



(REVIEW ARTICLE)



The Evolution of CRISPR Cas-9 and Its Impact on Plant Breeding for Creating Drought and Disease Resistant Crops

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Abstract

Traditional plant breeding methods have played a significant role in improving crop yield, quality, and disease resistance, but they often require multiple generations of selection, extensive laboratory work, and considerable time to achieve desirable traits. The development of CRISPR Cas-9 genome editing technology has introduced a more precise and efficient approach to plant breeding by enabling targeted genetic modifications without relying solely on conventional breeding techniques. This review examines the evolution of CRISPR Cas-9 technology and its impact on the development of drought tolerant and disease resistant crops. The paper first reviews traditional plant breeding approaches, including natural selection, artificial selection, mutagenesis, protoplast fusion, and polyploidy, before discussing how CRISPR Cas-9 addresses many of the limitations associated with these methods. Published studies involving crops such as rice, tomato, soybean, and tobacco are examined to illustrate the application of CRISPR Cas-9 for improving agronomic traits, enhancing stress tolerance, increasing disease resistance, and improving crop quality. The review also discusses challenges associated with the technology, including regulatory restrictions, ethical concerns, and the technical expertise required for successful implementation. Overall, the evidence suggests that CRISPR Cas-9 has the potential to accelerate plant breeding, improve food security, and support the development of climate resilient crops while reducing the time and uncertainty associated with traditional breeding methods.

Keywords: CRISPR Cas-9; Genome editing; Plant breeding; Drought tolerance; Disease resistance

1. Introduction

Before the influx of affordable personal computers in the 1980's, writing long scripts of research was tedious, time consuming, and difficult to store. The advent of personal computers reduced the reliance on typewriters and removed the burden of storing large documents in paper form. Like personal computers, CRISPR-Cas-9, (clusters of regularly interspaced palindromic repeats) has the potential to reduce the time needed for breeding generational lines, the tedious laboratory work involved in outcrossing plants, and the randomness associated with crossing plant through traditional breeding methods. (Li et al, [1]). The evolution of Crispr Cas-9 will improve plant breeding by reducing the generational cycles between plants, producing plants that are drought tolerant, producing disease resistant crops.

While the advent of Crispr has opened a door of possibilities for the plant breeding world, traditional plant breeding methods like protoplast fusion, mutagenesis, polyploidy etc. have been sustaining that world. To understand the ongoing plant breeding research, and the problems Crispr can solve, this paper will first discuss the traditional plant breeding methods from natural selection to artificial selection, the problems associated with these methods, and the ways Crispr technology can solve them. The second half of this paper will discuss examples of Crispr research that has solved real life problems, and the downsides/limiting factors of Crispr-Cas-9.

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Traditional breeding began with natural selection. (Hamdan et al [2]). This form of selection did not involve farmers or plant breeders. Forces of nature randomly reduced the genetic pool of plants resulting in some species being lost for several generations. Food crops like carrots, corn and cabbage have distinct morphology in the consumer market, but previous phenotypes of these plants had multiple variations. This reduction in diversity of plant morphology is evident when plants are inbred for multiple generations, and a new variant is born with different morphology from their species.

After natural selection, artificial selection occurred. This form of selection was carried out by farmers and plant breeders selecting what they want for their fields and what consumers were asking for “Cross breeding which is an age-old practice involves the intentional mating of different plant varieties to obtain offspring with desirable traits” (Hamdan et al,[2].) The culling process ensured less chance of an undesired trait coming back on the farm. This method worked for ensuring disease susceptible plants didn’t develop new offspring, short plants that produced less food didn’t produce offspring, plants with undesired taste didn’t produce new offspring and only the plants that had what the farmers and plant breeders wanted made it to the next generation. This form of breeding required less technical knowledge, and breeders could only control one plant. If the farmer wanted a new trait in the plant, there was only one species to select from. Things changed for traditional breeding when multi organism levels of genetic modifications emerged. Mutagenesis, protoplast fusion, polyploidy, and shotgun sequencing gave plant breeders the ability to leave a one-dimensional breeding approach and enter the realm of three-dimensional modification.

