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# Biotechnology in agriculture: A modern tool for crop improvement

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**Abstract**
Plant breeding focuses on improving the genetics of plants through hybridization, screening, and the selection of advanced lines. Traditional techniques provide advanced cultivars with desired features, but they take longer to obtain (6 to 12 years). Biotechnology technologies boost breeding procedures by shortening the time it takes to develop better cultivars. Tissue culture, transgenic techniques, and molecular breeding procedures, in addition to traditional methods, can be used to advance varietals. The use of genetic markers to identify traits of interest is the most common biotechnology technique for crop development. Using current biotechnology advances, variety with enhanced abiotic and biotic stress tolerance may be created in far less time and with greater precision. Recent biotechnology techniques can help produce varieties with enhanced abiotic and biotic stress tolerance in less time and with greater precision. Several advanced methods, like nanotechnology and bioinformatics tools, are being used for this purpose, ushering in a new era of genomics-assisted molecular breeding. Biotechnological methods in agriculture are becoming more efficient and productive thanks to advances in next-generation sequencing and high-throughput genotyping. The current study focuses on a broad overview of biotechnological methods in use, attempting to cover every area of biotechnology in crop improvement.

**Keywords:** Biotechnology, crop improvement, plant tissue culture, molecular breeding, transgenics

## Introduction
For almost a century, plant breeding has been a key factor in enhancing agricultural productivity. Desirable traits like disease tolerance, greater yield, and abiotic stress tolerance have all been inculcated in a crop genotype. Crop development is based on the characteristics of novelty, stability, uniformity, and usefulness, which a breeder can achieve by combining traditional breeding with biotechnology tools. Plant biotechnology is used to augment breeding for crop development. Thus, by combining plant breeding with biotechnology, the increasingly complex and time-consuming breeding technique may be easily handled. To optimize the chances of success, continuous varietal development through traditional breeding requires biotechnology. Genetic engineering and Tissue culture are the two main biotechnology technologies for crop enhancement. Biotechnology is more than genetic engineering when it comes to plant breeding since it addresses issues in all aspects of agricultural production and processing. This involves increasing and stabilizing yields, improving pest, disease, and abiotic stress tolerance, such as cold and drought, and improving the nutritional value of crops such as protein in pulses, among other things. Transgenic techniques, Plant tissue culture, and molecular breeding techniques are the three key areas of biotechnology in crop research. Plant tissue culture is the process of growing plant cells or tissues in synthetic media. It can be used for embryo rescue, micropropagation, haploid generation, protoplast culture, and Protoplast fusion. Another important use of biotechnology is the transfer of genes from one creature to another, which can be accomplished either directly (through physical or biochemical transfer) or indirectly (by genetic engineering) through Agrobacterium-mediated gene transfer. The molecular breeding approach, in which DNA markers are utilized to improve variety through marker-assisted selection, is the most popular and widely used strategy for crop improvement. Agriculture biotechnology features may aid in the development of better varieties in response to changing climate conditions [1, 2]. For the creation of abiotic and biotic stress-resistant varieties [3]. biotechnology mainly stresses how the use of molecular-level plant breeding is currently assisting in the discovery of new genes and associated roles, which might lead to new directions in fundamental plant biology study [4]. For boosting the rate of crop improvement, biotechnology focuses on integrating speed breeding with other current crop breeding methods, such as high-throughput genotyping, genome editing, and genomic

selection [5]. Genomic selection (GS) was highlighted, with the claim that it allows the identification of superior variety in less time, thereby speeding up the breeding cycle. Crop improvement using biotechnological technologies, such as high throughput genotyping and phenotyping, genomics aided breeding, and genome editing, maybe done more quickly [6]. Agricultural biotechnology has the potential to be the most powerful and useful to the poorest people. Several value-added starch-based bioplastics and starch-derived biofuels are made from cereal starch, and they are likely to become less environmentally harmful than any of those made from petrochemicals. As a result, the purpose of this post is to look at the many attempts made by biotechnology in the sphere of agriculture to satisfy its rising and diversified end-uses [10].

