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# Science Writing

# Year 11-12


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Government of South Australia
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# How can CRISPR-Cas9 technology affect the horticulture industry?

## Introduction

Climate change has negatively impacted agricultural practices as global temperatures increase causing more volatile environments. During the 2019-2020 financial year, 61 million tonnes of cereal and broadacre crops were produced in Australia with global productions at 2711.7 million tonnes (Australian Bureau of Statistics, 2021; FAO, 2022). Predicted increases of global populations from 7.3 to 9.7 billion by 2050 may impact current horticulture practices. A possible solution is Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 (CRISPR-Cas9), a biotechnology used to edit crop genomes. This could potentially design an action for sustainability by increasing crop yields at a global scale, enhancing nutritional content, and resist disease (Haque, et al., 2018). However, social and economic limitations and biodiversity risks still require evaluation.

## Scientific Background

CRISPR-Cas9 is a highly accurate and versatile method to edit segments of genetic code through addition or modification of a DNA sequence (Your Genome, 2022). A genetically modified organism (GMO) is an organism which contains an artificially altered genome which may or may not include DNA from foreign species. If the DNA combined or inserted through recombinant DNA technology is foreign, this creates a transgenic organism (Pray, 2008).

Genomic mapping is used to develop haplotypes to group regions of interest to identify links in DNA sequences (Edwards & Batley, 2009). For example, the phytoene desaturase (PDS3) gene is an essential carotenoid biosynthesis pathway in plants, which assist production of vibrant pigments in plants and vegetables (Naing, et al., 2019). A gene probe is a single-stranded DNA fragment with a complimentary nucleotide sequence to the gene of interest which may be fluorescently or radioactively labelled (Schleifer, et al., 1992). It can then bind to the target sequence and be identified under an ultraviolet light or an X-ray.

Artificial guide RNA (gRNA), a single stranded sequence of RNA, has complimentary nucleotide bases to the PDS3 gene (Asmamaw & Zawdie, 2021). A Cas-9 nuclease, predominantly isolated from *Streptococcus pyrogenes* (SpCas9) recognises a specific sequence of nucleotides known as a protospacer adjacent motif (PAM) sequence (Figure 1). This is required for a Cas9 nuclease to recognise and cut, typically located three to four base pairs downstream of the PDS3 gene. A recognition domain protein locates the gene while the nuclease binds, facilitating a double-stranded break in the DNA (Hassan, et al., 2021). After these are produced, the cell's natural repair mechanism enables the application of targeted base substitutions and gene insertion or deletion (Sedeek, et al., 2019). This occurs through non-homologous repair end joining pathways (NHEJ) which introduces point insertions or deletions to the editing site disrupting genes in the plant genome but is error-prone (Nahirñak, et al., 2022). If donor DNA is present, a homologous recombination (HR) pathway can be used where DSBs can precisely make point changes or replace larger sections of genes (Yin & Qiu, 2019).

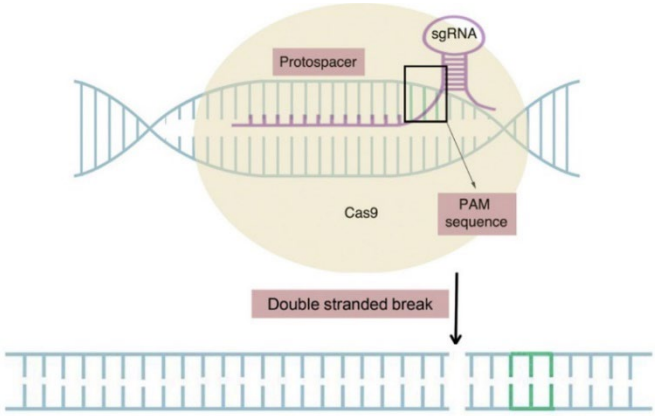


Figure 1: CRISPR Cas9 Mechanism (Antony Ceasar, et al., 2016)

The PDS gene is also widely used as a molecular marker as it is easily detected (Naing, et al., 2019). For example, they have been used to observe developmental genes in strawberry crops. By incorporating PDS into diploid and octoploid *Fragaria* populations using CRISPR-Cas9, a mutation in this gene resulted in an albino phenotype to be expressed at high rates (Wilson, et al., 2019). This was distinct from bleaching seen on untransformed tissue culture, hence showing that the marker pattern in the genotype allows scientists to effective test if a specific gene has been incorporated.