Plant breeders could take the gene of one plant and insert it into the gene of another plant to get the desired trait they wanted. “Protoplast fusion involves merging the protoplast of different plants and individual plant cells that have undergone cell wall removal to create hybrids that have combined characteristics”. (Hamdan et al, [2]). A bacterial that is harmful to a plant pest, for example bacillus bacteria is harmful to northern corn root worm, can be inserted into corn through protoplast fusion. The DNA of bacillus is extracted, and the protoplast of corn is grown in a tissue culture under controlled lab conditions. (Li et al, [3].) The protoplast of the corn is isolated from the rest of the tissue culture and the cell wall is removed to enable gene transfer from the gene gun to the protoplast and the DNA coated microparticles is from the bacteria is shot into the cell “these experiments emphasized isolation, transfection, and regeneration to improve the recovery of genome edited plants from the protoplast” (Li et al, [1]). The corn seed is inbred to prevent the mixing of genetic materials across generations, and thousands of seeds are produced. This method was used to create Bt corn which infects northern corn root worm/corn borers after the insect eats it, but the corn itself does not harm humans when consumed. Other traditional methods like mutagenesis and polyploidy have assisted transgenic breeding like protoplast fusion and enabled a multi-organized form of genetic editing.

Mutagenesis is an umbrella term for organisms that have undergone changes in their DNA. This definition includes CRISPR Cas-9 technology, but the traditional method of mutagenesis relied on chemical and physical alterations of DNA to produce changes in the organism “Mutational breeding which emerged in the late 20th century involves the induction of genetic mutations through radiation or chemical agents” (Hamdan et al, [2]). Chemical mutagenesis functions by adding chemicals that alter DNA structure of phosphate sugar bonds to the DNA of an organism and growing the plant/organism to record phenotypical differences in the plant. This method was left to chance, and the outcome could not be predicted.

According to (Hamdan et al [2]), polyploidy is the cross breeding of two distant plant species to create a line of infertile offspring “Polyploidy doubles the number of chromosomes in an organism, resulting in altered gene expression and the potential enhancement of traits”. (Hamdan et al, [2]). Polyploidy works when the infertile offspring produced has better traits than the viable offsprings of the initial parents, and the infertile offspring can be cloned (asexually reproduced) to create more genetic similar lines. Seedless watermelons can be made by doubling the number of chromosomes or daughter cells in the watermelon and crossing the doubled chromosome with a regular watermelon that has half the cells. This crossing will produce a line of watermelons that are seedless because the parents were incompatible. (Hamdan et al, [2]).

In 2012, Dr. Jennifer Doudna and Dr. Emmanuella Charpentier made a detailed and practical discovery of CRISPR Cas-9. While working on a laboratory experiment with E. Coli, scientists discovered that the bacteria could infect another microorganism and replace a part of the organism’s DNA with its own DNA. Doudna, Sternberg, [4]. This system was refined by both scientists to create a Cas9 system that enabled scientists to cut through an organisms CRISPR Cas-9 (lines of regularly interspaced palindromic repeats) and replace its code with a different protein/nucleic acid that would change the reading of the DNA sequence and thereby change the protein the organism codes for. (Ahmad et al, [5])

The Cas 9 system works with Cas9 Nucleases, a spacer, sgRNA, cytidine deaminase, and UGI. The DNA sequence consists of four base pairs that have complementary nucleic acids: adenine, guanine, cytosine, and thymine. The four base pairs have a complementary pair the nucleic acid must bind with. Adenine will only bind with thymine, and guanine will only

bind with cytosine. This configuration is unique to the DNA double helix one nucleic acid standing opposite its nucleic pair in a sugar phosphate solution (Ahmad et al, [5].) This arrangement is slightly different for the RNA molecule. While DNA is double stranded, helix RNA is a single strand from the DNA double strand. Nature saves time and energy by copying one strand of the DNA and sending it to the offspring during sexual and asexual reproduction; this is synonymous with a zip file in a computer. Genetic material compressed from a double helix to a single helix to save time. This zip file is unlocked by the new organisms attaching the complementary pair of the RNA to form a DNA.

