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Genetic Engineering in Vegetable Crops: Progress, Controversies and Future Implications

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Introduction

Genetic engineering or recombinant DNA technology has revolutionized agriculture by enhancing crop yields on limited land. It began with the discovery of recombinant DNA in 1973 and has since made significant advancements, such as the creation of the 1st genetically modified crop plant in 1982, field trials in 1986 and the approval of herbicide-resistant soybeans in 1990. The Flavr Savr tomato, insect-resistant corn and golden rice were introduced in 1994 and 1996, respectively. GMOs produced through this technology have applications in animal feed, processing and fuel generation, offering increased crop productivity, environmental sustainability, reduced inputs and targeted solutions to farming challenges.

Genetic Engineering Mechanism in A Nut-Shell

Genetic engineering involves several steps. First, the gene to be cloned or the target DNA, is isolated. This gene is then inserted into a vector, another piece of DNA that allows the gene to be taken up and copied by the recipient cell. The recipient organism's cells are then infected with recombinant vectors through transfection or virus infection. After infection, scientists identify which cells contain the recombinant vectors. The changed organism is then allowed to grow. Finally, gene expression occurs, resulting in the desired result.

One method used in genetic engineering is Agrobacterium-mediated transformation. This technique utilizes *Agrobacterium tumefaciens*, which transfers DNA from its tumour-inducing plasmid into plant cells. The transferred DNA, known as transfer DNA (t-DNA), contains genes that cause tumorous growth and benefit the bacteria by producing opine. Scientists could remove the tumour-causing genes and replace them with desired genes, effectively using the bacterium as a vector for genetic engineering.

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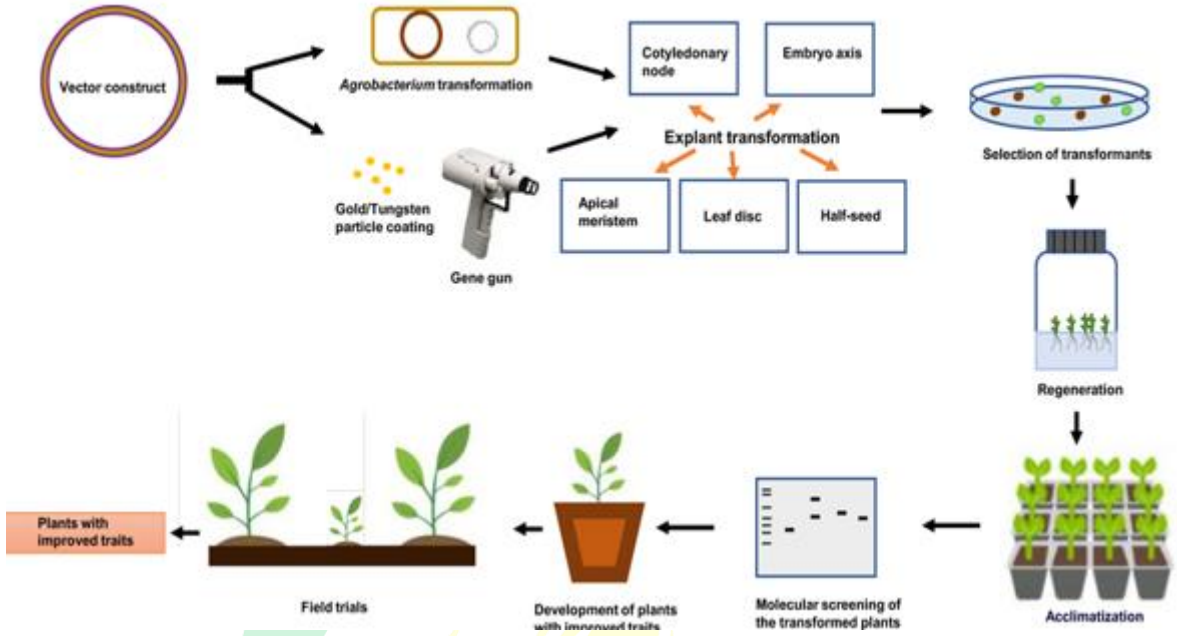

From the 1980s to 2015, genetic engineering relied on recombinant DNA technology, tissue culture, Agrobacterium-mediated cell transformation and microprojectile bombardment. Microprojectile bombardment, also known as biolistics, was developed to genetically engineer plant taxa that were thought to be non-transformable by Agrobacterium. This method uses microprojectiles coated with DNA to pierce plant cells and introduce foreign genes. Almost all plant taxa have been shown to be amenable to Agrobacterium-mediated transformation, while microprojectile bombardment allows for the genetic engineering of plant species that were previously non-transformable.

