



# Crop Breeding Technology: Recent Technological Advancements

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## ABSTRACT

Plant breeding is one of the oldest agricultural operations, dating back to dawn. Fruits, vegetables, decorative flowers, shrubs, trees, legumes, cereals, and pulses are just a few of the horticultural and agronomical crops that have been domesticated to meet human dietary and aesthetic needs. Crop varieties emerged as a result of selection in the early stages of human civilization. The extremely lengthy crop duration limits the creation of superior varieties in plant breeding given the several stages of crossing, selection, and testing that go into developing novel plant types. Most traditional and current crop improvement approaches rely on natural or experimentally induced genetic differences and need time-consuming characterization of progenies from numerous generations resulting from time-consuming genetic crossings. Since the discovery that nucleases from the bacterial CRISPR (clustered regularly interspaced palindromic repeat)-associated (Cas) system may be employed as easily programmable tools for genome editing, several aspects of plant biology have been dramatically changed. The present state of their utilization for crop breeding to add attractive new agronomical features into specific cultivars of diverse crop plants is examined in this study.

**KEYWORDS:** Crop breeding, Site-Specific Recombination (SSR), Zinc Finger Nucleases (ZFNs), Transcription Activations Like Effectors Nucleases (TALENs), CRISPR/Cas9

## 1. INTRODUCTION

The population of the world is increasing tremendously day by day. With this, demand for food is also increasing but the area of cultivable land and other agricultural resources is decreasing. So, to complete the increased demands for food and raw materials, we would require highly productive plant varieties. Modern high-yielding varieties are usually bred for high production.[1] Plant breeding is the science of changing the phenotypes of plants. Cereals are the most

important group of cultivated plants for food production. Cereals including, rice, wheat, maize, barley, millets, sorghum, and oats are also main staple foods in many parts of the world.[2] Due to high rise in population, the demand for food has been predicted to increase by 87% till 2050. The production of traditional agricultural yields, is highly dependent on climatic and weather conditions.[3] The population in India is also growing at an alarming rate of around 1-2% per year. This makes food-grain production to raise at the same or

even a higher rate an essential necessity to improve nutritional status of the masses.[4] In present times, the agricultural produce is high due to inputs like fertilizer, irrigation water, plant protection, cultural practices, and improved crop varieties. New age management practices are yet to be fully exploited along with the vast tracts of cultivated lands to be managed efficiently.[5] After the application of biotechnological practices, the yield of crop plants increase by 1 - 3% per year in addition to progression of plant yields per unit area.[6]

Before 10,000 BCE humans start farming and day after day farming was improved by artificial plant selection. The selection and breeding of plants were done on basis of higher nutritional values.[7] A crop's performance (e.g., yield) depends on its genotypes. The genotyping in many plants is observed usually aggregated to obtain single phenotypes.[8] The interaction between physical characteristics and genotype to an environment and crop management is known as phenotype. So genetic characteristics of an organism (plant) are set with the help of phenomics.[9] The observation of phenotypic variation depends upon shape and size of the genome, environment, and their interactions. With the help of genome-wide association studies and high throughput phenotyping facilities, phenomics may be an ideal tool for studies of plant genetics and breeding.[10]

Nowadays, scientists and researchers apply different techniques for improving crop yield, disease, insect, stress and salt resistance. A researcher trying to increase the amount of crop production by inbreeding, backcrossing or introgression breeding, hybrid breeding, mutation breeding, molecular marker-assisted selection, genetic engineering, gene editing and many more ways. Depending on the species, some plants may be fertilized by themselves. This is done to produce an inbred variety, which is same generation after generation. Because it preserves the original traits, it is useful in three ways: for research; as new, true-breeding cultivars; and as the par. [11] Hybrid breeding requires more time and resources than inbred breeding coz hybrid crop varieties vastly inbreed progenitors in economically important crop species. With the help of genomic selection, the quality of crops is improved in hybrid breeding. [12]

