Biotechnology in olericulture: Advancements and obstacles

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Biotechnology is the application of biological systems, organisms, or their derivatives to develop or modify products and processes for specific uses. It consists of a wide range of techniques, from the ancient use of yeast in bread making and fermentation to modern advancements in genetic engineering and synthetic biology. The history of biotechnology dates back thousands of years to the earliest forms of agriculture, where humans began selecting and breeding plants and animals for desired traits. Fermentation, a biotechnological process used to produce bread, beer, and wine, was practised as early as ancient Egypt and Mesopotamia.

The 19th and 20th centuries marked the classical era of biotechnology, characterized by a more scientific approach to techniques like selective breeding and fermentation. A significant milestone during this period was Louis Pasteur's work in the 1850s on microbial fermentation and pasteurization. However, the modern era of biotechnology truly began in the 1970s, following the discovery of the structure of DNA by James Watson and Francis Crick in 1953. The production of the first recombinant DNA molecule by Paul Berg in 1972, the creation of the first genetically modified organism (GMO), and the development of the first biotechnology-based pharmaceutical product, human insulin, in 1982, were pivotal advancements that have shaped the field as we know it today.

Despite its known applications, biotechnology has some fascinating lesser-known aspects. For instance, the fermentation of beer, dating back to around 7,000 BC in ancient China, is one of the oldest biotechnological practices. Biotechnology also began with the artificial selection of traits in crops and animals, such as the transformation of wild teosinte into modern corn about 9,000 years ago. In more recent times, NASA has explored the use of biotechnology for long-duration space missions, investigating genetically engineered bacteria to produce oxygen, fuel, and food in space environments. Moreover, the CRISPR Cas9 technology, a groundbreaking tool for genome editing first demonstrated in 2012, has ignited significant ethical debates, especially concerning its potential applications in human gene editing.

Biotechnology made a significant impact in the field of agriculture. Plant tissue culture, recombinant DNA technology, molecular markers, genome sequencing, and genome editing aim to solve problems associated with traditional plant breeding approaches, such as long-term trials, costly and less precise exercises, and the risk of tagging undesirable traits along with desirable ones. These methods also face challenges like inbreeding depression, ineffective phenotypic selection, and difficulties in gene transfer across species. In contrast, modern biotechnological techniques address these limitations by ensuring faster development, 100% successful gene transfer, and the preservation of beneficial traits without inbreeding inconveniences. They also enable precise trait selection, effective disease diagnosis, and gene transfer across species, making them more reliable and efficient than conventional methods.

Plant tissue culture and its bifurcations

Initially, plant biotechnology majorly revolved around Plant Tissue Culture wherein, the concept of 'totipotency' plays an important role. Totipotency is the capacity of a plant cell to regenerate into a whole plant.

Techniques such as micro-propagation, somatic hybridization, soma clonal variations, synthetic seed production, cryopreservation and genetic engineering stem from this very concept of plant tissue culture.

i. Micro propagation

Micropropagation is a set of techniques used in plant tissue culture to produce large number of genetically identical plants, offering several advantages over sexual reproduction. One widely used method is meristem culture, which involves cultivating the small, actively growing regions of a plant, typically the shoot tips. This technique is particularly effective in producing virus-free plants, as meristems are often free of viruses due to their rapid cell division. For example, meristem culture is used to produce healthy, disease-free potato plants, which is critical in ensuring high-quality crops. The variety of Kufri Jyoti in India was multiplied using meristem culture, ensuring that the plants are free from viral pathogens like Potato Virus Y (PVY) and Potato Leafroll Virus (PLRV). This method has significantly increased the yield and quality of potato crops.

Anther culture is another type of micropropagation that involves culturing the pollen grains of the flower to develop haploid plants. These haploids contain only one set of chromosomes, which can be doubled to produce homozygous

lines much faster than traditional breeding methods using chemicals such as colchicine. This has been particularly successful in crops like rice and barley, where it helps in rapidly fixing desirable traits, such as disease resistance or improved yield. The tomato variety VFNT Cherry has been improved using anther culture to increase its resistance to Fusarium wilt and nematodes.

