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Genetic Engineering: Agriculture for the 21st Century

Sybil S Waters, Karolina M Pajerowska-Mukhtar*

Department of Biology, University of Alabama at Birmingham, Birmingham, AL 35294, USA

Abstract For as long as man has been cultivating crops and raising animals, there have been modifications of the genomes of these plants and animals. The progress made in current genetic technology allows for modifying the genome very precisely, one gene at a time. Research has made possible targeted changes in varieties of plants that have enabled man to increase both yields and the quality of these crops. Crops developed through genetic engineering are commonly known as transgenic crops or genetically modified (GM) crops. This review will detail benefits of genetic modifications in crop production, and describe additional concepts in crop biotechnology that will make more direct contributions to food quality, environmental benefits, pharmaceutical production, and non-food crops.

Keywords Genetically Modified Organism (GMO), Transgenic Crops, Sustainable Agriculture

1. Introduction

As the population of the world continues to rise, so do concerns about the land capacity necessary to produce food for the nearly ten billion people that are predicted to inhabit the earth by the year 2050[1]. In considering how to generate sufficient crops for the ever-growing population, researchers, farmers, and policy makers are also weighing the potential environmental stresses that are predicted to increase with global warming. For example, as sea levels rise, croplands will be submerged; abiotic stresses such as salinity, drought, and UV radiation are all predicted to escalate[1, 2]. These stresses could pose serious threats to crop development. Other factors that adversely influence crop production include pests, which are becoming increasingly difficult to control as they develop resistance to some commonly-used herbicides[2]. Faced with the challenge to produce more crops with less land in the presence of increasing environmental stressors, many researchers have turned to genetic engineering.

Genetic engineering is a means of altering the phenotype of an organism through the modification of a few well-characterized genes[2]. This may include inserting a gene or group of genes that has been synthetically produced or isolated from another organism. This method is superior to gene modification from other means such as selective breeding for the reasons discussed below[3]. First, this method is usually much faster than other methods because it does not require waiting for multiple subsequent generations to be produced and subsequently bred[4]. Secondly, genetic engineering allows the transfer of just a few genes rather than the numerous uncharacterized genes that may be transferred through artificial selecting or grafting. Third, and perhaps most promising for future research, is the potential for genes from unrelated species to be inserted into organisms. For example, genes from bacteria may be inserted into the genome of plants[2].

This paper will discuss recent research in the area of agricultural genetic engineering, focusing first on the recent results of this research and then on the various groups of genes that may be modified to produce these results.

2. Agricultural Improvements from Gene Modification

2.1. Insect Resistance

One of the most economically successful instances of genetic engineering in agriculture is the development and use of *Bt* crops. Approximately 50% of cotton and 40% of corn planted in the United Stated has been genetically engineered to produce toxins from the bacterium *Bacillus thuringiensis (Bt)*, which is commonly found in soil[5]. These insecticidal toxins are known as Cry toxins. This naturally produced insecticide was once sprayed onto plants to kill caterpillar and beetle pests but is now produced inside the plant so that when the insects attempt to eat the crop they are killed[2]. In the Egyptian Nile, the stem borer, rather than the beetle or caterpillar, is a major pest in the rice fields. When a gene from *Bt* that led to expression of Cry toxins was introduced into the rice plant, significant mortality of the stem borer pests was recorded (some 100% after 4 days), thus protecting the crops and allowing them to grow; meanwhile the plants retained their normal phenotypes[6].

