Current Status and Future Potential of Plant Biotechnology

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Introduction

Plants have been continuously genetically modified and improved for centuries, initially by selecting seed from superior plants and reproducing these with continual selection and breeding. Traditional breeding methods have resulted in significant increases in productivity, with corn and wheat yields approximately doubling over the past 40 to 50 years. More recently, the ability to introduce DNA directly into crop plants has enabled the specific and selective genetic modification of plants through techniques commonly referred to as plant biotechnology, genetic engineering or recombinant DNA methodology. Numerous traits have been marketed or are being assessed for their potential to: (a) protect plants against insect damage, fungal, viral or bacterial diseases; (b) provide selectivity to preferred herbicides; (c) directly enhance crop yields; (d) increase nutritional value; (e) reduce naturally occurring toxicants or allergens or produce naturally decaffeinated coffee; (f) provide fruits or vegetables with superior flavor; (g) use plants as factories to make pharmaceutical products or to produce foods containing eatable vaccines; and many others.

History and Current Status

The first genetically modified plants were produced in 1983 using the bacterium *Agrobacterium tumefaciens*, which naturally transfers a specific portion of its own DNA into plants. This system was modified to enable the selective transfer and stable integration of specific genes of interest, like those encoding protection against insects or disease into plant cells and the regeneration of plants from these cells. Since the initial genetically modified plants were produced, techniques such as protoplast transformation and particle bombardment also have been used to directly transfer DNA into cells and stably insert the DNA into the plant genome. Today, over a decade and a half since the initial reports of the first genetically modified plants, almost all economically important plants have been genetically modified. By the end of 1997, more than 70 different crop species had been transformed and field tests had been conducted at more than 25,000 individual field sites, in at least 45 countries with at least 60 different crops (1).

Following extensive field testing, over 50 different products have been approved by the appropriate regulatory authorities for marketing in at least one country. Approximately 3 million acres of genetically modified plant products were grown globally commercially in 1996, with over 30 million acres planted in 1997, over 65 million acres in 1998, over 98 million acres in 1999 and the number of acres are expected to be maintained or to increase again in 2000. Thirteen countries (US, Argentina, Canada, China, Australia, South Africa, Mexico, Spain, France, Portugal, Romania, Bulgaria and Ukraine) grew nine crops (soybean, corn, cotton, canola, potato, squash, papaya, tomato and tobacco) commercially in 1999 (2). Approximately 55% of the soybeans, 45% of the cotton and 35% of the corn grown in the United States in 1999 were genetically modified, while over 70% of the soybeans grown in Argentina in the 1999/2000 season were genetically modified.

Insect Protection

Several different approaches have been or are being evaluated to control a variety of insect pests that cause tremendous damage to the most agronomically important crops. These pests typically had been controlled by use of a variety of chemical insecticides. Insecticides and associated management practices cost approximately $10 billion annually worldwide. Yet 20% to 30% of total crop product is still lost due to insect pests. Based on the importance and value associated with effective insect control and the availability of genes from the class microbes, *Bacillus thuringiensis* (*B.t.*), with insecticidal activity, genetically modified plants containing genes which provide protection from insect damage were among the first field tested and marketed. The effectiveness of this approach is reflected in the successful launches of insect protected potato, cotton and corn products (1, 2).

Approximately 1.8 million acres of genetically modified insect protected cotton varieties were grown in the U.S. in 1996, with approximately 2.2 million acres in 1997, 2.6 million acres in 1998 and 4.0 million acres in 1999 (2). In addition, insect protected cotton varieties were planted in Australia, Mexico, China and South Africa. These plants provide protection against the major insect pests in cotton. Cotton growers planting these varieties significantly reduced their use of chemical insecticides (3). while increased effectiveness in control of these insect pests resulted in a significant yield increase in cotton lint production while also resulting in increased numbers of beneficial insects due to increased specificity of the pesticidal protein. Insecticide use was reduced from an average of approximately 5.3 applications for conventional cotton varieties in the US major cotton growing areas in 1998 to an average of 1.4 applications for *B.t.* cotton, which resulted in a total reduction of approximately two million pounds of chemical insecticides eliminated (3). Reductions of insecticide use were even more impressive in China, where approximately 15 to 20 insecticide applications are typically used per growing season for cotton. Insecticide applications have been reduced by at least 80% by growing *B.t.* cotton in China (4).

