutrient-rich media under aseptic and controlled conditions. In this case, an explant can be anything that holds the potential to regenerate and has the ability to develop into a whole plant. The swift advancement in asexual *in vitro* propagation has made it feasible to efficiently propagate numerous crops that were previously challenging to propagate. This technique not only allows for the production of elite, disease-free propagules but also enables the secure and quarantined transfer of germplasm across international borders, aiding in their conservation. Currently, micropropagation can be applied to nearly all fruit and vegetable crops. In horticultural crops, plant tissue culture can be used as a model system for evaluating various biochemical, physiological, and genetic problems related to plants is commercially used in the propagation of economically important horticultural crops.

3.2 Embryo rescue technique

Embryo rescue technique involves culturing of the immature or immature embryo under aseptic conditions with an objective of growing a whole plant. This technique is usually employed by the plant breeders to the embryos that would normally abort and did not further undergo the ontogeny. This technique is widely used in seedless stenospermocarpic grapes breeding in which embryo get frequently abort other than this, embryo rescue can be employed in any crop which posses the barrier of embryo abortion. Interspecific, intergeneric and intervarietal crosses in papaya, mango, banana and seedless citrus can be generated using embryo rescue techniques. The first steps involved in the embryo rescue technique are the excision of the weak embryo followed by culturing them on nutrient medium.

3.2.1 Factors affecting embryo rescue technique

3.2.1.1 Stage of the embryo: Stage at which the embryo is excised is very crucial for the establishment of the culture. Embryos excised at early stages are hard to establish so the nurse tissue such as endosperm can be used to provide natural condition and nutrition to the embryo (Akmal, 2021) [6].

3.2.1.2 Culture medium: In general, Murashige and Skoog media and Gamborg’s B-5 are the two widely used nutrient medium for embryo rescue technique (Burbulis *et al*., 2004). During the initial phases, immature embryos that are heterotrophic obtain their nourishment from the endosperms and surrounding tissues. However, as these embryos mature, they transition to a partly autotrophic state, where they only require essential mineral salts and sucrose (Akmal, 2021) [6]. Also, sucrose is an important constituent of nutrient medium and act as an energy source which help in triggering embryo formation.

3.2.1.3 Light and temperature: Embryo rescue is greatly influenced by the light and temperature and their specifications differ from crop to crop.

3.3 Somaclonal variation

Somaclonal clonal variations can be defined as the genetic and epigenic changes that arise due to cytogenic abnormalities or gene mutations of *in vitro* regenerated plants (Krishna *et al*., 2016). The term "somaclonal variation" was first coined by Larkin and Scowkraft in the year 1981. One notable example is the *in vitro* production of Cavendish banana plantlets, where the range of variation extended from 6% to 38%. From a commercial perspective, variation in clonal propagated plants is not desirable which lead to a loss of genetic fidelity. However, from a breeding point of view, somaclonal variation offers ample opportunities for horticultural crops, especially since they are mostly vegetatively propagated. This is due to factors like longer juvenile phase as in perennial fruit crops, occasional inbreeding depression, self and cross incompatibility, narrow genetic base especially in ornamentals, etc (Jain, 2001) [46]. Somaclonal variations can result from various factors such as wounding, lighting conditions, imbalance of nutrients in culture media, exposure to sterilants, alternations in temperature and humidity. Numerous somaclonal variants have been developed in various crops, and a selection of them is listed in Table 1.

Table 1: Horticultural crops with improved traits developed through somaclonal variations