CRISPR Cas-9 interferes with the unlocking of the transferred DNA by changing the code the organism receives. This is made possible by RNA containing one odd nucleic acid, Uracil. The Cas 9 Nucleases and sgRNA cut through the spacer sequence, deaminates cytosine into uracil, UGI inhibits uracil, and excision occurs this converts adenine to Guanine in a complementary chain. After DNA is repaired, adenine combines with its DNA complementary pair thymine and an RNA that was initially coding for cytosine, and guanine will start coding for adenine and thymine “Site specific DNA double strands breaks (DSBs) which are induced by the Cas-9 protein under the control of the sg-RNA, trigger DNA repair mechanisms” (Ahmad et al, [5].) The zip file that was passed from parent to offspring will be extracted as a different amino acid code, which depending on what acid was changed could benefit the organism and increase the plants’ chance of survival.

Traditional plant breeding models are slow, random and cumbersome to carry out in a laboratory “the more knowledge we obtain from about the underlying genomic factors of yield and quality the more limitations of these traditional breeding approaches become apparent” (Wolter et al, [6].) Artificial selection requires the farmers/plant breeders to wait for multiple generations of inbreed plants before a cross can be made. Generation cycles of the plant could be 6 months and inbreeding 6 times could mean waiting 3 years for the result. Protoplast fusion and shot gun sequencing works by trial and error: shooting genes into the protoplast does not guarantee DNA acceptance by the new cell and the scientists need to grow the plant and make test crosses before confirming or disconfirming the gene transfer happened. Polyploidy relies on the ability of infertile offsprings to be cloned for multiple lines of the same organism, and mutagenesis can create deleterious genes that could adversely affect an organism without producing any positive benefits. (Hamdan et al, [2].)

According to Kim S.G [7], CRISPR Cas-9 can slow down ripening genes in plants by targeting qualitative loci’s that can reduce the ripening process. The implication of this is that crops being transported from other continents will arrive in the destination ripe instead of spoiled because their ripening process was hindered. CRISPR Cas-9 can be used to silence genes causing browning. (Kim S.G, [7]) Horticultural crops like cabbage carrots, flowers, and squash tomatoes are grown and chosen for physical appearance. Horticultural crops that have blemishes on their outer covering could lead to deductions during sales, and it is important for plant breeders to reduce the occurrence of browning and blemishes on these crops. CRISPR Cas-9 can alter oil composition in crops. An example of these alterations is in soybeans. The oil content of soybeans can be altered to increase the production of oil in the seeds and reduce the production of starch “For instance improved soybean with high oleic acid and low linoleic and linolenic acids was generated by removing several nucleotides in two fatty acid desaturated genes”. (Kim S.G, [7]) This technology is useful to farmers growing soy specifically for oil to increase the amount of oil that can be extracted from soybean seeds after blending. CRISPR Cas-9 also can enhance drought tolerance genes by increasing the expression of genes in identified areas of quantitative loci which will improve root formation, reduce height of the plant and increase transition from vegetative to reproductive stage of growth. Cases of CRISPR Cas-9 used for plant breeding research include rice, tomato to increase carotenoid formation, and tomato to improve inflorescence architecture. (Kim S.G, [7].)

Lippman and a laboratory team created a novel genetic improvement for tomatoes by improving the inflorescence architecture of the plant by generating weak transcriptional alleles “in tomato the group of Zac Lippman generated a biallelic promoter mutant for a trait regulating gene and transformed it with Cas 9”. (Schindele et al, [8].) The Arabidopsis genes, *sepallata4* and *fruitful* were combined to improve the inflorescence structure of the plant cis regulatory elements in *sepallata4* and *fruitful* were targeted to create alleles supporting expression levels which improved the architect structure of the crops “They improved inflorescence architecture by combining two natural mutations mediating reduced expression of the tomato homologs of the Arabidopsis genes *SEPELLATA4* and *FRUITFULL*. Inflorescent architecture in tomatoes is important because they control yield, weight, and number of tomatoes produced by the plant. Lippman’s team continued their research by discovering more transcriptional alleles. These alleles were created from biallelic mutants transformed with a multiplex CRISPR Cas-9 system and crossed with WT. the next generation is segregated for novel transcriptional genes and like the case of tomatoes for architectural inflorescence it can be used to improve another crop by merging the new genes together. (Schindele et al [8])