Embryo rescue is a micropropagation technique used when embryos are at risk of abortion due to incompatibility or other stress factors. By isolating and culturing the embryo in a nutrient medium, it is possible to develop viable plants that would otherwise not survive. This technique is vital in crossing distantly related species or varieties, such as in the production of certain citrus hybrids, where embryos often abort before they can mature naturally. In Brassica species, the hybrids often undergo embryo abortion due to genetic incompatibility. Embryo rescue allows for the successful maturation of embryos, leading to the development of new varieties which exhibits improved stress tolerance and higher yield.

ii. Somaclonal variations

Somaclonal variation is a phenomenon where genetic variation arises in plants that are regenerated from somatic cells through subculturing. This variation occurs because the cells undergo various treatments and changes during the in vitro culture process, such as exposure to growth regulators, nutrient imbalances, or prolonged culture periods. These factors can cause mutations, chromosomal rearrangements, or epigenetic modifications, leading to differences in the regenerated plants compared to the original parent plant. These variations can be in terms of size, shape, colour, disease resistance or other important agronomic traits. These variations have also given rise to certain desirable traits in crop plants. In tomatoes, somaclonal variation has led to the development of plants with increased resistance to soil-borne diseases like *Fusarium* and *Verticillium* wilts. Similarly, in eggplant, somaclonal variants have been used to create lines with better fruit quality and increased resistance to pests such as the Colorado potato beetle.

iii. Somatic hybridization

Somatic hybridization is a technique used to overcome limitations in traditional plant breeding by combining the genomes of two different plant species or varieties at the somatic cell level rather than through sexual reproduction. This method is particularly useful when traditional breeding faces barriers such as species incompatibility, sterility, or long breeding cycles. It takes place as follows:

Cell Fusion: Somatic cells (non-reproductive cells) from two different plant species or varieties are cultured in vitro. All the cell wall constituents such as cellulose, lipids and pectin are eliminated with the use of digestive enzymes such as cellulase, lipases and pectinases which leave behind the naked cytoplasm known as protoplast. It is then treated with agents or conditions that promote cell fusion, such as polyethene glycol (PEG) or electrofusion.

Hybrid Formation: The fused protoplasts, called somatic hybrids, combine the genetic material from both parent cells. Hence, often this technique is referred as protoplast fusion. These hybrids can then be induced to regenerate into whole plants. These somatic hybrids contain cytoplasm and nucleus of both species in contrast to the sexual hybridization where the hybrids contain only female parent cytoplasm. Besides, in somatic hybridization, the chromosome number is not reduced thereby forming the polyploids.

Cybrid Formation: In some cases, the nucleus of one species and the cytoplasm of other species are fused. The plants thus arose are termed cybrids.

Selection and Regeneration: The resulting hybrid plants are screened for desirable traits after they regenerate into whole plants. These plants exhibit new combinations of traits from both parent species.

One of the parent cytoplasm is often stained to ensure that the resulting hybrid consists of cytoplasm from both parents.

Pomato is a unique example of somatic hybridization where tomato (*Solanum lycopersicum*) and potato (*Solanum tuberosum)* are combined into a single plant. This hybrid showcases the integration of tomato's fruiting ability with potato's tuber production. By fusing somatic cells from both species in vitro, plants that produce tomatoes above ground and potatoes below ground was developed, optimizing space and combining valuable traits from both crops.

iv. Synthetic seed production

Synthetic seed technology is essential for efficiently propagating plants with complex or limited natural reproduction methods. This technology involves encapsulating plant tissues, such as somatic embryos or meristems, in a synthetic medium to create seeds that are easy to handle, store, and plant. The encapsulated seeds containing the explant develops into a plant, exemplifying the practical use of the concept of totipotency.

For instance, bell pepper often faces issues with uniform seed germination and disease transmission through traditional seeds. Synthetic seed technology can mitigate these problems by encapsulating somatic embryos or shoot tips derived from tissue culture into synthetic seed forms. This approach enables the mass production of disease-free,

genetically uniform plants, ensuring better crop consistency and quality. They can be also used to overcome lesser seed multiplication ratios in crops like cassava, sweet potato and yams.

v. Cryo preservation

Cryopreservation is a technique that preserves biological materials at extremely low temperatures, typically using liquid nitrogen at its boiling point, -196°C to halt all biological activity and preserve cells in a suspended state. This method often involves the use of cryoprotectants, such as dimethyl sulfoxide (DMSO), glycerol, or sucrose, which protect cells from damage during the freezing and thawing processes. In the case of crops like cassava, pronounced dichogamy, specifically protogyny is observed where female flowers can mature before male flowers causing asynchrony for controlled pollination. Cryopreservation here is especially valuable to carry out sexual mode of fertilization. By freezing and storing pollen when male flowers are available, breeders can ensure that viable pollen is available when female flowers finally mature. This approach enables successful hybridization between desirable clones and enhances the success of breeding programs.

