Chapter 3
Transgenics for New Plant Products, Applications to Tropical Crops

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Abstract Advancements in plant science and agricultural technology now allow the direct transfer of gene(s) from diverse origins into target crops for improvement, with the advantages of breaking cross-species barriers and saving time in comparison to conventional breeding and selection. Transgenic technology has been used and commercialized since 1994 to produce new crop products with herbicide tolerance, insect resistance, virus resistance, and improved post-harvest quality. These input traits are characteristic of first generation transgenic crops that continue to be widely adopted by farmers globally. Numerous transgenic crop new products, with increased emphasis on output traits such as improved and novel product quality (which are more appealing and directly beneficial to the consumers), are under development and field testing. Activities in developing crops with new and better agronomic properties and using plants as bioreactors to produce high value products are also on the rise. While tropical plant germplasm, with its rich biodiversity increasingly revealed through gene discovery through genomics and associated technologies, can offer novel genes and regulatory mechanisms for crop improvement, transgenic technology provides a complementary approach with new possibilities for improving tropical crops to assure food security and nutritional well-being of the people in the tropics.

3.1 Introduction

Plants are the primary source of food for humans and feed for their livestock. Through domestication and activities of breeding and selection, plants have been developed into crops that serve as the major source of dietary carbohydrates, proteins, lipids, vitamins, and minerals for humans and livestock. To enhance agricultural productivity, plant characteristics contributing to crop yield, quality, and production economics are identified and objectives established to increase total biomass, harvestable yield, nutritional quality, resistance to biotic and abiotic stresses, and to improve plant architecture, response to photoperiod and inputs, and processing characteristics. With continuous progress in plant sciences, plant genes were cloned

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and the first transgenic plant expressing a foreign gene was realized in 1983. Since then, a powerful new agricultural biotechnology, in addition to the conventional breeding and selection methods, has been available for application to crop improvement. This new technology has the advantages of breaking the cross-species barrier for gene introduction and is less time consuming for generating new traits/products when compared to conventional crop improvement methods. Through recombinant DNA and transgenic techniques, the new method offers unlimited source of genes and methods for their regulation that are more specific, precise, and time saving than are the traditional methods for crop improvement. With this promise and capability, the first ever transgenic tomato with improved post-harvest quality was approved for marketing in 1994; the first transgenic crop product with insect resistance was commercialized in 1996. By the year 2006, 11 years after the first commercialization of any biotech crop, 10.3 million farmers in 22 countries had planted 102 million hectares of transgenic crops, a 60-fold increase over the global biotech crop planting area in 1996. Transgenic soybean ranked top among crop species with 58.6 million hectares (m ha) (57%), followed by maize (25.2 m ha, 25%), cotton (13.4 m ha, 13%) and canola (4.5 m ha, 5%). In terms of transgenic traits, herbicide tolerance dominates (69.9 m ha, 68%), followed by Bt insect resistance (19.0 m ha, 19%) and stacked traits (herbicide tolerance plus insect resistance), 13.1 m ha (13%). From 1996 to 2005, global accumulated impact of transgenic crops in terms of net economic benefits to biotech farmers was 27 billion, and the accumulated reduction in pesticides was 224,300 million tons of active ingredients, equivalent to a 15% reduction in the associated environmental impact of pesticide use on these crops (James 2007).

The transgenic crop products currently on market, including the major traits of herbicide tolerance, insect resistance, and virus resistance, have all been generated by single gene transfer and manipulation to influence the performance of crop in the field and its production economics. These first generation biotech crops are more beneficial to the growers and developers with fewer inputs so that the traits involved are referred to as input traits (Castle et al. 2006), even though the environmental benefits of the traits benefit society at large. In the subsequent stage of crop biotechnology development, the transfer and manipulation of genes are targeted for increasing the quality of food, feed, and other products catering to the needs of the end users. Traits such as improved food nutritive content benefit the consumers more directly and are referred to as output traits, a characteristic of second generation biotech crops (Willmitzer 1999). Transgenic manipulation of output traits often involves more than a single gene and is thus a process of metabolic engineering. During the development of input traits, the concept of using plants as bioreactors to produce useful products arose. It has since been an area of active research and development in plant biotechnology. Recently, rapid advancements in genomics and related high throughput technologies have produced unprecedented opportunities for the discovery of genes and their regulatory mechanisms, which will accelerate the use of transgenic technology for crop improvement.