Advances in genome editing technologies, such as CRISPR/Cas9, have increased the precision with which genetic changes can be made in plant genomes. These technologies can complement and extend conventional methods of genetic improvement.

In nut shell, genetic engineering involves several steps, including isolating the gene to be cloned, inserting it into a vector, infection of recipient organisms' cells with recombinant vectors, identifying the appropriate cells, growth of the changed organism and gene expression. Agrobacterium-mediated transformation and genome editing are two essential techniques used in genetic engineering. Agrobacterium-mediated transformation utilizes a bacterium to transfer desired genes into plant cells, while genome editing involves the use of site-directed nucleases to mutate specific DNA sequences. These technologies have the potential to impact plant agriculture and improve crop traits significantly.

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Progress in Vegetable Crops

For Quality improvement

Calgene pioneered genetic engineering with the Flavr Savr® tomato, which was introduced in 1994. This innovative cultivar not only marked the beginning of genetically modified foods but also demonstrated significant flavour improvements and reduced softening. Calgene accomplished this feat by using an antisense strategy to inhibit tomato's polygalacturonase (PG) gene (Calgene Inc., 1994). DNA Plant Technology, Corp. (Endless Summer™) and Agritope, Inc. (SAMase®) used ethylene-blocking techniques to improve tomato quality (ABC Series: Tomato—DANR #72XX). Consumers in test markets responded positively to genetically modified tomatoes, including the 'Mac Gregor' variety, owing to their improved quality attributes (Smith *et al.,* 2005). Despite its initial success, the Flavr Savr tomato faced numerous challenges, including consumer resistance, productivity issues, diseases, and cost constraints, eventually leading to its withdrawal from the market.

Various genetic modifications have been tested in horticultural biotechnology to improve crop characteristics. Genetic loci associated with increased anthocyanin accumulation in tomatoes have been identified, including the *AftAft*/- and *atvatv* loci in Solanum chilense Anthocyanin fruit (Smith *et al.*, 2005). TALEN-mediated transformation of the *Vinv* gene in the potato domain has been used to improve cold storage and processing characteristics (Williams *et al.*, 2023). Similarly, CRISPR/Cas9 targeting of the *PL* gene, which is responsible for plant cell wall degradation, has shown promise for increasing fruit firmness efficiency (Davis *et al.,* 2022). Further modifications involving TALEN-mediated transformation targeting the *SBE1* and *INV2* genes in potatoes resulted in higher amylopectin content and lower cold sweetening (Thomas *et al.*, 2023). Similarly, CRISPR/Cas9 targeting of the *StGBSS* gene has increased amylopectin content, improving potato quality (Robinson *et al.,* 2022). likewise, CRISPR/Cas9-mediated *CaMBD* gene manipulation in tomatoes has increased gamma-aminobutyric acid (GABA) content in mutant plants (Turner *et al.,* 2023).

Biotechnology is used for more than just genetic modifications; it includes interventions to improve postharvest quality. For example, suppressing the ACC synthase and ACC oxidase genes in tomatoes slows fruit ripening and increases shelf life (Kumar *et al.,* 2004). In parallel efforts, Nambeesan *et al.,* (2010) expressed the *ySpdSyn* gene in tomatoes, resulting in fruits with longer shelf life, less shrivelling, delayed decay symptom development

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and increased lycopene levels, an antioxidant. Behboodian *et al.,* (2012) used RNA interference to silence the *ACO1* gene in tomatoes, resulting in lower ethylene production and an impressive extension of fruit shelf life to 32 days. Diretto *et al.,* (2013) also silenced the lycopene epsilon cyclase (*LCY-e*) gene in potatoes, which significantly increased carotenoid levels, particularly beta-carotene. Romer *et al.,* (2000) introduced the bacterial carotenoid gene (*crtI*) into tomatoes, increasing beta-carotene content. Fraser *et al.,* (2002) overexpressed the phytoene synthase gene (*crtB*) in tomatoes, resulting in elevated total carotenoid levels. Concurrently, Rosati *et al.,* (2000) altered the expression of the lycopene beta-cyclase (*beta-Lcy*) gene in tomato fruit, resulting in higher beta-carotene levels and a variety of colour phenotypes.