S. No.	Horticulture Crops	Improved characteristics	Reference
1.	Apple (*Malus* × *domestica* Borkh.)	Resistance from Fire blight of apple (*Erwinia amylovora*)	Chevreau *et al*., 1998 [20]
2.	Apple rootstocks M 26 and MM 106 (*Malus pumila* Mill.)	Resistance from apple collar rot (*Phytophthora cactorum*)	Rosati *et al*., 1990 [49]
3.	*Anthurium* sp.	'Orange Hot' derived from clone of 'Red Hot'	Henny and Chen, 2011 [39]
4.	Banana (*Musa acuminata* L.)	10 somaclones; GCTCV215-1 released for commercial planting	Hwang and Ko, 1992 [42]
		Var. CIEN-BTA-03, resistant to yellow Sigatoka	Giménez *et al*., 2001 [34]
		Semi-dwarf and resistant to *Fusarium* wilt TC1-229	Tang *et al*., 2001 [50]
5.	Begonia (*Begonia* × *elatior*)	Plant morphology and floral characteristics like number of flowers plant^{-1}	Jain, 1997 [48]
6.	Brinjal (*Solanum melongena* L.)	Stress tolerance	Ferdausi *et al*., 2009 [30]
7.	Blackberry	Thornlessness in var. 'Lincoln Logan'	Hall *et al*., 1986 [37]
8.	Capsicum (*Capsicum annuum* L.)	Yellow fruited var. Bell sweet	Morrison *et al*., 1989 [51]
9.	Carrot (*Daucus carota* L.)	Resistance from leaf spot (*Alternaria dauci*)	Dugdale *et al*., 2000 [27]
10.	*Citrus* spp.	Resistant from *Phoma tracheiphila*	Deng *et al*., 1995 [24]
		Salt tolerance in Rough Lemon	Kumar *et al*., 2010 [52]
11.	*Dieffenbachia sp.*	Unique and noticeable leaf patterning with taller, more expansive canopies and elongated leaves compared to the 'Camouflage' parent plants.	Shen *et al*., 2007 [53]
12.	Garlic (*Allium sativum* L.)	Resistance against '*Sclerotium cepivorum'*	Zhang *et al*., 2020 [18]
13.	Gerbera (*Gerbera jamesonii* Bolus)	Novel cultivars	Minerva and Kumar, 2013 [54]
14.	Ginger (*Zingiber officinale* Rosc.)	Tolerant to wilt pathogen	
15.	Grapevine (*Vitis vinifera* L.)	Resistant to *Botrytis cinerea* and *Plasmopara viticola*	Kuksova *et al*., 1997 [55]
16.	Kiwi fruit (*Actinidia deliciosa*)	5 somaclones, derived from cv. Tamuri, Salt tolerant	Anselmi *et al*., 2002 [8]
17.	Lemon grass (*Cymbopogon martinii*)	Increased oil content	Patnaik *et al*., 1999 [56]
18.	Mango (*Mangifera indica* L.)	Resistant to *Colletotrichum gleosporiensis*	Litz *et al*., 1989 [57]
19.	Pea (*Pisum sativum* L.)	Resistance to *Fusarium solani*	Horáček *et al*., 2013 [41]
20.	Peach (*Prunus persica* L.)	Somaclone S 122-1 was found resistant to bacterial canker (*Pseudomonas syringae* pv. *syringae*)	Hammerschlag, 2000 [38]
21.	Pear rootstock (*Pyrus communis* L.) 'Old Home × Farmingdale (OHF 333)'	Tolerance to the fire blight	Nacheva *et al*., 2014 [58]
22.	Potato (*Solanum tuberosum* L.)	Non-browning var. White Baron	Arihara *et al*., 1995 [9]
		Improvement in size, shape, appearance, content and yield of starch	Thieme and Griess, 2005 [59]
		Superior processing quality than cv. 'Russet Burbank'	Nassar *et al*., 2011 [60]
23.	Strawberry (*Fragaria* sp.)	Improvement in horticultural traits	Biswas *et al*., 2009 [11]
		Resistant from *Verticillium dahliae* Kleb	Zebrowska, 2010 [61]
		'Serenity', a lighter skin-colored, late variety, resistant to powdery mildew and *Verticillium* wilt somaclonal variant of the cv. 'Florence'	Whitehouse *et al*., 2014 [61]

3.4 Genetic engineering

Genetic engineering, popularly known as recombinant DNA technology, involves the alteration of genetic material to enhance or suppress the function of a gene or set of genes. This process includes the insertion of desirable gene(s) from any origin (microorganisms, animals, and plants) into the target plant. Unlike conventional methods that may involve the combination of both desirable and undesirable genes, recombinant DNA technology selectively incorporates only desirable genes, aiding in the development of abiotic and biotic stress-resistant varieties. Many wild strains of horticultural crops carry genes for resistance to abiotic and

biotic stresses, which can be effectively introduced into cultivated varieties through genetic engineering. Over the past two decades, the genetic transformation of fruit crops has primarily aimed at improving disease resistance against viruses, fungi, and bacteria, as well as increasing tolerance to abiotic stresses such as drought, frost, and salinity (Gomez-Lim and Litz, 2004, Gambino and Gribaudo, 2012) [35, 32]. Additionally, efforts have been made to modify plant growth habits and enhance fruit quality. However, there have been limited instances of field evaluation and commercial deployment of these transgenic plants. In horticultural crops, the first successful transgenic was the "Flavr Savr" tomato, in which the shelf life of the tomato was enhanced using biotechnology. It was the first crop to get approval for commercialization in the USA in 1994. Subsequently, many genetically modified (GM) crops were developed, such as GM papaya, specifically the Rainbow papaya, which was engineered for resistance against the papaya ringspot virus. However, in India, only one GM crop has been approved for commercial cultivation, which is Bt cotton. A variety of genetic engineering techniques are used few of which are described below:

3.4.1 Microbial Vectors

This technique involves transfer of the desirable genes using a vector which is *Agrobacterium* in most of the cases. *Agrobacterium* is the soil microbe which is known to cause crown gall disease in plants. During the infection of the host it transfers a portion of its own DNA into the host plant which was stably integrated with plant DNA. The plant read the foreign DNA and expresses the genes as its own. In genetic engineering, the DNA of *Agrobacterium* is replaced by the DNA of interest.

3.4.2 Microprojectile bombardment

This technique was first discovered by Klein *et al*. (1988), they observed that naked DNA could be successfully inserted in the target organism by shooting along microscopic pellets. This is an effective method of genetic engineering for crops in which *Agrobacterium* is not effective.