**Crop improvement scenario at a global level**

Development of gene-based markers, biofortification, nanotechnology, the use of tissue culture, molecular markers, and genetic engineering are some of the diverse uses of biotechnology in agriculture, particularly in crops. These instruments would aid in meeting the world's escalating food demands, which are expected to exceed 9 billion people by 2050 **[7].** Research and developmental (R&D) operations in genetics began in the 1960s, but the widespread practical use of transgenic crops did not begin until the 1980s, following the success of tobacco trials. crops like cotton, canola, maize, soybean, papaya, sugar beet, and pumpkin rendering with features like tolerance to the herbicide, and insect and virus resistance were later created and marketed. More than 50 more species of transgenic vegetables, fruits, and field crops were projected to be under development in laboratories and limited facilities in 2004 with a long-term objective of potential commercialization. There are anticipated to be more than 120 distinct transgenic activities in biotech crops throughout the world, a four-fold increase over the number of transgenic events now identified in commercially farmed genetically modified crops. India is the world's second-largest producer of food grains, with a wide range of cereals and pulses used mostly in the country. Food grain output in 2016-17 is expected to be 273.38 million tons, according to preliminary projections. According to the International Service for the Acquisition of Agri-Biotech Applications (ISAAA), India is ranked fourth in the world in terms of the area planted with genetically modified crops. Many nations have permitted field experiments for 21 Genetically Modified food crops, including Genetically Modified vegetables and grains, but commercial GMO food production has yet to be approved [8]. When there is little or no genetic diversity in nutrient content among plant types, the transgenic technique can be a viable option for developing biofortified crops (nutritionally improved food crops) [9]. Genes from many sources have targeted micronutrients, minerals, vitamins, certain necessary amino acids, and beneficial fatty acids to improve the nutritional status of food crops. soybean (greater unsaturated fatty acid), Maize (high lysine), cassava (Iron-rich and high provitamin A), and Golden rice (vitamin A) are some of the successful transgenic food crops. There have also been reports of biofortified cereal, fruits, vegetables, legumes, oilseeds, and fodder crops. Molecular breeding methods are the most effective for improving agricultural plant biotic and abiotic stress adaptability, and recent breakthroughs in high throughput phenotyping, sequencing, and genotyping platforms (phenomics) have changed molecular breeding into genomics assisted breeding (GAB). Marker-assisted selection and genomic selection are the most often employed methodologies for genomics-assisted breeding (GS). Marker aided backcrossing, gene pyramiding, mapping for linked targeted phenotypes by particular genes or QTLs, precise mapping of QTL region, and other techniques are all part of MAS. Genomic Selection, on the other side, predicts breeding value based on all available markers data for a population. Traditional approaches for developing superior inbred lines for commercial crop production are time-consuming and costly.

**Different approaches to crop improvement by various biotechnological tools**

**A. Tissue culture technique**

Tissue culture is the aseptic cultivation of cells, tissues, organs, or complete plants under regulated nutritional and environmental circumstances [11]. The very first reports on tissue culture came from the early twentieth century, while [12] undertook experiments to sustain mesophyll cell cultures based on postulates establishing the "totipotency of plant cells.". Plant tissue culture is the *in vitro* growing of live plant cells, tissues, or organs (seedlings, embryos, cells, protoplasts) on a nutritional medium in an aseptic environment. Plant tissue culture techniques include micropropagation, somatic embryogenesis, somaclonal variation, meristem culture, pollen culture, embryo culture, protoplast fusion, cryopreservation, and secondary metabolite production, depending on the plant part used as an explant (part of the plant used for regeneration). Tissue culture techniques have been developed based on two features of plant tissue: cell plasticity [11] and cell totipotency [13]. Cell totipotency is the genetically preserved ability of all live cells to generate a new genetically identical cell and, through cell divisions and differentiation processes, to create tissues, organs, systems, and full persons [14]. Cellular plasticity is the ability of plant and animal cells to multiply, divide, differentiate, and the production of a new individual that distinguishes them.

**i) Somaclonal variation**

*In vitro* cell and tissue culture is a powerful method for inducing somaclonal diversity since tissues may be cultivated with little development or individual cells can be cultured **[15].** This makes it simpler to accurately dose the mutagenic agents, pinpoint the mutagenesis activity in tissues with a higher potential for mutation, extend the exposure to mutagens, and reproduce plants with high effectiveness **[16].** The disadvantage is that some tissues mutate more easily when merely subjected to crop production regulators or other "regular" special additives [17]. It is important to evaluate the genetic stability of the plants either during tissue culture to determine whether variation induced by tissue cultures can be used to acquire new genotypes and whether this variation can be detrimental to maintaining the genetic accurateness of the *in vitro* proliferated material. Cytogenetic, biochemical, and molecular approaches can be used to determine the production of somaclonal variation after tissue culture [18, 19]. meristematic development, the genetic history of the cell (ploidy level of genotype), the formation of the growth media, the intensity of growth regulators, the category of explant sources, and DNA methylation pattern have all been found to cause somaclonal variation when during vitro culture [20, 21, 22]. As a result, genetic stability *in vitro* relies on the taxa and

procedures utilized.