Genetically modified *B.t.* corn plants protected against corn borer were planted commercially initially in 1996. A number of additional insect protected, genetically modified corn products entered the market in 1997 and acres have continued to expand rapidly in the US, Canada and Argentina (2). In addition to effective control of European corn borer, increased yields and reduced insecticide use, another key advantages of *B.t.* corn varieties is that those varieties which provide effective protection of corn ears from insect damage also drastically reduce the amount of secondary infection caused by the fungus *Fusarium*. The ability to control *Fusarium* results in up to 95% reduction of the level of the mycotoxin, fumonisin, under some conditions (5). Fumonisins are fungal toxins that produce morbidity and death in horses and swine (6) and have been linked in epidemiological studies to high rates of esophageal and liver cancer in African farmers (7). Comparable reductions in the levels of fumonisin have been demonstrated in the US, Italy, France and Argentina with additional studies being conducted to verify reductions in other geographies where corn is grown globally (5, 8).

The other major insect pest for corn is the corn root worm. Recent efforts have identified a novel *B.t.* gene which, when expressed in the roots of corn plants, enables the corn plant to protect itself against this major pest. By combining protection against both the European corn borer with protection against the corn root worm, the vast majority of chemical insecticides which have been traditionally used for corn production can be eliminated by the use of corn varieties containing both traits, which are combined by traditional breeding.

Research continues to develop insect protection in many other crops, e.g., tomato, rice, peanuts, soybeans and chickpeas through insertion of *B.t.* proteins. To date, hundreds of different *B.t.* genes have been isolated and characterized from a broad variety of *Bacillus* species. With this diversity of genes, many additional insect pests will be controlled and crops protected. In addition to the genes from *B. thuringiensis*, many other sources have been, and continue to be, evaluated for insecticidal proteins. Among the proteins that have been identified are those that interfere with the nutritional requirements of the insect. For example, protease inhibitors and alpha-amylase inhibitors continue to be tested individually and in combination to provide insect protection.

Virus Protection

Virus resistant genetically modified plants have been produced by using genes derived directly from the virus, so called pathogen-derived resistance (9). The first genetically modified plants, tobacco plants with increased resistance to tobacco mosaic virus (TMV), resulted from the expression of the TMV coat protein in tobacco plants (10). This coat protein-mediated resistance has been used extensively to confer viral resistance to numerous crops, including tobacco, tomato, squash, melon, papaya and potato. In 1987, TMV resistant tomato plants were the first transgenic tomato plants to be field tested. Squash varieties resistant to zucchini yellow mosaic virus (ZYMV) and watermelon mosaic virus (WMV) were approved in the U.S. in 1995 and marketed. These two viruses routinely reduce crop yields by 20% to 80% depending on production season and growing region. More recently, squash plants resistant to these two viruses plus a third destructive virus, cucumber mosaic virus (CMV), were developed (11). Resistance to papaya ringspot virus has essentially saved papaya production in Hawaii (12), while resistance to sweetpotato feathery mottle virus has the potential to increase sweetpotato yields in Kenya by up to 80% (13). Other viral-derived genes such as the viral replicase have been identified to provide effective control of viruses.

Fungal/Bacterial Disease Resistance

The identification of anti-fungal genes and the production of genetically modified plants that effectively control fungal disease lag significantly behind the production of insect and virus resistant plants. Recent advances, however, indicate that this will only be a temporary situation; genetically modified plants that are effective in controlling important plant diseases are being produced and evaluated (14).

Selectivity to Herbicides

Weeds are one of the major agricultural pests that can devastate a crop if not managed properly since weeds compete with crops and reduce yield, decrease harvest efficiency, decrease seed quality and serve as a reservoir for crop pests (15). Today herbicides are used on essentially 100% of the acreage of the major agronomic crops in developed countries. Traditionally, herbicides have been selected based on the weeds to be controlled and the natural resistance of the crop to the herbicide. Biotechnology provides an opportunity to modify crops so they tolerate selected herbicides with preferred environmental properties, such as glyphosate. These herbicide tolerant crops allow the farmer to apply herbicide to planted fields, killing weeds but leaving the planted crop unaffected. This ability provides increased flexibility and cost savings to growers. In addition, farmers can move from using pre-emergent, soil incorporated herbicides to post-emergent herbicides that are applied on an "as needed" basis. This strategy can reduce the number and total amount of herbicides used and enable the application of herbicides that bind tightly to the soil and are less likely to enter the ground water, thereby providing significant environmental benefits.