3.4.3 Electroporation

In this technique, plant protoplasts take up macromolecules from the surrounding fluid, enhanced by an electric impulse. Electroporation is a method that uses strong, quick bursts of electricity to open up cell membranes temporarily. When an electric field is applied, it temporarily weakens the cell membrane, allowing different molecules to enter the cells. Small molecules can get in by just moving through the weakened membrane, while larger ones, like DNA, can be pushed in using electricity.

3.4.4 CRISPR Cas9 gene editing

Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated 9 (CRISPR/Cas9) has emerged as the most widely utilized gene-editing tool over the past decade. It is renowned for its high efficiency, simplicity, and user-friendliness. The initial CRISPR sequence was discovered in *Escherichia coli* in 1987, identified as the gene responsible for the isozyme conversion of alkaline phosphatase (Ishino *et al*., 1987) [44]. The CRISPR/Cas system's principle is derived from the adaptive immune mechanism of type II prokaryotic organisms. The CRISPR/Cas systems can be categorized into three distinct types (I, II, and III), each employing unique molecular mechanisms to identify and cleave nucleic acid sequences. The protospacer adjacent motif (PAM) sequences, which are brief sequence motifs located next to the crRNA-targeted region on the invading DNA, are crucial in type I and type II systems, particularly during the phases of adaptation and interference.

In horticultural crops, CRISPR Cas9 can use to improve the quality of produce by improving taste, aroma, shelf life of produce. It can also be used to modifying the architecture of fruit trees, ornamental flowers, and trees; enhancing yield potential; and bolstering resistance to pests, diseases, and environmental stress in plants (Jain, 2015) [47]. CRISPR-Cas technology was utilized to knockout MPK20 (mitogen-activated protein kinase 20), resulting in the inhibition of transcription and the synthesis of protein products for several genes involved in the sucrose metabolism pathway (Chen *et al*., 2018) [19]. CRISPR Cas9 can augment nutrient content in fruits by altering essential genes associated with metabolic pathways.

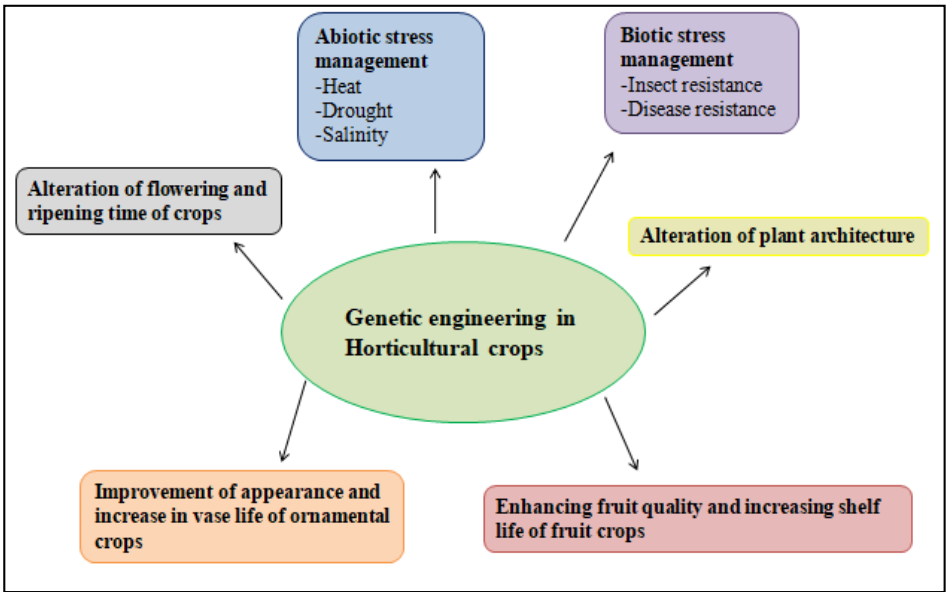

Fig 1: Quality trait improvement of horticultural crops through genetic engineering.

3.5 Molecular markers

Molecular markers are specific, easily detectable and measurable sequences of DNA that can serve as unique genetic signposts within an organism's genome (Ahmad *et al*., 2020) [5]. They are used to identify and track the presence or absence of particular genetic traits or variations in individuals or populations. Molecular markers can be widely used in horticultural crops for assessing genetic diversity, selecting disease resistance, evaluating the quality of planting material, choosing rootstock, assessment of genetic, facilitating marker-assisted breeding etc. Molecular tools for marker-assisted breeding (MAB) offer the potential to tackle challenges that have been difficult to address with traditional methods, as well as enhance the efficiency of routine breeding processes. However, MAB will be most effective when it is seamlessly integrated into the various components of breeding programs, serving as an integral part of the overall strategy rather than being treated as a supplementary technique. They can be successfully applied to detect alterations at molecular level.

3.5.1 Classification of molecular markers

Molecular markers are broadly classified into two categories i.e., Non-PCR based markers and PCR based markers, which are described as follows:

3.5.1.1 Non- PCR based markers

The initial genetic marker created and employed for identifying DNA variations and constructing genomic maps in humans was restriction fragment length polymorphism (RFLP) (Botstein *et al*., 1980) [12]. Subsequently, various molecular markers were developed for plant genetic analysis. RFLPs are utilized to assess genetic diversity and to investigate the relationships among closely related taxa (Dijkhuizen *et al*., 1996) [25].