Aside from natural phenomena such as formation of the new genes caused by cosmic rays and radiations of the Sun, mutations can also be induced by certain chemical

agents. Through the use of chemical or physical agents, genetic modification can be performed to improve crop species. This technique is referred to as mutation breeding. [13] Concerning certain other affluent countries, India's major crop yields are minimal. For comparison purposes, India's rice yield is 2.6 tonnes per hectare, compared to 4.7 tonnes in China, 3.7 tonnes in Brazil, 5.9 tonnes in United States of America (USA), or 9.5 tonnes in Australia; wheat production is 3.0 tonnes per hectare in India, 5.3 tonnes in China, and 3.1 tonnes in United States of America; maize and soybean yields are 2.5 and 0.75 tonnes per hectare in India, compared to 5.9 and 1.8 tonnes in China respectively. Farmers and other key stakeholders in India are no strangers to cutting-edge technologies for achieving better and more sustainable agricultural growth. [14]

## 2. NEW-AGE BREEDING PRACTICES (GENOME EDITING)

Now-a-days best way to improve crop production is with genome editing techniques. These genome editing practices allow change in gene expression patterns to get new insights into an organism's functional genomics. Site-specific recombinase or site-specific nuclease systems might be used to modify the genome. Confirmatory genotyping methodologies, off-target site analyses, and modification expression chances are all important considerations for gene editing. [15]

### A. Genome Editing Mediated by Site-Specific Recombinase (SSRs)

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### B. Genome Editing Mediated by Site-Specific Nucleases (SSNs)

ZFNs, TALENs, and CRISPR/Cas9—programmable site-specific nucleases—have given molecular researchers the power to modify plant and mammalian genomes site-specifically and irreversibly.[21] SSNs work by building endonucleases that can cleave DNA in a specific sequence in the genome. SSN attaches to the target sequence using a DNA binding domain or an RNA sequence. After SSN cleaves the target sequence, cellular DNA repair systems intervene, resulting in gene change at target locations.

### 2.2.1. Zinc-Finger Nucleases for Crop Improvement

ZFNs are SDNs with a DNA-binding domain (zinc finger) and a nuclease domain that has been created. Each zinc finger (ZF) is intended to identify a unique 3-bp DNA sequence. To detect a unique DNA sequence (12–18 bp), four to six separate ZFs are typically joined together. Because the FokI nuclease cleaves double-strand DNA as a dimer, two ZF proteins are needed to target the appropriate DNA sequence (24–36 nucleotides). [22] ZFNs may be programmed to bind and cleave nearly any DNA sequence, allowing for production of DNA double-strand breaks (DSBs) at specific locations.

In a wide range of species, the expression of genes producing ZFNs and concurrent cleavage at endogenous genomic loci has been established including humans, mice, pigs, plants, and insects. [23] To see if the modified ZFNs might promote homologous recombination and inclusion of particular DNA sequence alterations at SuR loci (HR). Three donor templates, each containing a missense mutation conferring herbicide resistance to one or more herbicides, were created. To identify donor template from native locus and spontaneous mutants from those created by recombination, silent nucleotide alterations were added into codons next to each mutation. [24] The generation of one or more DNA DSBs by an engineered endonuclease (also known as site-directed nucleases or sequence-specific nucleases) is central in most of the current genome editing attempts. ZFNs can promote NHEJ-mediated targeted transgene integration in tissue cultures. [25]

### 2.2.2. Transcription activator-like effectors nucleases (TALENs) based genome editing

The genome-editing revolution was sparked by TALE nucleases (TALEN), which followed in the footsteps of zinc finger nucleases (ZFN). [26] Because the assembly of tailored TALE repeat arrays can be difficult due to almost identical repeat sequences, a variety of platforms have been developed to aid in this process. These can be categorized into three groups: Cloning techniques based on restriction enzymes and ligation Methods of Golden Gate assembly and solid-phase assembly. [27] TALENs are beneficial in a variety of plant species, including Arabidopsis, tobacco barley, rice, and Brachypodium. TALE repeats modular character, along with efficient

ways for building repeated DNA sequences, have allowed TALENs to become one of the most important tools for plant genome editing. Gene in-sections is also possible using TALENs, in contrast to gene function impairment. [28] Zhang demonstrated that in *N. benthamiana*, a protoplast method may accomplish effective multiplexed gene knockdown. TALENs were delivered to four genes implicated in glycosylation pathways using the method. The entire plant may be easily grown from single protoplasts. Over half of the plants had at least one mutation at one site, and about 3% had all eight alleles knocked out, according to a non-selective screening of over 100 regeneration plants. [29]