For Biotic stress

Genetically engineered (GE) crops have introduced a variety of traits to improve resistance and nutritional value. Monsanto and Simplot Plant Sciences collaborated to create the Bt IR3 Potato, which is resistant to Potato Virus Y, Potato Leafroll and late blight pathogens, as well as having low acrylamide content and non-browning properties (Smith *et al.,* 2021). In terms of insect resistance, broccoli, cabbage, cauliflower, and swede have all been successfully engineered with *cry* genes from the bacterium *Bacillus thuringiensis* (Bt), protecting against pests such as the diamond back moth (Robinson *et al.,* 2018). Further, genes such as *avidin* from chicken egg white have been introduced into broccoli to improve insect resistance (Miller *et al.,* 2022). In chilli peppers, CRISPR/Cas9 targeting of the *CaERF28* gene, which is associated with anthracnose disease susceptibility, resulted in resistant mutant lines (Anderson *et al.,* 2022).

Genetic modification has also been used to improve pest resistance in various horticultural crops. Transgenic Bt tomato plants have been engineered to resist pests such as *Spodoptera litura* and *Heliothis virescens,* providing a sustainable and environmentally friendly pest control solution (Fischhoff *et al.,* 1987). Similar success has been achieved with transgenic brinjal expressing a synthetic *cry1Ab gene,* effectively resisting the lepidopteran insect *Leucinodes orbonalis* (Kumar *et al.,* 1998). Other crops, such as potatoes, cauliflower, cabbage and okra have been modified to resist pests by introducing specific genes, such as cry genes from *Bacillus thuringiensis* (Shelton *et al.,* 2002; Chakrabarty *et al.,* 2002; Paul *et al.,* 2005; Narendran *et al.,* 2013). Narendran *et al.,* (2013) introduced the *Cry1Ac* gene into okra, conferring insect resistance to the fruit and shoot borer.

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Carrots (*Daucus carota*) have been genetically modified to increase disease resistance. Punja and Raharjo (1996) transferred the tobacco *class I ChiC* gene to carrot plants, increasing resistance to *Botrytis cinerea*, the cause of grey mould disease. In cabbage (*Brassica oleracea var. capitata*), the *Cry1B-cry1Ab* fusion gene confers insect resistance to *Plutella xylostella* (Paul *et al.*, 2005). Certain Taiwanese cauliflower varieties contain a trypsin inhibitor gene to resist *Spodoptera litura* and *Plutella xylostella* (Ding *et al.*, 1998). Genetic modifications in brinjal (eggplant) *cv.* Pusa Purple Long includes the synthetic *cry1Ab* gene for resistance against *Leucinodes orbonalis* (Kumar *et al.*, 1998), the modified rice cystatin gene (*OC-IDD86*) for resistance against *Meloidogyne incognita* (Papolu *et al.*, 2016) and the *lucerne glucanase* gene for fungal resistance (Singh *et al.*, 2014). Currently, Bangladesh is the top producer of Bt-Brinjal.

Normal brinjal vs Bt brinjal

In 2008, Qing *et al.*, reported that transgenic cowpea expressing the α-amylase inhibiting protein (*αAI-1*) gene from *Phaseolus vulgaris* demonstrated insect resistance. Researchers also developed resistance to *Maruca vitrata* in cowpeas by transforming nodal cuttings with a plasmid carrying the *Cry1Ab* gene from *Bacillus thuringiensis*, with promising field trial results in Nigeria and Puerto Rico (Qing *et al.*, 2008).