3.5.2.1 PCR based markers

PCR-based molecular markers are pivotal tools in molecular genetics, leveraging the polymerase chain reaction (PCR) to amplify specific DNA sequences that serve as genetic landmarks for studying biodiversity, genetic diversity, and evolutionary relationships. These markers, including microsatellites, AFLPs, and SNPs, offer high resolution and reproducibility due to their locus-specific nature and ability to detect subtle genetic variations. PCR-based molecular markers in horticultural crops facilitate precise genotype identification and QTL mapping, crucial for enhancing traits like disease resistance and fruit quality. These markers are pivotal in guiding breeding efforts towards developing resilient varieties and ensuring the authenticity of horticultural products in commercial markets (Table 2).

Table 2: Utilization of molecular markers in horticultural crops

S. No.	Crop	Markers type	Trait identified	Remarks
1.	Apple	RFLPs, RAPDs, SSRs, ISSRs, SCARs, SNPs and DArTs	Genetic variability, identification of quantitative trait loci (QTLs) controlling flesh mealiness, construction of a genome map for woolly aphid to identify resistance genes, development of a high-density molecular marker map, and applications of genome-wide association studies (GWAS) in QTL analysis are crucial areas of research. Additionally, the MYB10 gene has been linked with a locus responsible for red flesh and foliage, while ACS and ACO, two genes associated with ethylene production, have been identified using gene-specific markers positioned on a molecular marker linkage map.	Bus *et al*., 2008 [15], Zhen *et al*., 2020 [18]
2.	Asparagus	ISSR and SSR	Genetic variability, QTL mapping	Caruso *et al*., 2008 [17], Chen *et al*., 2020 [18], García *et al*., 2022 [33]
3.	Banana	RAPDs, SSRs and ISSRs	Genetic variability and phylogenetic studies	
4.	Carnation	RAPDs, SRAPs and ISSRs	Mapping of plant chromosomes	Sharma *et al*., 2017 [23]
5.	Chrysanthemum	RAPDs, ISSRs and AFLPs	Genetic diversity, germplasm identification	Olejnik *et al*., 2021 [63]
6.	Citrus	RFLPs, RAPDs, AFLPs, SSRs, ISSRs, SNPs and DArTs	Identification of hybrids, phylogenetic studies and association of genome mapping to detect various QTLs	Curtolo *et al*., 2017 [22], Abouzari *et al*., 2020 [2]
7.	Cucumber	AFLPs, RAPDs, SCARs and SSRs	Genome mapping	Dar *et al*., 2017 [23]
8.	Grapes	AFLPs, RAPDs, SSRs, ISSRs, SNPs, SCC8, SCF27 and GSLP1	Sex expression, identification of seedless parents, identification of QTLs association with downy mildew resistance	Divilov *et al*., 2018 [26]
9.	Mango	AFLPs, RAPDs, SSRs and ISSRs	Identification of hybrids and cultivars	Galal *et al*., 2017 [31]
10.	Strawberry	RAPDs, SNPs, SSRs and SCARs,	Identification of genes, i.e., LOC101295509, Hsp70, and LOC101311180 involved in heat tolerance and DNA fingerprinting,	Ahmad *et al*., 2020 [5]
11.	Tomato	RAPDs, AFLPs, SSRs, ISSR, SCoT and SNPs	Hybrids identification and genetic diversity	Herison *et al*., 2017 [40], Abdein *et al*., 2018 [1]

3.6 Cryopreservation

Cryopreservation is an excellent technique used for the conservation of germplasm. It involves use of liquid nitrogen to maintain tissue at ultra low temperature (-196 °C), making it a long term preservation technique. Here, liquid nitrogen arrests all the metabolic and biochemical activities of plant tissue which makes it a long term storage technique. However, preserving plants using

cryopreservation is not easy, as most plants cannot tolerate the low-temperature stress. For this purpose, cryoprotectants are used to avoid extracellular and intracellular ice formation. The main purpose of cryoprotectants is to dehydrate the plant material to avoid crystallization of water. Presently, standardized protocols for cryopreservation are available for more than 200 plant species and over 10,000 accessions started from *in vitro* cultures are safely stored in liquid nitrogrn. More than 80% of these accessiond belong to five major crops i.e., potato (38%), cassava (22%), banana and plantains (11%), mulberry (12%) and (5%) garlic (Acker *et al*., 2017). Various types of planting material that can be stored through cryopreservation technique such as pollens, embryos, shoot primodia, zygote, roots, bulbs, callus, cell suspension culture, spores, dormant buds, nodal segments etc..

3.7 Achievements

Biotechnology has opened any gates and discovered many solutions in the past few decades. Traits like herbicide tolerance, virus resistance, insect tolerance, resistance against abiotic stress, quality improvement etc., were successful achieved using different tools and techniques of biotechnology. In horticultural crops, biotechnological achievements are mentioned in the Table 3.