The production of Cry toxins has been well described from the genetic level to translation via the ribosome[7]. At the level of transcription, scientists have identified five

* Corresponding author:
kmukhtar@uab.edu (Karolina M Pajerowska-Mukhtar)
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sporulation-specific transcription factors, which are tightly regulated and include σ^H (active in pre-division cell), σ^F and σ^G (active in the forespore), and σ^E and σ^K (active in the mother cell). Various promoters will employ these transcription factors; *BtI* promoter (downstream) for the *cry1Aa* gene is active between stages II and VI of sporulation and utilizes the transcription factor σ^E. An upstream promoter region, *BtII* is active later in sporulation and utilizes σ^K. After transcription, transcriptional terminators contribute to increased mRNA stability resulting in a half-life that is five times greater than that of most bacterial mRNA. At the level of translation, a perfect Shine-Dalgarno sequence has been identified, encouraging quick binding of ribosomes. This has been suggested to further protect the mRNA from degradation[5].

MON810, a variety of genetically modified corn produced by a Missouri, US-based plant breeding company Monsanto, is perhaps the best known example of a herbicide-resistant GM crop, marketed with the trade name YieldGard. This variety expresses the Cry1Ab insecticidal toxin under the control of enhanced 35S promoter from *Cauliflower mosaic virus* and incorporates the maize *Hsp70* intron. The toxin binds to receptors in Lepidopteran insects' guts, causing a quick decease, and is especially effective against corn borer (*Ostrinia nubilalis*). There are no binding sites for Cry1Ab on the surface of mammalian intestinal cells, therefore, livestock animals and humans are not susceptible to this protein. MON810 is grown in a number of countries worldwide, including USA, Canada, Australia, and China. An ongoing debate on the biosafety of MON810 was initiated when several European countries banned this variety[8], claiming various threats to humans, lower animals and ecosystems. Despite a publication arguing against this decision, and a statement from the European Food Safety Authority[9], MON810 is being continuously withdrawn from additional EU countries, such as Poland in April 2012.

2.2. Herbicide Tolerance

Weeds present a key threat to crops as they compete for light, water, and key nutrients. Herbicides along with tilling practices are used to remove the unwanted growth, but both of these can cause damage either to the crops themselves or the soil and surrounding water systems. Herbicide resistance of key crops could lead to less tilling, thereby resulting in improved water quality and less soil erosion[2]. Researchers have developed crops (soybean, alfalfa, cotton and corn) that are resistant to a key, non-toxic herbicide glyphosate[2]. These plants were engineered by the insertion of a gene from *Agrobacterium* that codes for CP4 EPSP, a glyphosate-insensitive form of a required enzyme, 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase[10]. These crops are known as RoundUp Ready crops and are currently marketed by Monsanto, the makers of the herbicide RoundUp (glyphosate)[11]; as of 2005, these transgenic crops represented 87% of soybeans, 61% of cotton and 21% of corn planted in the United States[10]. Newer research focuses on utilizing plastid genomes instead of nuclear genomes to produce herbicide tolerant plants[12].

2.3. Viral Resistance

Two specific cases, in which viral resistance was developed in plant species, provide examples of the wide range of possibilities in genetic engineering. The first example from the 1990s was in response to the papaya ringspot virus (PRSV) that had threatened to wipe out the Hawaiian papaya production multiple times in the past. Dennis Gonsalves, a local Hawaiian, engineered papaya to carry a mild strain of PRSV with a premature stop codon to prevent full expression of the coat protein[2]. The second example demonstrates a method scientists may use to identify gene candidates for further study. Researchers noted that resistance to Karnal bunt disease, which affects wheat, is increased when the wheat is treated with jasmonic acid (JA). JA-treated wheat displayed marked increases in expression of WC_2, WC_3 and WCMD cystatins, which makes them good candidates for engineering wheat strands with greater resistance to the virus[13].

While researchers work to find ways to guard plants against pathogens two issues have plagued them; one is the short-lived term of the resistance due to a mutation in the pathogen, and another is the loss of fitness and other negative pleiotropic effects associated with genetic changed in the resistance (*R*) genes[14]. Additionally, these genetic changes usually confer resistance to only one pathogen. In order to increase resistance while maintaining plant fitness, current efforts focus on a different gene target: pathogen-associated molecular pattern (PAMP) receptors[14]. Specifically, a receptor from *Arabidopsis* that recognizes a highly conserved bacterial elongation factor (EF-Tu) has been introduced to tobacco and tomato plants, both leading to broad-spectrum resistance against some of the most common enemies of plant growth[14].