Tropical and subtropical regions of the world house over 50% of its biodiversity (Kochhar 1981), offering the greatest wealth of forms and genes from wild and

cultivated plants. For crop biotechnology, tropical biodiversity represents a rich and invaluable source of useful genes and associated regulatory elements/mechanisms for crop improvement. Tropical and subtropical regions are also the location of many developing countries having dense populations and large food needs. Many of the staple foods in these regions are starchy roots and tubers that provide calories, but are poor in nutritional quality due to their low content of protein and micronutrients. Thus, while the tropics can offer the world a wealth of novel genetic elements for crop improvement, and recent advances in tropical crop genomics coupled with transgenic technology have the potential to contribute to improving the adequacy and nutritional state of food for the tropics, there remains much to be done for this to be accomplished.

This chapter will cover transgenic new products from crops (emphasizing those already commercialized or under development) and will analyze the future prospects of plant transgenics for improving tropical crop plants.

3.2 Transgenic Plant Products

3.2.1 Commercialized Products

Transgenic crops such as soybean, cotton, corn, canola, squash, and papaya with input traits of herbicide tolerance, insect resistance, and virus resistance are the major transgenic products commercialized since 1996. Plants with these traits meet the farmers' desire of high yields with fewer inputs such as reduced herbicide and pesticide use while having reduced environmental impact. Studies have shown that when applications of agricultural chemicals were reduced as a result of growing insect resistant cotton, incidences of farmers' health problems were also reduced (Huang et al. 2002). Further, the first generation transgenic products were generated by relatively simple transgenic manipulation of single genes. All these factors contribute to the success of first generation transgenic crops.

3.2.1.1 Herbicide Tolerance

Weeds decrease crop yields, accounting for a 13% loss of total world crop production. Annual herbicide production and sale is the largest component in agrochemical business. In the US, the annual cost of herbicides is approximately US $5 billion. Developing crops having the herbicide tolerance input trait was thus one of the earliest targets of research and development on agricultural biotechnology, and the transgenic product became one of the first and most widely adopted commercialized biotech accomplishments. Most of the herbicide-tolerant crops now on market were engineered through two approaches: 1) the herbicide target molecules (either enzymes or other cell components) were engineered for over production, or to become insensitive to the herbicide, and 2) the crops were engineered with gene(s) or a pathway to degrade or detoxify the herbicide.

a. Glyphosate-tolerant crops: Glyphosate is a broad-spectrum, leaf applicable, non-selective, non-toxic to animals, organic phosphate herbicide that is easily degraded in soil. Glyphosate inhibits the enzyme 3-enolpyruvateshikimate- 5-phosphate synthase (EPSPS) of the aromatic amino acid synthesis pathway. A modified *Agrobacterium* gene encoding EPSPS (named CP4 EPSPS) was developed, and the gene CP4 *epsps* was introduced into soybean, generating transgenic soybeans tolerant to herbicide glyphosate (Padgette et al. 1966). Because glyphosate herbicides are relatively inexpensive and broadly toxic to nearly all broadleaf and grass weeds, and the use of herbicide-tolerant crops allow reduced- and no-till practices, transgenic glyphosate-tolerant soybeans are welcome by farmers and widely adopted. Currently over 85% of US soybeans and 56% of soybeans globally are glyphosate tolerant. Similar approaches have been applied to cotton, canola, and corn and these transgenic new products are increasingly adopted by farmers (Castle et al. 2006).
b. Crops tolerant to other herbicides: Phosphinothricin or bialaphos-based herbicides (glufosinate) are broad-spectrum and non-selective organic phosphate herbicides that break down rapidly in the soil. These herbicides strongly inhibit glutamine synthase activity in plants, resulting in the accumulation of toxic ammonium in the cells that kills the plants. To engineer glufosinate-tolerant crops, two approaches can be used, either over-express the glutamine synthase gene, or introduce a gene to deactivate the herbicide. The enzyme phosphinothricin acetyltransferase (PAT or BAR) modifies phosphinothricin into an inactive form through acetylation. The *pat* gene was isolated from *Streptomyces viridichromogenes* while the *bar* gene from S. *hygroscopicus.* Using either of these two genes, phosthinothricin-tolerant transgenic cotton, corn and canola were developed (De Block et al. 1987). Bromoxynil herbicides inhibit electron transport in photosynthesis. Introduction of nitrilase can detoxify bromoxynil. A gene encoding BXN nitrilase from *Klebsiella pneumoniae* (Stalker et al. 1988) was introduced into cotton and canola to generate resistance. However, transgenic crops with tolerance to these two classes of herbicides, especially the bromoxynil herbicides, are not as popular as those that are glyphosate tolerant since glyphosate costs less and controls more weed species (Castle et al. 2006). Using the same molecular approaches, tolerance has been generated in plants against the sulphonylurea and imidazolinone herbicides, which inhibit the branched-chain amino acid biosynthesis pathway, by introducing a mutant acetolactate synthase (*ALS*) gene that is resistant to the herbicides. For the herbicide atrazine, which inhibits photosystem II, resistance can be engineered by introducing a mutant gene for Q8 protein, or by introducing the gene encoding glutathione-S-transferase to detoxify the atrazine.