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## Application

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**Increasing crop size and yields**

Countries with poor agricultural yields, like Haiti where up to 4.4 million people experience food insecurity, could benefit from scientific application of CRISPR-Cas9 to increase crop abundance (World Food Programme, 2022). CRISPR-Cas9 was used to produce larger tomato crops through isolating and disrupting the CLV3 gene which controls meristem size by initiating peptide ligands (Zhu, et al., 2021). This resulted in larger transgenic crops than wild-type plants (Figure 2) (Wang, et al., 2019). As this technology advances, a larger number of edible plant species' genomes can be edited to increase crop size. Applications would increase crop yields, increasing the amount of food available, decreasing consumer costs.

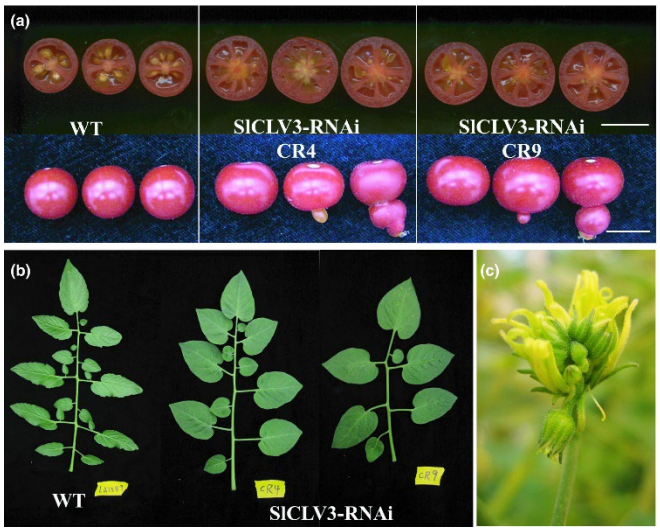


Figure 2: Difference in phenotypic expression between wild-type and modified CLV3 tomatoes and leaves (Chu, et al., 2019)

**Enhancing Nutritional Content**

Editing specific loci on genes could potentially enhance the nutritional content of crops, reducing impacts of malnutrition in upwards of 690 million people worldwide. Rising levels of carbon dioxide have been found to reduce the protein, zinc, and iron levels in rice, legumes, and vegetable species (Soares, et al., 2019). The global population will be deficient in protein and zinc by 1.3% and 1.9% respectively by 2050 according to current projections undertaken by Myers and Smith, 2018. Increasing beneficial vitamins in easily accessible foods could aid physical wellbeing of individuals. For example, overexpressing the OsZIP7 gene in rice crops increased zinc concentrations by 25% (Ricachenevsky, et al., 2018). Additionally, lysine content increased by 35% in rice seeds with a balance of the other amino acids without detected consequences (Wong, et al., 2015).

Meat is nutritious and contains several amino acids, fatty acids, minerals, and vitamins which are difficult to obtain in other food sources. Increasing the nutritional content of plant-based sources using CRISPR-Cas9 would enable individuals who do not regularly consume animal products to receive critical macro and micronutrients, aiding bodily functions (Geiker, et al., 2021). Through this, excess medical costs to increase nutrient levels with supplements and impacts of nutrient deficiencies may decrease. This would not alter possible implementations of familiar foods in the diet but may counteract the effect of a focused diet and reduce physical and mental stress regarding intake of essential nutrients.

**Disease Resistance**

Scientific understanding of CRISPR-Cas9 can be used to genetically implement traits to crop species, benefitting local economies. Disease effecting crops are expected to become more widespread due to increasing atmospheric carbon dioxide causing physiological changes in plants (Hunjan & Lore, 2020). Additionally, higher temperatures allow for faster replication of pathogens in crops which may become more susceptible to disease (Karavolias, et al., 2021).

One such example for the successful use of CRISPR-Cas9 was the application of the BSR-K1, a gene coding for a protein which binds to mRNA of defense-related OsPAL genes (Guo, et al., 2021; Zhou, et al., 2018). Rice leaf blight (*Xanthomonas oryzae)* is a bacterial disease which thrives in hot, humid conditions. Infected crops exhibit grey-white lesions on their leaves with seedlings often dying within three weeks (Yen, 2020). Yield losses due to *X. oryzae* range from 8% to 32% (Liu, 2018). However, as OsPAL mRNA accumulates in the mutant crop containing BSR-K1, this results in the ability to breed rice with broader disease resistance (Zhou, et al., 2018). As rice is a staple food for 3.5 billion people, reducing the impact of disease on valuable crops would significantly increase the amount of preserved crop yields. This would decrease the economic burden placed on farmers regarding damaged crop, leading to more stable income streams and job security. Increased yields also promote food security for farmers and consumers in communities.