2.4. Nitrogen Use Efficiency

Billions of dollars are spent each year on fertilizer that contains ammonia, a form of nitrogen that has already been fixed for utilization by plants. However, plants only utilize 30 - 40% of the applied nitrogen. Increasing their nitrogen use efficiency by only a few percentage points could save millions each year. Studies being carried out in Arabidopsis have identified a *GNC* gene, member of a GATA transcription factor family, that may play a role in nitrogen use; overexpression of this gene led to positive effects on plant growth. However, in rice, a negative pleiotropic phenotype was observed, suggesting the need for greater understanding of nitrogen fixation and mobilization pathways for more targeted gene modifications[15].

2.5. Biomass Increase

Many countries are searching for possible alternate sources of fuel. One possible source is biofuels, such as ethanol from corn[16, 17]. As such, there is a push to increase biomass productivity on lands already in use for purposes in addition to human consumption. Multiple genes and systems have been targeted for these changes, including overexpression, loss of function, and gene shuffling to produce changes in photosynthetic genes, lipid-biosynthetic enzymes, transcription factors, and other target pathways. Other researchers have focused on genes located outside the nucleus, in the chloroplasts. In tobacco chloroplasts expressing a desaturase gene taken from potato or cyanobacteria, marked increase in leaf lipids was noted[12].

2.6. Stress Resistance

As bleak forecasts of global warming and increasing environmental stressors continue, researchers search for ways to prepare plants for the coming onslaught. One possible target for increased stress resistance is a group of molecules called polyamines (Pas), which include spermidine (Spd), spermine (Spm) and putrescine (Put), their precursor. Pas have anti-stress effects through acid neutralization and antioxidant properties as well as membrane stabilizing ability[18]. Plants genetically engineered to overexpress the genes coding for these molecules have shown improved stress tolerance, even to multiple stressors such as salinity, drought, and heat[1].

Other studies have taken a different approach by focusing on specific stressors and attempting to identify gene targets for future engineering. Leakey et al. placed soybeans under the stress of elevated CO_2 levels and studied changes in both phenotype and gene expression[19]. Over the two years, they monitored changes in more than 37,000 RNA transcripts. Increases were mainly noted in transcripts associated with carbohydrate metabolism and respiration. While this research did not lend itself to immediate changes in soybeans, it provided a foundation for further studies.

2.7. Healthy Crops

Genetic engineering has opened the door to possible changes that a decade ago would have read like a page from a science fiction novel[20]. Researchers are now working to engineer crops with health benefits, including crops that grow vaccines and antibiotics. As geneticists turn more attention to non-nuclear genomes, many are beginning to see the chloroplasts as a potential machine for producing pharmaceuticals, such as vaccines and antibiotics, in addition to well-studied starches. Preliminary studies have already shown that genetic engineering can be used to accomplish this purpose, but expression levels have been quite variable. Additionally, oral vaccine experiments have been minimal and confined to small animal models[12].

Perhaps less lofty than growing pharmaceuticals is the goal of making our current plants more advantageous through the production of bioactive peptides or increased vitamin content. Rice, a ubiquitous source of nutrition around the world, has very few significant physiological properties, unlike soybean. In an attempt to increase the positive effects of rice consumption and improve world health, researchers have developed rice seeds that express beta-conglycinin from soybeans. Regions of this protein have bioactive properties including hypotensive, hypocholesterolemic and memory-enhancing activity, thus demonstrating the potential for this strategy to contribute to improving health in the 21st century, especially in less developed nations where rice and other starches serve as a main source of nutrition[21]. Another example of genetic engineering targeted to rice is the so-called “golden rice”, overaccumulating β-carotene, a precursor for vitamin A that has been shown to prevent or reduce occurrence of blindness, common among poorer populations of Asia[22-24]. The amount of “golden rice” consumed in a typical third world diet could provide about fifteen percent of the recommended daily allowance of vitamin A, sufficient to prevent blindness.