### 2.2.3. CRISPR/ Cas9 SYSTEM for Crop Improvement

CRISPR advancement as a genome-editing technology in today's world may be traced back to its inception in late 1980s and a decade of intense testing since 2005. The CRISPR/ Cas9 microbial adaptive immune system, as well as its advancement to date, is the result of efforts from many researchers all over the world. A series of exhaustive reviews cover every facet of CRISPR/ Cas technology in great depth. [30] Zinc finger nucleases and transcription activator-like effector nucleases were most common genome editing tools until 2013. Both are manmade fusion proteins made up of a designed DNA-binding domain linked to restriction enzyme FokI's nonspecific nuclease domain, and they have been employed effectively in a variety of species, including plants. [31] CRISPR/ Cas system is a naturally occurring and acquired immune defence mechanism in bacteria and archaea that protects them against foreign DNA material, mostly viruses. This also allows scientists to consider a novel technique to alter a gene by utilizing the immune system's inherent defences. [32] As previously stated the use of meiotic mutants increases crossover rates but does not affect crossover distribution. As a result, this method cannot activate a large portion of the genome for recombination. As a result, targeting these areas directly to create DSBs during meiosis and promote homologous recombination is an apparent technique. In theory, there are two ways to accomplish this goal: employing a programmable DNA nuclease for DSB induction or using its DNA-binding capabilities to direct the natural DSB-inducing machinery to the appropriate target site. [33] Following breakthrough of CRISPR/ Cas9-mediated

plant genome editing, scientists have concentrated on developing more effective delivery systems, ideally into germline cells to avoid requirement for tissue culture and regeneration following editing. Aside from that, the goal has been to create genome-edited plants devoid of foreign DNA. Floral-dip, nanoparticle-mediated delivery, and magnetoreception-mediated delivery technologies have all been successfully used in some plants to produce altered plants even without a plant tissue culture facility. [34] Effective CRISPR/ Cas9 genome editing in plants necessitates the use of a suitable vector system (codon adjusted Cas9 gene and promoters for Cas9 and sgRNA), as well as efficient target locations and transformation methods in the relevant plant species. [35]

### 3. SIGNIFICANCE OF PLANT BREEDING

The majority of the world's impoverished and malnourished people reside in rural parts of developing countries, where agriculture provides food, cash, and jobs. Data from throughout the world demonstrate a strong link between poor agricultural output and high rates of malnutrition. Global studies have also demonstrated that increasing small-scale farmers' income is one of the effective means to reduce poverty. New plant breeding technologies (NPBTs) such as genome editing, may contribute significantly to global food security with judicious deployment and scientifically informed management. [36] Plant breeding is a cyclic process in which breeders generate diversity, most commonly by trying to make crosses within the diversity generated over a variable number of years, depending on the crop, methodology and type of variety to be produced. [37] Aside from genetic selection, low-cost screening of huge populations before more advanced yield testing based on traditional MAS and/ or phenotyping can lead to development of desired characteristics as disease resistance. However, due to unfavorable genetic correlations between traits evaluated in early generations and traits evaluated later, selection imposed in early generations or before more extensive phenotyping may reduce gain from selection for traits evaluated in more advanced stages of testing such as grain yield and quality. [38] Due to the effect that direct and indirect selection has on decreasing genetic diversity before inter-mating, significant selection for early blooming in early generations would

lead to reduced sensitivity to selection for agricultural production in coming generations.

### 4. PRESENT OPPORTUNITIES IN PLANT BREEDING

Life on our planet is directly and/ or indirectly dependent on plants. Plants play an essential role as medicines, as sources of oxygen, as pollution controllers, as suppliers of food, manure, rubber, paper, fiber raw material, shelter raw materials, and so on. Plant Breeding is a powerful tool to meet up present environmental challenges on the grounds that the yield obtained is enhanced both in terms of quality and quantity resulting in cost efficiency. Plant breeding has a long history with major revolutionary technologies. Ecologists and land-use planners may be interested in plant breeding objectives and increasing public and/ or private support for improving plants for specific benefits of plant breeding. Cereals, pulses, oilseeds, roots/ tubers, and plantains are all included in plant breeding for one or the other reasons. It involves producing more products with less input and less dependency of water, nutrients, pesticides, fertilizers, and fossil energy, which in turn reduces the final cost of its production.