Genetic engineering

Genetic engineering is a transformative tool in biotechnology that allows precise alterations in an organism's DNA to achieve desired traits, providing solutions where conventional technologies might fall short. Techniques such as CRISPR-Cas9 enable targeted gene editing, allowing for the development of crops with improved yields, resistance to pests and diseases, or enhanced nutritional content. Unlike traditional breeding, which can take years to introduce these traits, genetic engineering significantly reduces the development time. It also offers unmatched precision, minimizing unintended changes to other genes and ensuring the desired traits are expressed. This precision is critical when addressing complex issues such as creating plants that can withstand climate change or combating specific agricultural challenges. Additionally, genetic engineering can introduce traits that are otherwise impossible to achieve through traditional methods, such as the production of biofortified crops that address nutritional deficiencies.

i. *Bacillus thuringinesis* (Bt) toxins

The discovery of Bt (*Bacillus thuringiensis*) toxin revolutionized pest management in agriculture by providing an effective and environmentally friendly alternative to chemical insecticides. Bt toxin, a natural insecticide produced by *Bacillus thuringiensis*, targets specific insect pests by disrupting their gut cells, leading to death. Through genetic engineering, scientists introduced the genes responsible for Bt toxin production into various crops, resulting in Bt crops like Bt cotton, Bt corn, and Bt brinjal (eggplant). These genetically modified plants express the Bt toxin continuously, offering season-long protection against pests like bollworms and borers.

Bt brinjal, developed to combat the fruit and shoot borer, was introduced in India as a potential solution to reduce pesticide use and increase yields. However, its commercialization has faced significant controversy and regulatory hurdles. Concerns in India include potential impacts on non-target organisms, the development of pest resistance, and the socioeconomic effects on smallholder farmers. Despite these challenges, Bt brinjal has been cultivated successfully in Bangladesh, where it has shown positive results in reducing pesticide use and improving farmers' income.

ii. Engineering for Parthenocarpy

Genetic engineering for parthenocarpy enables the development of seedless fruits by manipulating hormonal pathways critical for fruit development. The process involves introducing specific genes that regulate auxin production, a hormone essential for fruit development. A key gene used in this approach is *iaaM*, which encodes the enzyme indoleacetamide mono-oxygenase. This enzyme catalyzes the conversion of tryptophan into indoleacetamide, an auxin precursor. Once indoleacetamide is formed, it is further converted into active auxin (indole-3-acetic acid, IAA), which triggers fruit development in the absence of fertilization. By incorporating the *iaaM* gene into plants such as brinjal, parthenocarpy can be induced, resulting in seedless fruit production. For instance, in genetically engineered tomatoes, the *iaaM* gene-driven increase in auxin levels within the ovary leads to the development of seedless fruits without pollination. This approach not only ensures fruit set in challenging growing conditions but also produces high-quality, consistent, and marketable seedless produce.

iii. Increased shelf-life

Antisense RNA technology is a sophisticated genetic tool that regulates gene expression by silencing specific mRNA molecules, effectively preventing them from being translated into proteins. This method involves the introduction of an antisense RNA sequence that is complementary to the target mRNA, forming a double-stranded RNA complex that blocks translation. The technology has been utilized in various applications, including the development of the Flavr Savr tomato. In this case, antisense RNA was used to suppress the expression of the polygalacturonase gene, which is responsible for fruit softening. By inhibiting this gene, the tomatoes had an extended shelf life and retained their firmness for longer

periods, making them more suitable for transport and storage.

iv. Ethylene modification

Modification of ethylene production in plants is a critical area of research in agricultural biotechnology, particularly for controlling fruit ripening and stress responses. Ethylene is synthesized from methionine through a series of enzymatic steps. Methionine is first converted into S-adenosyl-methionine (SAM), which is then converted into 1-amino-cyclopropane-1-carboxylic acid (ACC) by the enzyme ACC synthase. Finally, ACC is converted into ethylene by ACC oxidase. However, genetic modifications can alter this pathway to reduce ethylene production, thereby extending shelf life and delaying ripening. One approach involves the introduction of the enzyme SAMase, which diverts SAM away from ethylene synthesis by converting it into methylthio-adenosine and homoserine, thus reducing the substrate availability for ACC production. Another modification includes the introduction of ACC deaminase, an enzyme that converts ACC into α-ketobutyrate and ammonia, further preventing the formation of ethylene.