3.2.1.2 Insect Resistance

Insect pests cause approximately a 13% loss of world crop production even though the annual worldwide expenditure on insecticides amounts to US $8 billion. Crops

are affected and damaged by diverse species of insects, often with specificity to crops at a certain stage of development or specific organs of the crops. To engineer new crop products resistant to insects, genes conferring this input traits must first be identified. Most of the resistance genes discovered thus far target the digestive system of insects, either as an anti-feedant or as a toxin. Examples of such genes are those encoding proteinase inhibitors that block insect digestive enzymes, or induce hypersecretion of digestive enzymes leading to depletion of essential amino acids; amylase inhibitors that inhibit carbohydrate digestion, which in turn, affect insect larval development; lectins that can bind to the midgut epithelial cell; and chitinase that may affect the formation of chitin, a structural component of insects. Many of these genes have been introduced into a variety of crops where they have demonstrated different degrees of plant protection. However, it is the gene encoding a crystal protein (Cry) or delta-endotoxin in *Bacillus thuringiensis* (*Bt*), a gram-positive, spore forming soil bacterium, that has received the most attention and reached the highest level of usage in producing insect-tolerant transgenic crops. During sporulation, *Bt* produces crystal proteins that are highly toxic to a broad range of insects, but are not harmful to mammals. The Cry protein has a molecular weight about 130 kDa, although some truncated forms also occur. In the insect midgut, the Cry protein is processed into an active N-terminal 65–70 kDa truncated form that causes the death of insect. Cry proteins consist of a family of homologous forms exhibiting diversity in insecticidal specificity. Numerous Cry proteins and their genes have been identified and cloned, and used to generate crops for resistance to specific insect pests (Schuler et al. 1998, De Maagd et al. 1999, De Maagd et al. 2003, Whalon and Wingerd 2003, Federici 2005). Insect resistant cotton and corn transformed with the Bt genes *cry1Ac* and *cry1Ab*, respectively, were commercialized in 1996 and have since been widely adopted by farmers. It is worth noting that in addition to these private sector efforts, the public institution, the Chinese Academy of Agricultural Sciences, China, has also developed insect resistant cotton, by combining Bt gene *cry1Ac* with the trypsin inhibitor gene *CpT1* from cowpea, that is widely adopted by farmers in China (Wu and Guo, 2005).

3.2.1.3 Disease Resistance

Like weeds and insect pests, plant diseases also cause about a 13% loss of the total world crop production. Plant virus infections lead to a range of diseases causing significant economic damage to most of the world's major crops (Agrios 1997). Since there are no effective chemical viricides available, effort has been made from the inception of plant biotechnology to apply this new technology to develop virus resistance crops. Several approaches have been demonstrated to confer virus resistance to target crops, including the genes encoding viral coat proteins (CPs), replicases, movement proteins, proteinases, defective interfering RNA, and satellite RNAs. Recent studies suggest that plant protection against the viruses is, in most cases, by an RNA-based post-transcriptional gene silencing mechanism (O'Brien and Forster 1994, Cooper et al. 1995, Lomonossoff 1995, Dawson 1996, Baulcombe 1996, Fuchs and Gonsalves 1997, Beachy 1997, Malpica