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## Limitations

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**Social Risks**

Although there are many benefits to genomic modification in plants, this technology requires consistent monitoring to limit possible negative consequences. A few years is typically required to develop genetically altered crop species and undertake regulation processes. In Australia, CRISPR-edited crops are not subjected to harsh regulations if foreign DNA is not incorporated, possibly allowing for more rapid distribution (Zhang, et al., 2021). However, it is important for scientists to evaluate possible risks of consumption to human wellbeing before commercial sale. Testing the performance of modified crops is also critical for several generations to

monitor their resistance to volatile climates and to observe if enhanced genomic traits can be sustained over an extended duration (Zaidi, et al., 2020). Ethical issues also arise where introducing new genetic material into organisms is deemed as immoral and disrupts nature's pathway. Individuals may also abstain from GMOs for religious reasons as humans are 'play[ing] God' (Phillips, 2008).

**Costs for Technology**

There are economic limitations to CRISPR-Cas9 which may hinder the viability of gene modification. Experts in America currently believe that genomic edited crops may reach consumer markets at $10.5 million USD in five years. However, if crops are more strictly regulated, increased costs of $24.5 million USD could ensue and may take up to 14 years to reach markets (Lassoued, et al., 2019). For example, regulatory approval costs for insect-resistant maize incurred a total of approximately $7 to $15 million USD to produce and import in ten countries (Table 1) (Kalaitzandonakes, et al., 2007). Current start-up costs may be too extensive, requiring funding from various sources. Hence, the effectiveness of CRISPR-Cas9 on crops produced need to be considered to evaluate the cost-benefit relationship of the technology.

| Cost categories | Range of costs incurred ($) |
|---|---|
| Preparation for hand-off of events into regulatory | 20,000–50,000 |
| Molecular characterization | 300,000–1,200,000 |
| Compositional assessment | 750,000–1,500,000 |
| Animal performance and safety studies | 300,000–845,000 |
| Protein production and characterization | 162,000–1,725,000 |
| Protein safety assessment | 195,000–853,000 |
| Nontarget organism studies | 100,000–600,000 |
| Agronomic and phenotypic assessments | 130,000–460,000 |
| Production of tissues | 680,000–2,200,000 |
| ELISA development, validation and expression analysis | 415,000–610,000 |
| EPA expenses for PIPs (e.g., EUPs, tolerances) | 150,000–715,000 |
| Environmental fate studies | 32,000–800,000 |
| EU import (detection methods, fees) | 230,000–405,000 |
| Canada costs | 40,000–195,000 |
| Stewardship | 250,000–1,000,000 |
| Toxicology (90-day rat)—when done | 250,000–300,000 |
| Facility & management overhead costs | 600,000–4,500,000 |
| Total | 7,060,000–15,440,000 |

Table 1: Compliance costs in Argentina, Australia, Canada, China, European Union (EU), Japan, Korea, Philippines, Taiwan and the United States for insect-resistant maize
(Kalaitzandonakes, et al., 2007)

**Biodiversity Risks**

Editing crops to express a particular phenotype using CRISPR may result in more biologically similar proteins within an organism (Landry, 2015). Maintaining high biological variability within a species' genome provides crops with increased adaptability to changing environments. For example, between 1845 and 1850, the fungal pathogen *Phytophthora infenstans* infected potato crops throughout Ireland (Fraser, 2003). Farmers planted sections of DNA from a parent potato which limited genetic variation leading to higher proportions of crop death (Figure 3). This led to mass starvation with 25% of the Irish population being displaced or killed (Fraser, 2003). Similar issues may arise if crop species do not contain sufficient genetic variation, requiring monitoring by scientists in the future.

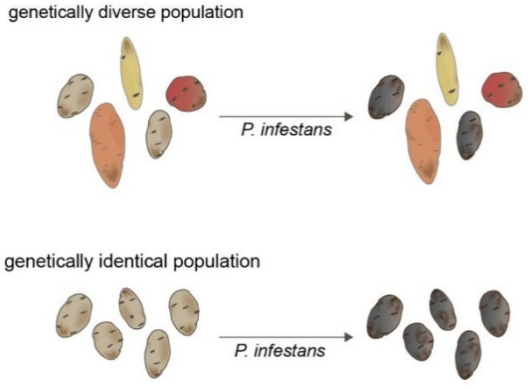


Figure 3: Reduced DNA variability in potato crops leading to reduced adaptability to *Phytophthora infenstans* (Fraser, 2003)

## Conclusion

Editing plant DNA using CRISPR-Cas9 technology has various social and economic benefits. It shows great promise to make crops more robust in environments impacted more severely by climate change. The ability to insert or delete gene sequences to staple crops and enhance the nutritional content targeting loci on specific genes potentially benefits the health of individuals facing food instability increasing crop yields. However, due to the relatively novel discovery of the technology's applications, scientists may need to monitor health and biodiversity risks, and ethical concerns of gene edited crops before they become available to the public.

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