v. Male sterility

Male sterility (MS) is a crucial trait in hybrid seed production, particularly in vegetable crops, as it eliminates the need for labour-intensive manual emasculation, where male flower parts are removed to prevent self-pollination. Male sterility (MS) can be transferred into non-MS plants using recombinant DNA (rDNA) technology, which involves the precise insertion of male sterility genes into the genomes of target plants. This process typically starts with identifying and isolating male sterility genes, such as *barnase* from *Bacillus amyloliquefaciens*. These genes are then cloned into a plant transformation vector, which is introduced into the non-MS plants using methods like Agrobacterium-mediated transformation or gene gun. The *barnase* gene encodes a ribonuclease enzyme that specifically degrades the tapetum, a tissue essential for pollen development, thereby causing male sterility. To control this sterility, the barstar gene is introduced, which produces an inhibitor that binds to barnase, neutralizing its activity in non-targeted tissues, thus preventing unintended cell damage. Additionally, the bar gene, which provides resistance to the herbicide phosphinothricin, is linked to the male sterility construct. This allows for the selective survival of MS plants. After spraying with phosphinothricin, only the MS plants expressing the *bar* gene survive, ensuring that the sterile plants can be easily identified and maintained in breeding programs. This approach enables the development of new hybrids by crossing these male-sterile plants with compatible fertile lines, eliminating the need for manual emasculation and ensuring high efficiency in seed production.

vi. Starch enhancement

Enhancing starch content in crops like potatoes can significantly boost their value for food and industrial uses. To achieve this *GBSS* (Granule-Bound Starch Synthase) is a key gene that helps produce amylose, a type of starch. By enhancing the activity of GBSS, potatoes can produce more amylose and, therefore, more starch. At the same time, reducing starch breakdown is crucial. This is done using antisense technology, which involves introducing an antisense promoter and an antisense alpha-amylase gene. Alpha-amylase is an enzyme that breaks down starch into sugars. The antisense alpha-amylase gene inhibits the production of this enzyme, slowing down starch breakdown and allowing more starch to accumulate in the potato tubers. By combining these methods of boosting GBSS activity and reducing alpha-amylase activity potatoes with higher starch content can be produced, improving their utility for various applications.

vii. Nutrient enhancement

Grain Amaranthus (*Amaranthus hypochondriacus*) is notable for its high protein content, which is significantly richer in essential amino acids compared to other grains. Specifically, the protein in *A. hypochondriacus* contains 2 to 4 times more essential amino acids, such as methionine, lysine, leucine, and threonine, than those found in conventional grains. This enhancement in protein quality makes it a valuable nutritional resource. To harness these benefits, the gene responsible for high-quality protein, known as AmA1 (Amaranthus Albumin1) from amaranthus was transferred into potato plants. This genetic modification aims to enhance the nutritional profile of vegetables and fruits by increasing their content of essential amino acids, thereby improving their dietary value.

Genome editing

Genome editing is a cutting-edge technology that allows precise alterations to the DNA of an organism, enabling the addition, removal, or modification of specific genes. The principle of genome editing relies on the use of engineered nucleases, which are molecular scissors that make targeted cuts in the DNA at specific locations. The most widely used genome editing technology is CRISPR-Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats associated protein 9). This system uses a guide RNA (gRNA) to direct the Cas9 nuclease to a specific DNA sequence, where it creates a double-strand break. The cell's natural repair mechanisms then fix this break, allowing for the precise insertion, deletion, or modification of genetic material.

One significant example of genome editing in vegetables is the enhancement of disease resistance in tomatoes. Researchers have used CRISPR-Cas9 to edit the SlMlo1 gene in tomatoes, which is responsible for susceptibility to powdery mildew, a common fungal disease. By knocking out this gene, the edited tomatoes exhibit increased resistance to the disease without the need for chemical treatments. Another example is the modification of the ALS (acetolactate synthase) gene in lettuce to confer resistance to herbicides like sulfonylureas. Through genome editing, the ALS gene was altered to create a version of the enzyme that is no longer inhibited by the herbicide, allowing the lettuce plants to survive herbicide application while weeds are controlled.

Genetic markers

Genetic markers are specific DNA sequences within a genome that can be used to identify particular genes or genetic variations associated with specific traits. The principle behind genetic markers lies in their ability to co-segregate with a trait of interest, allowing researchers to predict the presence of a gene or trait based on the presence of the marker. There are different types of genetic markers, including simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and amplified fragment length polymorphisms (AFLPs). These markers are identified through various molecular techniques, such as polymerase chain reaction (PCR) or sequencing, which amplify and detect the specific DNA sequences associated with the marker.