**ii) Embryo rescue**

The embryo rescue procedure is used in breeding programs and genetic selection for people who have an early abortion after fertilization [23]. It is also effective for saving species that have lost the ability to reproduce sexually owing to biotic or abiotic conditions that inhibit germinating seeds [24]. Plants that grew through genetic crosses that do not yield viable seeds are recovered using *in vitro* propagation of matured and/or immature embryos [25]. For example, given that the approach is particularly efficient in a wide variety of plant species, such as cereals [26, 27]. this tissue culture technology has significant use in breeding programs.

**iii) Micropropagation**

Micropropagation is the bulk creation of clonal progeny in the laboratory from extremely small parts of the plant (0.2-10 mm), accompanied by their implantation in the soil in greenhouse conditions. And over 500 million plants of various types are currently grown annually using micropropagation across the world. Planting materials such as bananas, strawberries, citrus, and timber trees such as Dalbergia may undoubtedly boost the yield potential in terms of vegetatively propagated plants. Micro propagated plants were true to type, free of disease, high quality, and super-elite seed production material. This technology has enormous promise for making the environment cleaner and greener.

**B. Transgenics**

Transgenic plants are those whose DNA has been altered using genetically engineered procedures. The goal is to impart to the plant a new characteristic that does not exist in nature in the species. A transgenic plant has a genetic trait that has been added intentionally. The transgene is then introduced gene sequence; it might be from a similar plant or a completely other species. The goal of putting a gene combination into a plant is to make it as helpful and productive as feasible. This technique offers benefits such as increased shelf life, greater yield, higher quality, insect resistance, heat, cold, and drought tolerance, and resilience to a range of abiotic or biotic challenges. The "Gene Gun" approach, also known as the "Micro-Projectile Bombardment" or "Biolistic" method, is most widely utilized in maize and rice species. In this procedure, DNA is bonded to tiny particles of tungsten or gold, which are then fired into plant cells or specific plant cells using a cannon at high pressure. The accelerated particles penetrate the cell wall as well as the membranes. Inside the nucleus, the DNA splits from the plated metal and integrates into the plant genome. This approach has been used effectively for many crops, particularly monocots such as wheat and maize, for which Agrobacterium transformation has become less successful. This method is both clean and safe. The main downside of this procedure is that major injury can occur [28]. It employs soil-dwelling microorganisms called Agrobacterium tumefaciens. It is capable of infecting plant tissue with a portion of its DNA. A tumour-inducing plasmid integrates a fragment of DNA that infects a plant into a plant chromosomal (Ti plasmid). The Ti plasmid can control the plant's cellular machinery and utilize it to replicate its own bacterial DNA several times. Ti plasmids are huge circular DNA particles that reproduce independently of bacterial chromosomes. 3 This plasmid is significant because it has t DNA areas where a researcher may introduce a gene that can be transmitted to a plant cell via a process called the "floral dip." [29]. Arabidopsis thaliana and tobacco are the most genetically modified plants in the study because of well-developed transformation procedures, simple propagation, and well-studied genomes. They are used as models for other plant species. Transgenes have also been employed for polluted soil bioremediation. Transgenic plants harbouring bacterial enzyme genes have been used to remove mercury, selenium, and organic pollutants such as polychlorinated biphenyls from soils [30]

**C. Molecular Breeding Approaches**

Because of the combined effect of technical, economic, and societal considerations, crop species and crop improvement institutes have adopted molecular plant breeding methodologies at varying speeds. Early scientific challenges were cereal crop species' resistance to Agrobacterium-mediated transforming and a lack of understanding regarding genetic regulation of features that had already been identified as essential breeding objectives. Plant transformation of practically all key agricultural and horticultural species has been greatly aided by ongoing study and technological development [31]. Similarly, genomic research in species of plants and other creatures has yielded a wealth of knowledge on gene structure and function, as well as a significant number of DNA markers for use in plant genetics. Regardless of these assets, genetic specific of strong QTLs remains elusive unless breeding programs and accompanying information management are reformed to completely incorporate knowledge about pedigrees, morphologies, and marker genotypes that can be used to maximize responsiveness to selection [32, 33]. Even with such integration, modifying regulatory function remains a scientific problem for molecular breeding since determining the sequence foundation for regulatory alterations and predicting their phenotypic impacts is challenging [34]. Even though molecular breeding now is considered an essential component of large companies' current crop improvement efforts for major crops, the broad utilization of modern molecular methods to plant breeding remains a topic of contention among some practicing plant breeders in the government sector, especially for minor crops [35, 36]. There are at minimum three additional reasons supporting this viewpoint, in addition to the real scientific and economic considerations that have delayed or impeded the use of molecular techniques in attaining specific plant breeding objectives. To begin, molecular plant breeding needs knowledge and experience in molecular biology as well as plant breeding. Educational attempts to provide such multidisciplinary training were developed in the early 1990s, but are currently restricted to a very small set of academic institutes with historic strengths in plant genetics [37, 38, 39]. A second factor for some plant breeders' reluctance to adopt biotechnology is issues with transgenic crop acceptance among some governments and consumer groups, as demonstrated by the recall of wheat cultivars with glyphosate herbicide-resistant transgenes [40]. Finally, enthusiasm for the promise of molecular plant breeding prompted changes in financing at public institutions to improve intellectual services and capability for molecular genetics and genomics research, which, unfortunately, frequently came at the expense of plant breeding [41]. This emphasis may have been necessary temporarily to lay the groundwork for 21st-century plant biology, but there is now growing research linking molecular methods with breeders' objectives required to fully realize the