Lesser dependency on factors as water, nutrients, pesticides, fertilizers, and fossil energy helps the society to stabilize rural economies and manage public health in a better way. New age plant breeding techniques are developed in a manner to reduce any adverse environmental effects with maintaining the same productivity and sustainability. This also makes it an excellent career opportunity to the young researchers by providing high-impact, altruistic results to improve farmers' with better profits, product security and positive environmental impact. Plant breeding in agriculture involves improving the health and quality by increasing soil organic matter, preventing nutrient and chemical runoff, decreasing erosion of soil, and maintaining biodiversity. Larger and improved root systems can sequester carbon and nitrogen which in turn helps in diversification of a nation's forests. Selecting and breeding such trees having pest and drought tolerance improves the survival in poor conditions leading to environmental benefits as management of storm water, cooling of evapo-transpirational air, soil, and water quality is improved, which leads to global climate change with

great opportunities.

Farmers and farm groups associated with organic and sustainable agriculture movements have supported publicly funded breeding for the diversity of products. Ideally, these partnerships should begin before the development and implementation of breeding objectives. By selective breeding, germplasm growing under local environmental conditions, individual cultivars can be optimized for small regional areas of production that fit prevailing environmental and weather patterns. These breeding techniques lead to an optimum cropping system. The new breed of plants has augmented properties as better survival, higher carbon/nutrient absorption resistance to soil-borne diseases and pests. Selective breeding of perennial crops and tree species can provide higher cellulosic feedstock with increased yield and energy values as compared to their naturally occurring counterparts. Cellulosic biofuels provide one approach for mitigating the impacts of global warming associated with fossil-fuel combustion. [39] The study of plant metabolism with abiotic, biotic stresses, naturally occurring changes, and identify herbicide mode of action by comparison of herbicides with known and unknown targets is studied under "Metabolomics". Plant breeding and metabolomics together aide the study of improvements in the new varieties and its application is increasing day by day. [40] Countries with unfavorable flora habitat conditions have increased their crop yields by twofold by adapting newer plant breeding techniques. [41] Also, speed breeding techniques apart from providing the yield in minimal resources do favor disease resistance and reduction in crop produce; which in turn leads to the development of homozygous lines for accelerated breeding, regulation of soil moisture, increase density of plant populations, and modifying carbon dioxide levels by market-preferred traits. [42,43] Plant breeding techniques, helps to increase produce with minimal resources, disease free varieties, lesser effects of global climatic conditions which are adept to biotic and abiotic stress factors with development of optimum cropping systems as per the local requirements. This multidisciplinary approach of plant breeding can be fruitful not only to the individuals but adds a great positive potential to national and worldwide ecosystems.

## 5. CONCLUSION

Modern agriculture consists mainly of cross-breeding, mutation breeding, and transgenic breeding, requiring extra time and efforts. These techniques more often involve in untargeted initiatives resulting in low yield or no yield varieties, which might not be able to cater the ever growing food needs of the present world. On the contrary, newer methods as Marker-assisted breeding, transgenic techniques etc have the potential to address this problem by improving crop selection efficiency, creation of desirable characteristics via exogenous transformation into elite cultivars. A large number of crops with enhanced features viz. high yield, improved shelf life, newer colors and textures of fruits and vegetables, ornamental flowers, etc have been selected and bred successfully.

In traditional breeding techniques, long breeding cycles, large heterozygosities, a lack of varying degrees of precision in hybridization, and low frequencies of favorable mutations hinder the adaptability. On the contrary, newer breeding techniques have made new varietal development resource-intensive, when the genomic data for the given plant species is not available.

A few latest genome-editing technologies, notably CRISPR/Cas systems, promise to be more efficient and accurate in editing genes. However, in many circumstances, the traditional technologies might be as straightforward and efficient as transgenic approaches, allowing new kinds to be created without introducing foreign genes into the plant genome preserving the natural varieties. As a result, new crop types developed using the traditional approaches may be classified as non-transgenic crops, making them more acceptable in nations where transgenic plants are widely not acceptable.

### Conflict of interest statement

Authors declare that they do not have any conflict of interest.

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