Following the same path, efforts were launched towards increasing iron assimilation in populations largely relying on rice as staple food to fight iron-deficiency anemia. Three parallel approaches were applied, including (i) transfer of a ferritin gene from *Phaseolus vulgaris* into rice grains, increasing their iron content up to two-fold[25], (ii) transfer of a thermotolerant phytase to increase iron bioavailability by breaking down phytate, a substance naturally present in rice grains that inhibits iron uptake, and (iii) overexpression of the endogenous cysteine-rich metallothionein-like protein that is considered a major enhancer of iron absorption[26].

2.8. Global Implementation

Transgenic crops are currently in all stages of testing, from gene transfer and laboratory testing to confined field tests to successful commercialization. Golden rice, for example, has undergone over five years of laboratory testing and is now being field testing in both Bangladesh and at AgCenter Rice Research Station in Louisiana, USA. In both of these tests, the golden rice performed equivalent to the control seed while producing beneficial carotenoids[27]. Another strain of golden rice (Golden IR64) demonstrated similar results in greenhouse tests in the Philippines[27]. A variety of drought-resistant maize that contains the cold-shock protein B (CspB) from *Bacillus subtilis* has been trial tested in various African countries, including Tanzania, Uganda, Kenya, and Mozambique[28]. Sixteen different drought tolerant varieties are currently undergoing National Performance Trials (NPT) in Kenya with other countries expected to follow later this year[29]. *Bt* crops are some of the most widely used in the United States and their implementation is increasing around the world. In Kenya, field trials of *Bt* cotton are going into their third season. Preliminary results have shown a marked decrease in targeted pests[30]. *Bt* crops are showing promise in Africa and India as well. In South Africa, *Bt* maize, soybean and cotton are currently being released for commercial production following a five-year field trial of *Bt* cotton, which demonstrated about 40% higher yields than their non-transgenic counterparts and resulted in a 42% decrease in pesticide spraying cost. In Burkina Faso, *Bt* cotton has been in field tests since 2003, demonstrating 30% higher yields and a reduction in spraying cost[31]. In India, the commercialization of *Bt* cotton has resulted in doubling of the cotton production, with 50% of that increase attributed specifically to the *Bt* crop. On the horizon for India, *Bt* rice is currently in field tests and *Bt* brinjal (eggplant) has been approved for seed production, with field tests to begin soon thereafter[32]. A large number of other GM crops are also currently in field trials, including sweet potatoes, banana, cowpeas, sorghum, wheat and sugarcane in Africa, cabbage, cauliflower, groundnut, okra, and tomato in India[31, 32]. What once seemed like a far-fetched dream is quickly becoming a reality here in America and around the world.

3. Biotechnology at the DNA Level

In order to produce the changes in phenotype discussed above, there are a number of different groups of genes that may be targeted. Some of these pathways are well understood and have been applied in commercial production. Others are only in the beginning stages and currently produce many undesirable pleiotropic traits, requiring more research to understand and eliminate the negative side-effects. A few of these gene targets are discussed below.

3.1. Photosynthetic Genes

Targeted mainly in an attempt to increase biomass, there are numerous photosynthetic genes that have been the subject of genetic modification. For example, plants that normally utilize a C_3 pathway have been transformed through the insertion of C_4 pathway enzymes such as PHOSPHOENOLPYRUVATE CARBOXYLASE (*PEPC*) and PYRUVATE ORTHOPHOSPHATE DIKINASE (*PPDK*). Rice with these modifications had 30 to 35% higher yield[4]. Another unique example was the use of gene shuffling in the area that codes for RUBISCO ACTIVASE (RCA), which limits photosynthesis at high temperatures. This shuffling led to more thermostable varieties of Arabidopsis that demonstrated higher rates of photosynthesis when exposed to moderate temperature stress[4].