Table 3: Achievements of biotechnology in horticultural crops:

S. No.	Crop	Enhanced trait of interest	Reference
1.	Apple (Arctic apple)	Anti browning property by suppressing the polyphenoloxidase activity using antisense RNA technology	Carter, 2012 [16]
	Arctic Apples (Arctic Golden Delicious)	PGAS PPO suppression gene resulted in non browning and antibiotic resistance	Carter, 2012 [16]
2.	Carnation (Moon series)	Sulfonylurea herbicide tolerance , Modified flower color	Lu *et al*., 2003 [64]
3.	Cowpea	Lepidopteran insect resistance , Antibiotic resistance using *Cry1Ab* from *Bacillus thuringiensis* subsp. kumamotoensis	Fatokun, 2009 [29]; Ishiyaku, 2010 [45]
3.	Eggplant	Lepidopteran insect resistance , Antibiotic resistance using *Cry1Ac*	Acciarri *et al*., 2002 [3]
4.	Lettuce	Salt resistance	Park *et al*., 2005
5.	Papaya (Sun Up and Rainbow)	Resistant to papaya ring spot virus (PRSV)	Gonsalves *et al*., 2004 [36]
6.	Potato	Resistant to potato tuber moth using gene *Cry1Ab*	Kumar *et al*., 2010 [52]
		Vitamin-C biofortified potato developed by Introgression of *GDP* gene to enhance tuber ascorbate content	Bulley *et al*., 2012 [13]
		Potato cv. Innate 1.0 having improved processing quality developed by manipulation of genes *VInv* and *Asn1* to provide resistance to CIS and acrylamide formation	Waltz *et al*., 2015 [65]
		Potato cv. Elizaveta Plus and Lugovskoi Plus developed through Bacterial Cry3A gene to provide resistance to CPB	Korobko *et al*., 2016 [66]
7.	Rose	Modified flower color	Katsumoto *et al*., 2007 [67]
8.	Tomato (Flavr Savr)	suppression of the tomato polygalacturonase (PG) gene	Sheehy *et al*., 1988 [68]

3.8 Limitations of biotechnology

Biotechnology has brought significant advancements to horticulture, but it also has limitations and challenges that are mentioned below:

3.8.1 Regulations hurdles: Strict regulations surrounding genetically modified organisms (GMOs) can hinder the development and commercialization of biotech horticultural crops. Navigating complex regulatory processes can be time-consuming and expensive.

3.8.2 Consumer acceptance Some consumers are wary of GMOs and may be hesitant to purchase genetically modified horticultural products. Public perception and acceptance can affect market demand.

3.8.3 High cost: Developing and testing biotech horticultural crops can be costly due to the need for extensive research, regulatory compliance, and field trials.

3.8.4 Time consuming: Genetic modification and other biotechnological approaches often require years of research and development before a new crop variety can be brought to market.

3.8.5 Environmental concerns: There are concerns about the potential environmental impact of biotech crops, including unintended effects on non-target organisms and ecosystems.

3.8.6 Intellectual property issues: Biotech innovations are often protected by patents, which can limit access for smaller-scale farmers and researchers, potentially reducing genetic diversity.

3.8.7 Cross-breeding challenges: Cross-breeding genetically modified crops with wild or conventional varieties can sometimes result in the spread of modified genes to unintended populations.

3.8.8 Unpredictable outcomes: The complexity of genetic interactions means that unintended consequences can arise when introducing new traits or genes, potentially affecting crop performance or other characteristics.

3.8.9 Ethical concern: Some ethical concerns surround the manipulation of genetic material in horticultural crops, particularly when it involves modifying traits for non-food purposes, such as ornamental features.

3.9 Future prospect
Plant biotechnology in horticultural crops offers significant promise for the future. It can help create disease and pest-resistant varieties, improve tolerance to biotic and abiotic stresses, enhance nutritional content, increase crop yield and quality and support precision agriculture, reducing the environmental impact of farming. Additionally, biotechnology can address issues such as food waste by creating non-browning traits, aid in phytoremediation to clean up contaminated soils, and cater to consumer preferences with unique traits. However, challenges such as stringent regulations, ethical concerns, consumer acceptance, global trade barriers and the need for research funding must be addressed to fully realize the potential of plant biotechnology in horticulture. The future of these technologies will depend on scientific advancements, regulatory decisions and societal acceptance.

4. Conclusion
The future of plant biotechnology in horticulture is promising, with potential to develop disease and pest-resistant varieties, improve tolerance to biotic and abiotic stresses, enhance nutritional content, and increase crop yield and quality. These advancements support precision agriculture and reduce environmental impact. Biotechnology can also address food waste, aid in phytoremediation, and cater to consumer preferences. Continued research and innovation are essential to fully harness these technologies for sustainable horticultural crop production.

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6. Conflict of Interests
The authors declare that they have no conflicts of interest.