et al. 1998, Waterhouse et al. 2001). The use of genes or gene sequences derived from viral genomes to confer virus resistance in transgenic plants is known as pathogen-derived resistance (PDR, Sanford and Johnston 1985). The first transgenic virus resistant crop commercialized was squash resistant to watermelon mosaic virus (WMV) and zucchini yellow mosaic virus (ZYMV) through the introduction and expression of the coat protein genes of WMV2 and ZYMV (Fuchs and Gonsalves 1995). Other crops with resistance to a variety of viruses have also been developed. However, the most successful and widely known virus-resistant transgenic product, jointly developed in 1997 by the public institutions Cornell University, University of Hawaii, and the United States Department of Agriculture, along with an industrial entity, Upjohn Company, is papaya, which is resistant to papaya ringspot virus (PRSV) through expression of the PRSV coat protein gene. Farmer and consumer acceptance of the PRSV resistant transgenic papaya varieties (Sunrise and Rainbow) has contributed greatly towards reviving the papaya industry in Hawaii that had been decimated by this virus (Ferreira et al. 2002).

3.2.1.4 Improved Post-harvest Quality

Ripening is a normal maturation process of many fruits and vegetables. Delayed ripening by transgenic technology will allow farmers more flexibility in marketing their products and offer consumers produce near maximum freshness. Delayed ripening benefits both farmers and consumers and thus can be classified as both an input and output trait. Transgenic approaches developed to control the ripening process include aspects of ethylene metabolism including the suppression of 1-aminocyclopropane-1-carboxylic acid (ACC) synthase, which converts S-adenosylmethionine (SAM) to ACC during ethylene synthesis, or suppression of ACC oxidase, which catalyzes the oxidation of ACC to ethylene, by anti-sense technology, or by introduction of a truncated copy of the synthase gene (Theologis et al. 1993, Ayub et al. 1996), and the reduction of the amount of ACC available for ethylene synthesis by an ACC deaminase transgene, which converts ACC to alpha-ketobutylic acid (Klee et al. 1991). Control of fruit softening during maturation was also demonstrated by the suppression of polygalacturonase (PG) enzyme through antisense technology (Sheehy et al. 1988), or by introduction of a truncated copy of the PG gene to delay the cell wall pectin from degradation during fruit maturation. Tomato engineered with improved post-harvest quality by the PG technology received approval for marketing in 1994, under the trade name Flavr-Savr^{TM}, marking the first ever transgenic crop approved for marketing.

3.2.2 Products Under Development

Numerous proof-of-concept transgenic plant products have been generated and reported by academic and industrial laboratories throughout the world. While certain of them may in the future turn into new products for commercialization, it is not the aim of this chapter to account for these events. Instead, prototype products that

have been applied for field tests in the US, indicating that they are further down the path towards application, are summarized here. As the United States is most active in plant transgenics research, development, and application, this information will provide insight relevant to the status and development of plant transgenic activities.

The database of APHIS/USDA's Biotechnology Regulatory Services (www.isb.vt.edu/cfdocs/ISBlists1.cfm) reveals, as of August 10, 2007, an accumulation of 16,814 field test permits have been approved since 1987. Among these plant transgenic activities, a total of 866 phenotypes are involved that can be grouped into 10 categories: agronomic properties (AP), bacterial resistance (BR), fungal resistance (FR), herbicide tolerance (HT), insect resistance (IR), marker gene (MG), nematode resistance (NR), other (OO), product quality (PQ), and virus resistance (VR). The category of HT ranks top, with 4,330 permits approved for field testing, representing 26% of all approved permits, followed by IR (3,698, 22%) and PQ (3,079, 18%), while NR comes in last (42, near 0%) (Figure 3.1). Thus transgenic products with input traits, mainly HT and IR, have dominated the scene the last 20 years. However, when the number of permits for each phenotype category approved for field testing is compared over 10 year intervals (between 1988 and 1997 and 2006), a declining trend is noted for all major input traits including HT, IR, and VR, while the output trait PQ and category of AP are on significant rise during this 20 year period (Figure 3.2).