For abiotic stress

Genetic modification has helped improve abiotic stress tolerance in a variety of crops. Zhang *et al.*, (2011) aimed to enhance drought tolerance in tomato plants by introducing the *Sly-miR169c* gene. This genetic modification reduced the expression of target genes, resulting in increased drought resistance. Similarly, Subramanyam *et al.*, (2011) used genetic modification to improve salt tolerance in chilli pepper plants. They introduced the tobacco *osmotin* gene via Agrobacterium-mediated gene transfer, resulting in transgenic pepper plants with higher levels of chlorophyll, proline, glycine betaine, ascorbate peroxidase (APX),

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superoxide dismutase (SOD), glutathione reductase (GR) and relative water content (RWC). These transgenic peppers exhibited improved salinity tolerance, thriving in up to 300 mM NaCl.

Fan *et al.*, (2012) used the *SoBADH* gene from *Spinacia oleracea* in the sweet potato cultivar *Sushu-2* to improve salinity tolerance, oxidative stress resistance and low-temperature resilience. The transgenic sweet potato plants had better cell membrane integrity, increased photosynthetic activity, less reactive oxygen species (ROS) production and activated ROS scavenging mechanisms. Furthermore, Lim *et al.*, (2016) improved salinity stress tolerance in cherry tomatoes by introducing the *GalUR* gene, resulting in higher fruit ascorbic acid levels. These transgenic tomatoes showed increased tolerance to abiotic stresses caused by viologen, NaCl and mannitol, as well as increased expression of antioxidant genes like *APX* and *CAT*.

Genetic modifications have been employed beyond tomatoes and sweet potatoes to enhance stress tolerance in various crops. Subramanyam *et al.*, (2011) improved salinity tolerance in chilli pepper by introducing the *osmotin* gene, whereas Han *et al.*, (2015) increased salt tolerance in bottle gourd by introducing the *AVP1* gene, which regulates ion transport and homeostasis. Modifications to watermelon (*Citrullus lanatus*) include knocking out the *CIPDS* gene for albino phenotype, introducing the *Na^+/H^+ antiporter* gene for salinity tolerance and incorporating the *cAPX* gene for chilling and salinity tolerance, among others. These genetic modifications demonstrate a versatile approach to fortifying crops against various environmental stresses, contributing to agricultural systems' sustainability and resilience.

Controversies

The debate on genetic engineering in food crops is complex, with advocates arguing for increased agricultural productivity and food security. Opponents, however, raise concerns about ecological and long-term health effects. The safety of GM foods for human consumption remains a contentious issue, with conflicting studies causing concern. Environmental concerns include herbicide-resistant weeds and genetic contamination of non-GM crops. Social and ethical issues arise from corporate control of seed patents, concentration of power in the food industry and access to GM technology by small-scale farmers. Labeling and regulation of GM foods also cause discord, with proponents advocating for transparency and opponents fearing confusion. In essence, the genetic engineering of food crops presents a complex interplay of

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science, ethics, economics and environmental stewardship, challenging policymakers and society to navigate these intricacies and find common ground amidst the contentious discourse (Prescott *et al.*, 2005; Ellstrand *et al.*, 2013). Critics worry that releasing GMOs into ecosystems may lead to unintended consequences, such as transferring modified genes to wild relatives, potentially creating "superweeds," or altering natural ecosystems (Ellstrand *et al.*, 2013). Social and ethical dilemmas also emerge in the context of genetic engineering in agriculture, with concerns about corporate control of seed patents and the consolidation of power within the agribusiness industry fueling debates about fair access to technology and corporate influence (Howard, 2009).

Conclusion

As the world continues to face the challenges of food security and sustainability, genetic engineering plays a vital role in shaping the future of agriculture by addressing these pressing issues through scientific innovation and technological advancements. Genetic engineering in vegetable crops offers substantial promise for addressing the critical challenges faced by small-scale farming communities, including enhanced crop resilience, increased yields and improved nutritional content. However, its widespread adoption must be accompanied by rigorous regulation and ethical considerations to ensure environmental sustainability and equitable access for small-scale farmers. The recent amendments of guidelines of GOI, SDN1 & SDN2 in CRISPR/Cas system has been allowed to be used as genetic engineering without any implications of transgenic guidelines. The future implications of genetic engineering in vegetable crops depend on continued research tailored to local needs, fair distribution of benefits and responsible management of potential ecological and societal impacts, all essential for harnessing its full potential in advancing sustainable agriculture in small farming contexts.

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