Soybeans, canola, cotton and corn tolerant to glyphosate, oilseed rape and corn tolerant to phosphinotricin, and cotton tolerant to sulfonylureas are examples of genetically modified, herbicide tolerant crops that are entering the marketplace (15). For example, glyphosate tolerant soybeans were introduced in 1996 and because of exceptional weed control, crop safety, cost savings and ease of use these soybean varieties were rapidly adopted in countries where they have been approved for marketing. For example, over 90% of the soybeans which are being grown in Argentina in the 2000/2001 growing season are tolerant to glyphosate.

Food Quality Enhancements

Although many of the initial genetically modified plant products have improved agronomic traits, the first plant biotechnology product marketed in the U.S., the Flavr Savr™ tomato, is a product with food quality enhancements. Many additional products with improved quality or nutritional properties are under development.

Oils provide the second most important source of food energy for humans. Plant biotechnology efforts have focused largely on altering the fatty acid composition of canola, soybeans and sunflowers, all major sources of dietary oils, to provide either more stable sources of specific oils or oils with enhanced nutritional characteristics (16, 17). The first modified oil product to be marketed is a high laurate canola oil which serves as an alternative for oils with similar composition, such as tropical oils. A genetically modified soybean variety with increased levels of oleic acid, a mono-unsaturated fatty acid, and reduced levels of poly-unsaturated fatty acids, also has been produced. This product exhibits increased oxidative stability and significantly reduces the need for and costs of hydrogenation during oil processing. Elimination of chemical hydrogenation eliminates the production of *trans*-fatty acids, which have been linked to cardiovascular disease.

Foods that provide sufficient quantities of the essential amino acids are critical to meet dietary needs, especially in developing countries where foods low in one or more of these amino acids are often consumed. Approaches to increase the amount of lysine in cereals and methionine in legumes are being assessed (18). These amino acids may be introduced in their free form (e.g. as lysine or methionine) or they may be added as part of proteins, particularly seed storage proteins that are present at relatively high levels in the grain and are rich in these essential amino acids. For example, total lysine content of both canola and soybean was increased two and five fold, respectively, by expressing non-feedback inhibited forms of two key enzymes in lysine biosynthesis. A two to three fold increase in the total lysine content of corn also was obtained using the same approach.

Numerous research efforts to increase the levels of key vitamins and minerals in genetically modified crops are on-going. In developing countries where malnutrition is a major concern, enhancing these components in staple foods may improve nutritional status and reduce the risk of disease. These projects include increasing the levels of beta-carotene, the precusor of vitamin A, vitamin D, iron and other nutrients. The most exciting of these research programs is the production of a variety of rice with significantly increased levels of both beta-carotene and iron (19). The World Health Organization estimates that vitamin A deficiency affects over 250 million children worldwide, causing night blindness and vulnerability to disease, and leading to over 200 million deaths per year. Plants genetically improved to contain increased amounts of beta carotene, the precursor of vitamin A, may play an important role to address this problem. This high beta-carotene, high iron rice could also help address iron deficiency anemia, another widespread nutritional problem. Consumption of 300 grams of this genetically modified rice, which contains increased levels of beta carotene and iron, could satisfy the RDA for both vitamin A and iron. A canola variety with greatly increased levels of beta-carotene in the oil also has been developed (20).

Plant biotechnology also is being used to reduce or eliminate a number of food components, particularly components which are undesirable. For example, the level of one of the major allergenic proteins in rice has been reduced by approximately 80% by researchers in Japan (21). Other efforts to modify the amino acid sequences which are responsible for eliciting the allergenic response also have proven successful. Efforts to reduce or eliminate other undesirable components of foods, e.g., glucosinolates in canola meal, protease inhibitors in beans, glycoalkaloids in potatoes and mycotoxins in corn are being evaluated. Components such as caffeine from coffee beans can be eliminated to provide a coffee with no caffeine without using chemicals (22).