The development of disease resistant tomato varieties using markers linked to the Pto gene, which confers resistance to bacterial speck disease caused by Pseudomonas syringae have been widely used in breeding programs. By using markers closely associated with the Pto gene, breeders can efficiently select tomato plants that carry this resistance, even in the absence of the pathogen, greatly speeding up the breeding process and improving accuracy.

SNP markers in the breeding of cucumbers for powdery mildew resistance have also gained fruition by identifying and utilizing markers associated with resistance genes, such as pm1 and pm2 to develop cucumber varieties that are more resilient to diseases, improving crop yields and reducing the need for chemical treatments.

Flipside

Biotechnology, despite its vast potential, has several significant drawbacks that complicate its widespread adoption. One of the primary challenges is the high cost involved in research, including expensive equipment and laboratory facilities, which limits access to this technology. Additionally, the stability of transgene expression is not always thoroughly assessed, leading to concerns about the long-term effectiveness and safety of genetically modified organisms.

The requirement for tissue-specific promoters, such as those needed for male sterility (MS), parthenocarpy, and ripening, adds another layer of complexity. These promoters must be carefully designed to ensure that gene expression occurs only in the desired tissues, which is both challenging and costly. Controlled gene expression technologies, like controlled promoter technology, are essential to regulate when and where a gene is expressed but these technologies are still under development and not always reliable.

Ecological disturbances are another major concern, as GMOs can potentially disrupt local ecosystems. For example, undesirable gene flow from GMOs to wild relatives can lead to the creation of ‘super weeds’ that are resistant to herbicides such as Phalaris minor in wheat and *Echinochloa* in paddy which can cross with the genetically modified herbicide-resistant crop plants in the field to impart the quality of herbicide resistance thus, making it difficult to manage them. Here, the introduction of GMOs inadvertently affects non-target species, leading to a reduction in biodiversity.

Terminator gene technology, also known as Genetic Use Restriction Technology (GURT), is designed to prevent the reuse of genetically modified (GM) seeds by ensuring they produce sterile offspring. This system involves a series of genetic components that stay muted when the seeds are initially treated with tetracycline before they are sown which ensures their fertility. The seeds produced from these modified plants in the subsequent generation lack the toxin-inhibitory quality that can only be activated through the use of tetracycline. This leads to a series of genetic proceedings that activate toxins that interrupt the function of the *LEA* (Late Embryogenesis Abundant) gene which leads to the death of the seed. This technology ensures that GM seeds cannot be replanted, forcing farmers to purchase new seeds each season, raising ethical and economic concerns, particularly for small-scale farmers in developing countries.

Traitor gene technologies rely on the application of proprietary chemicals that are not disclosed to the public or regulatory bodies. These chemicals are designed to trigger the expression of inserted genes or to control the functionality of the genetically engineered traits, such as enhancing resistance to pests or improving growth characteristics. The lack of transparency regarding these chemicals raises significant concerns about safety and environmental impact, as the long-term effects of such substances on ecosystems and human health

remain unknown. Additionally, the secrecy undermines the ability of independent researchers and regulatory agencies to thoroughly assess the risks associated with these technologies, potentially leading to unforeseen ecological and health issues.

Finally, consumer acceptance remains a significant hurdle, as seen in the case of Bt brinjal in India, where public skepticism and regulatory concerns have delayed its commercialization despite potential benefits.

Discussion

Biotechnology has emerged as a pivotal force for social and economic development by offering innovative solutions that address some of the most pressing global challenges. One of the most significant contributions of biotechnology is in the field of agriculture, where techniques such as genetic engineering, CRISPR-Cas9, and micropropagation have revolutionized crop production. For example, genetically modified Bt crops have drastically reduced the reliance on chemical pesticides, leading to higher yields, improved farmers' income and reduced environmental impact. In addition, micropropagation techniques like meristem culture have enabled the production of disease-free plants, enhancing food security. Furthermore, biotechnology is crucial in addressing nutritional deficiencies, as seen with the development of biofortified crops that offer enhanced nutritional content. Through these advancements, biotechnology not only supports sustainable agricultural practices but also drives economic growth by creating new markets, improving productivity, and enhancing the quality of life, particularly in developing regions.

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