3.2. Introduction of Single Base Mutations

Instead of targeting an entire gene, some research has focused on slight modifications (alteration of one or two nucleotides) to produce changes in protein activity. In an attempt to increase the positive biological activity of the soybean beta-conglycinin peptide, one Threonine was replaced with Phenylalanine or Tryptophane. Prior to actually performing the change, computer modeling was utilized to insure correct folding and the modification was expressed in *E. coli* to check for activity. This research demonstrates that small changes in the genome, such as single nucleotide substitutions, can lead to significant changes in peptide activity and ultimately phenotype[21].

3.3. Polyamine Coding Genes

As discussed previously, polyamines are a group of molecules thought to be involved in stress responses. These ubiquitously present molecules have been targeted in plants to increase stress resistance[18]. Modifications in the genes that are involved in the polyamine pathways have led to increased stress resistance to salinity, heat and cold, drought, and other stresses; a decrease in tolerance has been seen in plants that lack these polyamines or a portion of the synthetic pathway. A list of the polyamine-related genes that have been targeted as well as the specific results is reviewed in[1].

3.4. Non-Nuclear Genes

Since a transgene integrated in the nuclear genome stays viable in the male gamete and is dispersed via the pollen, transgene out-cross incidence to wild relatives may be as high as 38% in sunflower and 50% in strawberries[33, 34]. Recently, there has been a negative public perception against GMO crops due to the documented transfer of transgenes to wild relatives of a few plant species. Maternal inheritance of a transgene, and thus effective elimination of transgene escape via pollen, is possible if a transgene is integrated into the chloroplast genome[35]. Not surprisingly, therefore, plastids have recently become a popular target for exploration in genetic engineering. These present promising targets as their genomes (about 60 genes) are much smaller than the nuclear ones and can easily incorporate new genes or gene clusters[12]. Moreover, expression level of the foreign protein in the chloroplast is enhanced several 100 fold compared to the nuclear-integrated transgene. Modification of plastid DNA was first accomplished in 1988; however, it has not yet been utilized in commercial crops at a broader scale. One successful modification was the expression of a transgene in plastid that led to herbicide resistance, including resistance to glyphosate, isoxaflutole, and sulfonylurea[12]. Another exciting application of plastid genetics was the construction of luminescent plants by the expression of the bacterial luciferase pathways, involving six genes[12]. This demonstrates the ability to translate bacterial proteins into plastids and the conservation between plastid and prokaryotic translation mechanisms. One limitation of using plastid-encoded herbicide resistance is their large number within the cell that makes it difficult to obtain a uniform population of plastid genomes. While challenging, plastid genetic modification has been accomplished, providing promise for future gains.

4. Controversies and Concerns

As the amount of genetic plant engineering grows, so do concerns about its safety to humans and the environment as well as its quality and regulation. While the benefit/cost ratio of *Bt* crops is the highest for any agricultural innovation in the past 100 years[2], many question whether or not the greatest costs are yet to be seen in relation to human health. In a study published in 2009, de Vendomois et al. compared the effects of three genetically modified (GM) corn varieties to their non-GM counterparts; the results of five and fourteen week rat-feeding trials showed some significant differences between the groups, including variations in urine phosphorus and ion levels, serum glucose and triglyceride levels[36]. The authors, however, caution that these are only "signs of toxicity" and not "toxic effects," and encourage repeated trials in several animal species[36, 37]. In 2008, the European Food Safety Authority (EFSA) published a study that stated "no biologically relevant differences between control and test animals" have been detected, thus approving the crops for human consumption[38]. Since that report, various trials have been completed that tentatively support that stance while calling for further research. Walsh et al. studied the effects of GM corn, specifically MON810, on the immune response of pigs during and following a 110-day-long feeding trial. The experiment detected no gene-specific antibody response[39], further supported by similar studies in pigs[40] and mice[41]. The trials did detect higher lymphocyte and leukocyte counts in all *Bt*-fed pigs at some point in the trial[40]. Another study demonstrated significant increase in leukocyte count in mice fed *Bt* triticale but still within the normal limits for these animals[42]. Despite these changes, Walsh et al. states that no adverse immune response is anticipated due to the fact that T-cells, spleen weight, and fecal bacterial structure all remained unchanged[40]. These varied conclusions demonstrate the necessity of larger and more prolonged trials. Additionally, human epidemiological studies could contribute greatly to this discussion.