While these trends may reflect the more mature, steady, and maintenance state of those major input trait products, it is clear that increasing interest is turning toward the output traits that are more appealing to consumers. The data on major individual phenotypes approved for field testing (45 permits or more), though generally in agreement with the category distributions, provide further details on the individual phenotypes under different categories (Table 3.1). For example, both glyphosate and phosphinothricin tolerance are dominating phenotypes, however, the former is far more active in development, attesting to certain extent of its popularity in the market. Alteration of carbohydrate, oil, and protein quality and

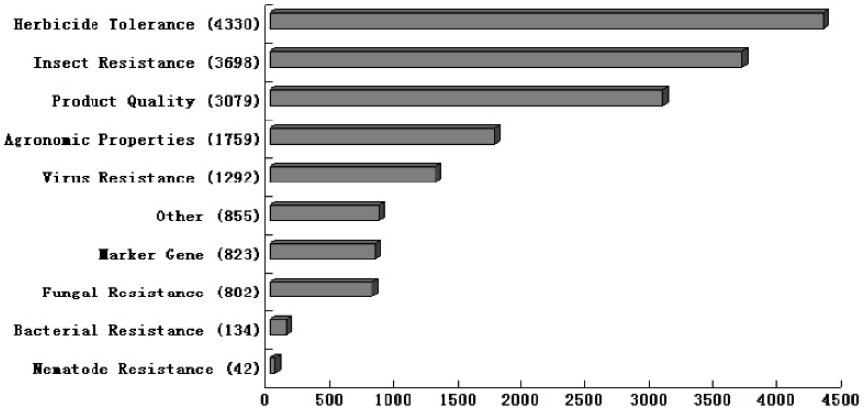

Fig. 3.1 Number of Permits for Each Phenotype Category Approved for Field Testing

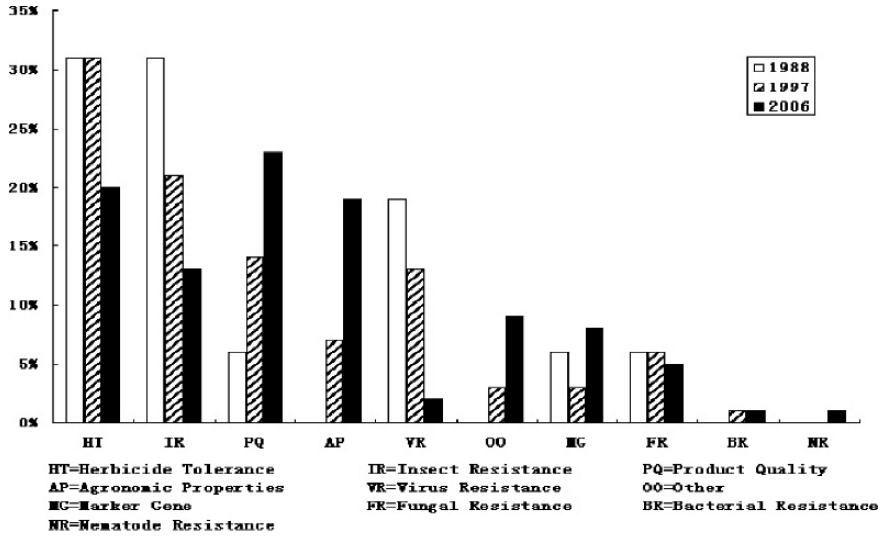

Fig. 3.2 Number of Permits (%) for Each Phenotype Category Approved for Field Testing

Table 3.1 Number of Permits for Each Phenotype Approved for Field Testing (Listed are Numbers $\geqq 45$)

Phenotype	Number	Phenotype	Number	Phenotype	Number
Glyphosate Tolerant	2506	European Corn Borer Resistant	139	Phytophthora Resistant	70
Lepidopteran Resistant	1736	Lysine Level Increased	138	Storage Protein Altered	70
Coleopteran Resistant	1356	Fertility Altered	134	Salt Tolerance Increased	68
Phosphinothricin Tolerant	1114	Altered Amino Acid Composition	124	Tryptophan Level Increased	67
Visual Marker	586	Growth Rate Altered	123	Solids Increased	64
Yield Increased	432	Zymv Resistant	120	Isoxazole Tolerant	63
CBI	403	Wmv2 Resistant	118	Lepidopteran Resistant/ Coleopteran Resistant	62
Carbohydrate Metabolism Altered	256	Fruit Ripening Altered	113	Bromoxynil Tolerant	58
Oil Profile Altered	252	Novel Protein Produced	108	Prsv Resistant	55

Table 3.1 (continued)