7. References
1. Abdein MA, Abd El-Moneim D, Taha SS, Al-Juhani WS, Mohamed SE. Molecular characterization and genetic relationships among some tomato genotypes as revealed by ISSR and SCoT markers. Egyptian Journal of Genetics and Cytology. 2018;47(1):1-17.
2. Abouzari A, Solouki M, Golein B, Fakheri BA, Sabouri A, Dadras AR. Screening of molecular markers associated to cold tolerance-related traits in Citrus. Scientia Horticulturae. 2020;263:109145.
3. Acciarri N, Restaino F, Vitelli G, Perrone D, Zottini M, Pandolfini T, *et al*. Genetically modified parthenocarpic eggplants: improved fruit productivity under both greenhouse and open field cultivation. BMC Biotechnology. 2002;2:1-7.
4. Acker JP, Adkins S, Alves A, Horna D, Toll J. Feasibility study for a safety back-up cryopreservation facility. Independent expert report: July 2017.
5. Ahmad R, Anjum MA, Balal RM. From markers to genome-based breeding in horticultural crops: an overview. Phyton-International Journal of Experimental Botany. 2020;89(2):183-204.
6. Akmal M. Embryo Culture and Embryo Rescue in Brassica. Brassica Breeding and Biotechnology. London, UK: Intech Open; c2021. doi: 10.5772/intechopen.96058.
7. Anonymous. Increasing fruit and vegetable consumption to reduce the risk of noncommunicable diseases; c2023. Available at: https://www.who.int/tools/elena/interventions/fruit-vegetables-ncds#:~:text=WHO%20Recommendations,the%20risk%20of%20certain%20NCD. Accessed on: 23-04-2024.
8. Anselmi S, Donat E, Manes F, Caboni E. *In vitro* selection of Actinidia deliciosa clones tolerant to NaCl and their molecular and *in vivo* ecophysiological characterisation. Acta Horticulturae. 2002;618:77-83.
9. Arihara A, Kita T, Igarashi S, Goto M, Irikura Y. White Baron: a non-browning somaclonal variant of Danshakuimo (Irish cobbler). American Journal of Potato Research. 1995;72(11):701-705.
10. Bigey F, Segond D, Friedrich A, Guezenec S, Bourgais A, Huyghe L, Sicard D. Evidence for two main domestication trajectories in Saccharomyces cerevisiae linked to distinct bread-making processes. Current Biology. 2021;31(4):722-732.
11. Biswas MK, Dutt M, Roy UK, Islam R, Hossain M. Development and evaluation of *in vitro* somaclonal variation in strawberry for improved horticultural traits. Scientia Horticulturae. 2009;122(3):409-416.
12. Botstein D, White RL, Skolnick M, Davis RW. Construction of a genetic linkage map in man using restriction fragment length polymorphisms. American Journal of Human Genetics. 1980;32(3):314-331.
13. Bulley S, Wright M, Rommens C, Yan H, Rassam M, Lin-Wang K, Laing WA. Enhancing ascorbate in fruits and tubers through over-expression of the l-galactose pathway gene GDP-l-galactose phosphorylase. Plant Biotechnology Journal. 2012;10(4):390-397.
14. Burbulis N, Kupriene R, Zilenaite L. Embryogenesis, callogenesis and plant regeneration from anther cultures of spring rape (*Brassica napus* L.). Acta Universitatis Latviensis, Biology. 2004;676:153-158.
15. Bus VGM, Chagné D, Bassett HCM, Bowatte D, Calenge F, Celton JM, *et al*. Genome mapping of three major resistance genes to woolly apple aphid (*Eriosoma lanigerum* Hausm.). Tree Genetics & Genomes. 2008;4(2):223-236.
16. Carter N. Petition for determination of nonregulated status: Arctic™ Apple (*Malus* x *domestica*) events GD743 and GS784. United States Department of Agriculture—Animal and Plant Health Inspection Service; c2012.
17. Caruso M, Federici CT, Roose ML. EST-SSR markers for asparagus genetic diversity evaluation and cultivar identification. Molecular Breeding. 2008;21:195-204.
18. Chen H, Guo A, Wang J, Gao J, Zhang S, Zheng J, *et al*. Evaluation of genetic diversity within asparagus germplasm based on morphological traits and ISSR markers. Physiology and Molecular Biology of Plants. 2020;26:305-315.
19. Chen L, Yang D, Zhang Y, Wu L, Zhang Y, Ye L, *et al*. Evidence for a specific and critical role of mitogen-activated protein kinase 20 in uni-to-binucleate transition of microgametogenesis in tomato. New Phytologist. 2018;219(1):176-194.
20. Chevreau E, Brisset MN, Paulin JP, James DJ. Fire blight resistance and genetic trueness-to-type of four somaclonal variants from the apple cultivar Greensleeves. Euphytica. 1998;104(3):199-205.