Plants as Production Factories

Plants provide an unmatched capacity to produce products with a maximum efficiency and a minimum of energy - thus, in an extremely cost effective manner. As illustrated in the previously described examples of insect control, plants can be bioengineered to produce their own insecticidal proteins using carbon dioxide, water and nitrogen, thereby reducing the need for expensive and energy intensive chemical insecticide manufacturing plants. A potato variety developed to contain genes that produce proteins that protect it from both the primary insect pest (Colorado potato beetle) and the major viral pest, potato leaf roll virus, is an excellent example. The combination of these two traits enables potatoes to be grown without the application of chemical insecticides to control these two major pests and will provide significant environmental and economic savings. Only water, carbon dioxide and nitrogen are required for the genetically modified potato plant to protect itself from these pests. In contrast, extensive raw materials and energy in the form of oil are required to produce the approximately 5 million pounds of chemical insecticides that are currently used to control these pests annually in the U.S. Approximately 2.5 million pounds of waste are generated annually in the production process. Less than 5% of the applied insecticide actually reaches the target pest. Using the plant to produce these pesticides is therefore much more energy efficient and environmentally acceptable.

Given these efficiencies in using plants as production factories, efforts are underway to produce protein-based pharmaceutical products and other compounds in plants. One of the most exciting applications of plant biotechnology is the ability to produce edible vaccines in plants. For example, transgenic potatoes containing the non-toxic binding subunit of the *Escherichia coli* enterotoxin (23) as well as transgenic potatoes which express the capsid protein of Norwalk virus (24) have been produced. The expressed proteins function as an oral immunogen in humans. Efforts are underway to introduce such vaccines into banana, which would be eaten raw and thus serve as an ideal source to deliver this technology to developing countries, where inexpensive vaccines are urgently needed.

Advanced Genomics

The tools of molecular biology can also be used to accelerate the rate by which traditional breeding can be used to produce in plant varieties by identifying and effectively transferring regions of genomic DNA which confer useful traits, like disease or yield to current commercial germplasm. With the considerable attention given to the development of the initial draft of the human genome, drafts of the rice genome also has been developed and is being used to identify key genomic DNA which confers important characteristics to rice and other plants. For example, a region of DNA which enables yield increases in corn has been identified from an old ancestor of corn. When this portion of the genome from this ancestral corn variety was bred into high yielding commercial varieties of corn, yield increases of approximately 15% were obtained. Many other traits can and will be identified which will be transferred from old varieties of crops to improve the disease resistance, quality or yield of our current crop varieties.

Conclusions

The benefits of genetically modified plant products are being realized as the first products establish their place in the market. Initial benefits were realized primarily by farmers who produce crops with increased yields and quality while using more environmentally friendly practices, such as decreased tilling and approaches which require less pesticide use. Although consumers may not see these benefits first-hand, nevertheless, they translate into a more stable and abundant food supply and an improved environment. Future generations of genetically modified plant products will include products with enhanced nutritional qualities that will directly improve the diets of people globally. Finally, the possibilities of using plants as clean, efficient factories for the production of medicines, biodegradable plastics and other important compounds are beginning to be explored.

References

1. James, C 1997 Global status of transgenic crops in 1997. ISAAA Briefs No. 5. ISAAA: Ithaca, NY.
2. James, C 2000 Global Review of Commercialized Transgenic Crops: 1999. ISAAA Briefs No. 17. ISAAA: Ithaca, NY.
3. Gianessi, LP and JE Carpenter. 1999. Agricultural Biotechnology: Insect Control Benefits. National Center for Food and Agricultural Policy.
4. Xia, JY, JJ Cui, LH Ma, SX Dong, XF Xui. 1999 The role of transgenic Bt cotton in integrated insect pest management. Acta Gossypii Sinica 11:57-64.
5. Munkvold, GP, Hellmich RL, Rice, LG 1999 Comparison of fumonisin concentrations in kernels of transgenic Bt maize hybrids and non-transgenic hybrids. Plant Dis. 83:130-38.
6. Norred, WP 1993 Fumonisins-mycotoxins produced. J Tox Environ Health 38:309-28.
7. Marasas, WFO, K Jaskiewicz, FS Venter, DJ van Schalkwyk 1988 Fusarium moniliforme contamination of maize in oesophageal cancer areas in the Transkei. S Afr Med J 74:110-14.
8. Masoero, F, M Moschini, F Rossi, A Prandini, A Fietri. 1999. Nutritive value, mycotoxin contamination and *in vitro* rumen fermentation of normal and genetically modified corn (CryIA(B)) grown in northern Italy. Maydica 44:205-209
9. Kaniewski, WK and EC Lawson. 1998 Coat Protein and Replicase