Another prominent research on CRISPR Cas-9 was carried out in rice. According to (Schindele et al, [8]) rice can be edited for eight agronomically important genes. The ability of rice to produce multiplexing systems of sextuple, septuple,

and octuple mutants increases the possibility of clonal reproduction of infertile hybrids while skipping the cumbersome process of polyploidy. Rice can be edited from the REC8, PAIR1, OSD1, AND MTL genes to produce superior hybrids that could outcompete the current rice breeding lines. (Schindele et al, [8].) Rice genes are also being searched by plant breeders for qualitative loci's that could increase the softness and glossiness of the rice. The gene that codes starch formations controls the softness of the rice; rice with less starch is softer than rice with more starch. The gene that codes for glossiness is connected to the gene for waxy rice, the gene controls the chain of starch molecules in the grain which determines how waxy/glossy the rice will appear "the highly efficient multiplexing system was employed for clonal reproduction of F1 rice hybrids by simultaneously targeting three meiotic genes and one gene involved in fertilization" (Schindele et al, [8])

From the case studies of tomatoes and rice, CRISPR has shown a possibility of reducing mutational risks by reducing generational cycles, the gene knock ins and knock outs of single nucleic acids, eliminating the need for clones by directly editing the gene of an organism thereby ensuring the next generation of plants through sexual reproduction can produce viable offsprings. (Kim S.G, [7]). These benefits have, however, been slow to reap due to regulatory hindrance on CRISPR Cas-9, ethical concerns for editing biological organisms and technical know-how involved in carrying out the research "despite the promises it holds the development of GM crops has been accompanied by controversies, concerns, and regulatory issues". (Ahmad et al [5])

Regulatory bans on genetically modified crops and by extension CRISPR Cas-9 crops, have delayed the production of more genetically modified crops. European countries have banned research on some aspects of gene editing and refused to buy crops from countries that use genetically modified plants for breeding. These sanctions imposed by multiple countries on GM food have reduced the desire of farmers to try GM foods which directly reduces the interest of plant breeders to make more improved lines of hybrids. According to (Ahmad et al [5],) the European union bans food containing 0.9% of GM technology while Japan bans food containing more than 5% of GM technology. Regulations and sanctions also require scientists to get special licenses to work with CRISPR Cas-9 technology which reduces interest in the scientific community "...such as the European Union where the authorization of new varieties developed by genome editing techniques is subjected to time and cost intensive admission procedures". (Wolter et al [6].)

Ethical concerns for CRISPR Cas-9 Is the second reason research have been lagging. Since the inception of CRISPR Cas-9 in 2012, Dr Jennifer Doudna one of the leading scientists is CRISPR Cas-9 research and author of *A Crack in Creation*, has been advocating the need to first make laws controlling the use of research before the knowledge fell into wrong use. Doudna, Sternberg, [4]. The technical know-how required for gene editing using the CRISPR Cas-9 system is vast and limited to PhD level of research "opposed to traditional breeding approaches, improving crops by genome editing requires a much higher degree of genomic and bioinformatic knowledge" (Wolter et al, [6]) Knowledge is protected and multiple fields of study including biology, chemistry, data analysis agronomy etc. are required to fully understand the science behind the cas9 system.

2. Conclusion

In conclusion, plant breeding methods have helped farmers improve the quality and quantity of crops produced. These plant breeding methods are not sustainable for the changing climate conditions and the expected increase in world population. Better breeding techniques are needed to increase the level of food production and provide higher yield for crops. The reports that CRISPR-Cas 9 had the ability to locate nucleic acids and edit them for a different acid created a light at the edge of the tunnel. CRISPR Cas-9 could solve the problems of traditional plant breeding: case studies with tomato carotenoids, architectural inflorescence of tomatoes and waxy rice have shown that CRISPR Cas-9 can improve crop yields. CRISPR Cas-9 technology can also improve plant breeding by reducing the waiting times between generation cycles, reducing drought tolerance, and creating disease resistant plants. These promises have been slow to realize due to regulations, ethical concerns, and technical know-how. Plant breeders can use CRISPR Cas-9 to create improved lines of crops that will help alleviate hunger, provide alternatives for the effects of climate change, and ensure food security in developing countries.

Compliance with ethical standards

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Disclosure of conflict of interest

The author declares no conflict of interest.

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