21. Choudhary B, Nasiruddin KM, Gaur K. The status of commercialized Bt brinjal in Bangladesh. ISAAA Brief. 2014;47:1-14.
22. Curtolo M, Cristofani-Yaly M, Gazaffi R, Takita MA, Figueira A, Machado MA. QTL mapping for fruit quality in Citrus using DArTseq markers. BMC Genomics. 2017;18:1-16.
23. Dar AA, Mahajan R, Lay P, Sharma S. Genetic diversity and population structure of *Cucumis sativus* L. by using SSR markers. 3 Biotech. 2017;7(1):1-12.
24. Deng ZN, Gentile A, Domina F, Nicolosi E, Tribulato E. Selecting lemon protoplasts for insensitivity to *Phoma tracheiphila* toxin and regenerating tolerant plants. Journal of the American Society for Horticultural Science. 1995;120(6):902-905.
25. Dijkhuizen A, Kennard WC, Havey MJ, Staub JE. RFLP variation and genetic relationships in cultivated cucumber. Euphytica. 1996;90:79-87.
26. Divilov K, Barba P, Cadle-Davidson L, Reisch BI. Single and multiple phenotype QTL analyses of downy mildew resistance in interspecific grapevines. Theoretical and Applied Genetics. 2018;131:1133-1143.
27. Dugdale SJ, Mortimer AM, Isaac S, Collin HA. Disease response of carrot and carrot somaclones to *Alternaria dauci*. Plant Pathology. 2000;49(1):57-67.
28. Ereky K. Biotechnologie der Fleisch-, Fett-, und Milcherzeugung im landwirtschaftlichen Grossbetriebe: für naturwissenschaftlich gebildete Landwirte verfasst. Berlin, Germany: P. Parey; c1919.
29. Fatokun C. Designer (Cowpea) Plants; c2009. Accessed on 10/12/2010 from http://r4dreview.org/2009/03/designer-cowpea-plants/ 2009.
30. Ferdausi A, Nath UK, Das BL, Alam MS. *In vitro* regeneration system in brinjal (*Solanum melongena* L.) for stress tolerant somaclone selection. Journal of Bangladesh Agricultural University. 2009;7(2):155-160.
31. Galal OA, Galal HA, Aboulila AA. Genetic variability and molecular characterization of some local and imported mango cultivars in Egypt. Egyptian Journal of Genetics and Cytology. 2017;46(1):1-16.
32. Gambino G, Gribaudo I. Genetic transformation of fruit trees: current status and remaining challenges. Transgenic Research. 2012;21(6):1163-1181.
33. García V, Castro P, Die JV, Millán T, Gil J, Moreno R. QTL analysis of morpho-agronomic traits in garden asparagus (*Asparagus officinalis* L.). Horticulturae. 2022;9(1):41.
34. Giménez C, De Garcia E, De Enrech NX, Blanca I. Somaclonal variation in banana: cytogenetic and molecular characterization of the somaclonal variant CIEN BTA-03. *In vitro* Cellular & Developmental Biology - Plant. 2001;37:217-222.
35. Gomez-Lim MA, Litz RE. Genetic transformation of perennial tropical fruits. *In vitro* Cellular & Developmental Biology - Plant. 2004;40:442-449.
36. Gonsalves D, Gonsalves C, Ferreira S, Pitz K, Fitch M, Manshardt R, *et al*. Transgenic virus-resistant papaya: from hope to reality for controlling papaya ringspot virus in Hawaii. APSnet Features. 2004; https://doi.org/10.1094/APSnetFeature-2004-0704.
37. Hall HK, Skirvin RM, Braam WF. Germplasm release of 'Lincoln Logan', a tissue culture-derived genetic thornless 'Loganberry'. Fruit Varieties Journal. 1986;40:134-135.
38. Hammerschlag FA. Resistant responses of peach somaclone 122-1 to *Xanthomonas campestris* pv. *pruni* and to *Pseudomonas syringae* pv. *syringae*. HortScience. 2000;35:141-143.
39. Henny RJ, Chen J. Tropical foliage plant development: origin of new cultivars. ENH1092. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida; c2011; http://edis.ifas.ufl.edu.
40. Herison C, Sutjahjo SH, Sulastrini I, Rustikawati R, Marwiyah S. Genetic diversity analysis in 27 tomato accessions using morphological and molecular markers. AGRIVITA Journal of Agricultural Science. 2017;40(1):36-44.
41. Horáček J, Švábová L, Šarhanová P, Lebeda A. Variability for resistance to *Fusarium solani* culture filtrate and fusaric acid among somaclones in pea. Biologia Plantarum. 2013;57(1):133-138.
42. Hwang SC, Ko WH. Breeding for resistance to fusarial wilt of Cavendish banana by using tissue culture method. Special Publication Taichung District Agricultural Improvement Station. 1992;29:229-237.
43. Irfan M, Kumar P, Ahmad MF, Siddiqui MW. Biotechnological interventions in reducing losses of tropical fruits and vegetables. Current Opinion in Biotechnology. 2023;79:102850.
44. Ishino Y, Shinagawa H, Makino K, Amemura M, Nakata A. Nucleotide sequence of the *iap* gene, responsible for alkaline phosphatase isozyme conversion in *Escherichia coli*, and identification of the gene product. Journal of Bacteriology. 1987;169(12):5429-5433.
45. Ishiyaku MF. Domestication of Agricultural Biotechnology in Nigeria: Challenges and Opportunities; a paper presentation to the one-day sensitization workshop organized by the ARCN, IAR of the Ahmadu Bello University, Zaria. 2010;6:1-10.
46. Jain SM. Tissue culture-derived variation in crop improvement. Euphytica. 2001;118:153-166.
47. Jain M. Functional genomics of abiotic stress tolerance in plants: a CRISPR approach. Frontiers in Plant Science. 2015;6:375.
48. Jain SM. Micropropagation of selected somaclones of Begonia and Saintpaulia. Journal of Biosciences. 1997;22(5):585-592.
49. Rosati P, Exacoustos C, Puggioni GF, Arduini D, Mancuso S. Ridotta crescita fetale in rapporto alla localizzazione nel corno uterino: modello sperimentale nel coniglio. Minerva Ginecol. 1990;42:19-23.
50. Tang FF, Xing X. Will the growth of multi-channel retailing diminish the pricing efficiency of the web?. Journal of Retailing. 2001 Sep 1;77(3):319-333.
51. Morrison NA, Shine J, Fragonas JC, Verkest V, McMenemy ML, Eisman JA. 1, 25-dihydroxyvitamin D-responsive element and glucocorticoid repression in the osteocalcin gene. Science. 1989 Dec 1;246(4934):1158-1161.
52. Kumar V, Aksoy L, Donkers B, Venkatesan R, Wiesel T, Tillmanns S. Undervalued or overvalued customers:

Capturing total customer engagement value. Journal of service research. 2010 Aug;13(3):297-310.
53. Shen J, Zhang J, Luo X, Zhu W, Yu K, Chen K, *et al*. Predicting protein–protein interactions based only on sequences information. Proceedings of the National Academy of Sciences. 2007 Mar 13;104(11):4337-4341.
54. Minerva G, Kumar S. Micropropagation of gerbera (*Gerbera jamesonii* Bolus). Protocols for micropropagation of selected economically-important horticultural plants. 2013:305-316.
55. Kuksova VB, Piven NM, Gleba YY. Somaclonal variation and *in vitro* induced mutagenesis in grapevine. Plant cell, tissue and organ culture. 1997 Apr;49(1):17-27.
56. Patnaik BV, Narayana PA, Seetharamu KN. Numerical simulation of vortex shedding past a circular cylinder under the influence of buoyancy. International journal of heat and mass transfer. 1999 Sep 1;42(18):3495-3507.
57. Litz BT, Keane TM. Information processing in anxiety disorders: Application to the understanding of post-traumatic stress disorder. Clinical Psychology Review. 1989 Jan 1;9(2):243-257.
58. Nacheva E, Pevıcharova G, Petkova V, Samalıev H. Pavelsko–a new Bulgarian potato variety suitable for chips. Türk Tarım ve Doğa Bilimleri Dergisi. 2014;1(Özel Sayı-2):1554-1558.
59. Thieme R, Griess H. Somaclonal variation in tuber traits of potato. Potato Research. 2005 Jun;48:153-165.
60. Nassar NN, Hassan A, Pereira-Almao P. Metal oxide nanoparticles for asphaltene adsorption and oxidation. Energy & Fuels. 2011 Mar 17;25(3):1017-1023.
61. Zebrowska M. *Mathematical modelling of autophagy pathway* (Doctoral dissertation, National University of Ireland Maynooth); c2011.
62. Whitehouse H, Lanman JA. The ties that bind us: Ritual, fusion, and identification. Current Anthropology. 2014 Dec 1;55(6):674-95.
63. Olejnik A, Śliwiński G, Karczewski J, Ryl J, Siuzdak K, Grochowska K. Laser-assisted approach for improved performance of Au-Ti based glucose sensing electrodes. Applied Surface Science. 2021 Mar 30;543:148788.
64. Lu J, Plataniotis KN, Venetsanopoulos AN. Regularized discriminant analysis for the small sample size problem in face recognition. Pattern recognition letters. 2003 Dec 1;24(16):3079-87.
65. Waltz TJ, Powell BJ, Matthieu MM, Damschroder LJ, Chinman MJ, Smith JL, *et al*. Use of concept mapping to characterize relationships among implementation strategies and assess their feasibility and importance: results from the Expert Recommendations for Implementing Change (ERIC) study. Implementation Science. 2015 Dec;10:1-8.
66. Korobko V, Korobko A, Chernyaev A. Isoperimetric properties of the torsion rigidity of convex sections. Procedia Engineering. 2016 Jan 1;150:1648-1656.
67. Katsumoto Y, Fukuchi-Mizutani M, Fukui Y, Brugliera F, Holton TA, Karan M, *et al*. Engineering of the rose flavonoid biosynthetic pathway successfully generated blue-hued flowers accumulating delphinidin. Plant and cell physiology. 2007 Nov 1;48(11):1589-1600.
68. Sheehy RE, Kramer M, Hiatt WR. Reduction of polygalacturonase activity in tomato fruit by antisense RNA. Proceedings of the National Academy of Sciences. 1988 Dec;85(23):8805-8809.