Mediated Resistance to Plant Viruses. In: Hadidi A, Khetarpal RK, Koganezawa H (eds.), Plant Virus Disease Control, APS Press, St. Paul, Minnesota, pp. 65-78.
10. Powell-Abel, P, RS Nelson, B De, N Hoffmann, SG Rogers, RT Fraley, RN Beachy. 1986. Delay of disease development in transgenic plants that express the tobacco mosaic virus coat protein gene. Science 232:738-743.
11. Tricoli, D.M., K.J. Carney, P.F. Russell, J.R. McMaster, D.W. Groff, K.C. Hadden, P.T. Himmel, J.R. Hubbard, M.L. Boeshore, J.F. Reynolds and H.D. Quemada. 1995. Field evaluation of transgenic squash containing single or multiple virus coat protein gene constructs for resistance to cucumber mosaic virus, watermelon mosaic virus 2 and zucchini yellow mosaic virus. *Bio/Technology* 13, 1458-1465.
12. Manshardt, R. 1999. The Development of Virus Resistant Papaya in Hawaii. ISAAA Brief NO 11:9-11. ISAAA: Ithaca, NY.
13. Kaniewski, W., D. Maingi, M. Kaniewska, S. Flasinski, J. Lowe, D. Kirubi, C. Macharia, F. Wambugu, R. Horsch and M. Hinchee. 2000. Engineered resistance to sweetpotato feathery mottle virus in sweetpotato. Fifth Triennial Congress of the African Potato Association. Kampala, Uganda, pp. 37.
14. Shah, D.M., C.M.T. Rommens and R.N. Beachy. 1995. Resistance to diseases and insects in transgenic plants: progress and applications to agriculture. Trends in Biotechnology 13:362-368.
15. Duke, S.O. (Editor) 1996. Herbicide-Resistance Crops: Agricultural, Environmental, Economic, Regulatory and Technical Aspects. CRC Press, Inc., Boca Raton, Florida.
16. Budziszewski, G.J., K.P.C. Croft and D.F. Hildebrand. 1996. Uses of biotechnology in modifying plant lipids. Lipids 31:557-569.
17. Murphy, D.J. 1996. Engineering oil production in rapeseed and other oil crops. Trends in Biotechnology 14:206-213.
18. Falco, S.C., T. Guida, M. Locke, J. Mauvais, C. Sanders, R.T. Ward and P. Webber. 1995. Transgenic canola and soybean seeds with increased lysine. Biotechnol 577-82.
19. Gura, T. 1999. Biotechnology: New genes boost rice nutrients. Science 285: 994-995.

20. Shewmaker C, J.A. Sheehy, M. Daley, S. Colburn and D.Y. Ke. 1999. Seed-specific overexpression of phytoene synthase: increase in carotenoids and other metabolic effects. `Plant J. 20:401-12.
21. Matsuda, T. Application of transgenic techniques for hypo-allergenic rice. Proceedings from the international symposium on novel foods regulation in the European Union - integrity of the process of safety evaluation. Berlin, Germany 1998; 311-314.
22. Kato, M., K. Mizuno, A. Crozier, T. Fujimura and H. Ashihara. 2000. Caffeine synthase gene from tea leaves. Nature 406:956-957.
23. Tacket, C.O., H.S. Mason, G. Losonsky, J.D. `Clements, M.M. Levine and C.J. Arntzen. 1998. Immunogenicity in humans of a recombinant bacterial antigen delivered in a transgenic potato. Nat. Med. 4:607-609.
24. Tacket, C.O., H.S. Mason, G. Losonsky, M.K. Estes, M.M. Levine and C.J. Arntzen. 2000. Human immune responses to a novel Norwalk virus delivered in transgenic potatoes. J. Infect. Diseases 182:302-305.