Others are concerned about the unknown effects of gene flow from these genetically modified plants to surrounding areas via pollen distribution. One highly publicized and criticized study claimed that GM crops had contaminated wild corn in Mexico[43]. However, later studies did not detect any evidence of transgenes in these wild corn populations[44]. In the late 1990s, a paper was published citing harmful effects of *Bt* plants to the larvae of the monarch butterfly in a lab study; further studies have found the threat to be insignificant as there is little likelihood of these high levels of exposure[45]. One suggestion for alleviating some of the concerns of gene flow from genetically modified species is by concentrating modifications on plastid genes, which are not passed to offspring through pollination[12]. Another major limitation of the current crop biotechnology is the limited stability of the transgene. Frequently, the beneficial effects of the novel gene are diminished or even completely disappear after several generations, usually as a result of gene silencing[46-48]. One possible strategy to circumvent this problem is the recent innovation of a method for synthetic clonal reproduction through seeds[49]. Such seed production via apomixis would constitute a major step towards making hybrid crop plants that can retain favorable traits from generation to generation.

A yet another field of concern is the increasing monopoly on GM crops, imposed by certain breeding companies. For example, about 9 out of 10 soybean seeds carry the Monsanto's Roundup Ready trait, as does cotton and corn. Monsanto sells 90% of the world genetically engineered seeds and exclusively produces Roundup Ready soybean seed for the commercial market[51]. This monopoly over the seed market has become quite controversial as no seed company could survive without selling Roundup Ready seeds. Moreover, Monsanto has designed a system that literally traps farmers to continue using their product, as it is illegal to save patented seeds. Additional controversy was added to this story when it was discovered that some farmers' crops became contaminated with this patented gene without their own knowledge due to bird- or wind-crosspollination and hundreds of unaware farmers were sued by Monsanto on the grounds of patent infringement[52].

5. Conclusions and Future Directions

Looking into the future filled with unknowns of global warming, rising sea level, and a growing population, genetic engineering holds promise for sustaining the earth's inhabitants. The benefits of GM crops seem to be a welcome change to the struggling economy and definitely deserve a chance to prove their worth. Genetic modification has already been shown capable of increasing insect and herbicide resistance in numerous commercial crops[5, 6, 12]. Early research has shown the possibility of plants with greater biomass, increased levels of vitamin A, greater resistance to stress and even the production of foreign proteins like vaccines and antibiotics[2, 12, 19].

All of the above mentioned areas, however, will all require a wide-range of additional testing, from a better understanding of plant synthetic pathways to large-scale field testing. In addition to research, time must also be invested to develop government policy, address public concerns and monopoly issues as the application of genetic modification grows.

Finally, the GM crops represent a still relatively new technology and continuous research is needed to ensure that they are tested thoroughly enough to be made generally available to the public. We should not forget about potential far-reaching implications resulting from disturbing the natural balance of an ecosystem by introducing genetically modified organisms into it. The optimal strategy for the 21st century agriculture is a balanced combination of genetic engineering and an increasingly popular trend termed permaculture, which relies on farming crops based on relationships found in natural ecologies to help redesign and diversify crop layouts, leading to increased sustainability[53, 54].

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