potential of recent advances in science and technology and genomics [39].

**Applications**

**i) Disease-free plants**

Disease-free plants are a highly practical use of biotechnology, and they may be created via the micropropagation method. Bananas are one example of such plants. Bananas are often farmed in places where they are a significant source of income/employment and/or food. Micropropagation is a method of regenerating disease-free banana plantlets from the tissues of healthy banana plants. It has all of the advantages of being a novel approach that is reasonably affordable and simple to apply [42].

**ii) Fortification of crops**

In underdeveloped nations or countries where food is scarce, fortified crops emerge as a good food source that is fortified with nutrients for growing malnourished children. 'Potato' is one such example of a fortified crop. This genetically engineered potato is commonly farmed and utilized in India, and it has roughly one-third one more protein than a regular potato. Furthermore, this genetically engineered potato has high amounts of all necessary amino acids, including lysine and methionine. This 'Potato' has the potential to be a very valuable food source in nations where potatoes are a key staple meal [47]. Golden rice is another example of such a crop. This genetically engineered rice has a greater beta-carotene concentration Cowpea grains and leaves are said to be eaten as side or relish dishes. Cowpeas are eaten as a staple cuisine in many nations. In Tanzania, genetically modified cowpea types have been cultivated [43, 44].

**iii) Pest resistant crops**

Pest assault is a fairly prevalent problem in a variety of different plants all over the world, these crops may include feed plants or other crops grown for food. Bt-Cotton is one example of such a crop. Bacillus thuringiensis (Bt) genes are placed in cotton crops to promote the synthesis of certain proteins. A variety of insects are very poisonous to the protein. With the use of biotechnology, the created Bt-cotton results in reduced insect assault, resulting in much-increased production [45].

**iv) drought-resistant crops**

Targeted and short gun approaches are two distinct but important strategies in genetic engineering. These strategies are used to create transgenic plants that can confer drought tolerance [46].

**Future Prospects**

Organismal genome sequences are critical for understanding the actions of different genes and determining evolutionary links. The discovery of genetic and molecular techniques underlying agronomic features will aid in the breeding process, resulting in superior varieties with higher quality and yield, tolerance to adverse environmental circumstances, and disease resistance. DNA sequencing is a functional test, and as it becomes quicker and less expensive, there will be an increasing number of applicants and uses for that in plant breeding. Our capacity to examine differences in complete genomes of species in a particular timeframe and at a far lower cost. Crop genome sequencing gives vital information on genomic structure and organization. It provides a plethora of chances for life science study, including biological evolution, developmental biology, biochemistry, genetics, and molecular biology. Agriculture science has been in the midst of a third technological revolution in Genotyping in recent years. Although traditional breeding techniques have raised agricultural output and yield greatly, new ways are necessary to further enhance crop productivity to fulfil the world's expanding food demand. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)/Cas9 (CRISPR-associated Protein9) gene-editing method has shown considerable promise in terms of rapidly modifying genomes. The genome-editing method (CRISPR)/Cas9 (CRISPR-associated protein9) has shown significant potential for swiftly resolving growing difficulties in agriculture. highlighted the promise of CRISPR/Cas9 for improving crop resistance against new pests and abiotic challenges in tropical regions. It is capable of precisely altering the genomic sequence of any creature, including plants, to obtain the desired characteristic. Several techniques, including optimising promoter to activate and express Cas9 and utilizing alternative fluorescence reporters and selection markers, have recently been investigated to optimise plant transformation by CRISPR/Cas9. The CRISPR/Cas gene-editing method may create heritably, targeted alterations while simultaneously addressing concerns about the entry of foreign DNA sequences by producing transgene-free plants. Rice is the most researched crop, followed by maize, tomato, potato, barley, and wheat. The continuous progress of biotechnology technologies will undoubtedly aid in increasing agricultural yield while ensuring sustainability.

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