Phenotype	Number	Phenotype	Number	Phenotype	Number
Pvy Resistant	232	Imidazolinone Tolerant	103	Nitrogen Metabolism Altered	53
Drought Tolerant	229	Fusarium Resistant	92	Ear Mold Resistant	53
Seed Composition Altered	210	Pharmaceutical Proteins Produced	89	Glyphosate Tolerance	50
Colorado Potato Beetle Resistant	189	Fruit Ripening Delayed	89	Bruising Reduced	49
Cmv Resistant	179	Sclerotinia Resistant	86	Sulfonylurea Tolerant	48
Protein Quality Altered	158	Protein Altered	84	Lysine Level Alterered	47
Male Sterile	147	Gene Expression Altered	76	Lepidopteran Resistance	45
Plrv Resistant	140	Oil Quality Altered	74	Drought Tolerance	45

properties are major activities in the category of product quality, while developing plants as bioreactors to produce novel proteins is also notable. A total of 1,395 genes and 301 institutes, of both public and private sectors, are involved in the research and development of these prototype transgenic products. These statistical data document the very active efforts and innovation directed toward the development of new crop products through transgenic technology and reveal the transgenic new products that may appear in the future (www.isb.vt.edu/cfdocs/ISBlists1.cfm; www.agbios.com/dbase.php?action=synopsis).

3.2.2.1 Products and Approaches Relevant to Tropical Crops

While the input and output traits and the transgenic technology involved (reviewed above) are relevant to both temperate and tropical crop improvement, some products and transgenic approaches are of special interest to tropical crops.

Post-harvest Quality

Banana, papaya, pineapple, and mango are important climacteric fruits of the tropics. The proven transgenic technology to delay fruit and vegetable ripening, as reviewed in Section 3.2.1.4, can be applied to benefit these tropical fruits that are globally favorites of many, by enhancing their shelf life and retaining their freshness, appearance, flavor, and nutrition for long distance export.

Nutritional Quality

Crops provide the proteins, vitamins, and minerals needed for human nutrition. However, in certain staple crops, especially roots and tubers that are major staple foods in the tropics, plant proteins are often low in content and deficient in essential amino acids. When these staple crops are used as sole or major source of dietary proteins, such deficiencies will cause adverse effects on human nutrition and health (Young and Pellett 1994). In general, cereal proteins are low in lysine (1.5–4.5% vs. 5.5% of WHO recommendation) while legume, root, tuber, and most vegetable proteins are deficient in the sulfur amino acids (methionine and cysteine, 1.0–2.0% vs. 3.5% of the WHO reference protein) (Sun 1999). Several transgenic approaches have been attempted to enhance the essential amino acid contents in plants, including enhancing the protein bound fraction by (i) modifying the protein sequence (De Clercq et al. 1990, Dickinson et al. 1990, Zuo 1993, Takaiwa et al. 1995, Marcellino et al. 1996, Tu et al. 1998, Katsube et al. 1999); (ii) producing a synthetic protein (Jaynes et al. 1986, Yang et al. 1989, Kim et al. 1992, Keeler et al., 1997, Zhang et al. 2003); (iii) expressing a heterologous protein (Sun and Larkins 1993, Sun et al. 2000, Sun and Liu 2004); and (iv) manipulating homologous protein expression (Coleman et al. 1997, Singh et al. 2000, Maruta et al. 2001, Lai and Messing 2002), and (v) increasing the pool of a specific free essential amino acid, such as lysine, through metabolic engineering (Mazur et al. 1999, Galili et al. 2002, Galili et al. 2002, for review). Significant enhancements, especially for methionine and lysine contents, have been demonstrated in the target transgenic plants through these approaches, for a review see (Sun and Liu 2004).

Vitamins and minerals are essential food components for human health. Deficiency in dietary micronutrients such as vitamin A, iron, iodine, or zinc, will result in micronutrient malnutrition and various deficiency diseases. An adequate and diverse diet of fruits, vegetables and animal products is the best solution in obtaining sufficient micronutrients. However, for people, especially children, in many poor developing countries who rely solely or mostly on a single staple food crop, such as rice, sorghum, cassava, or banana, will suffer from nutrient deficiency diseases, a major source of morbidity and mortality worldwide (Toenniessen 2002). Biofortification through transgenic technology to increase the micronutrient contents in staple crops is a promising approach. Transgenic and associated molecular technologies have been demonstrated, or are under investigation and development, to enhance the synthesis and bioavailability of vitamins and minerals in plants, for example iron and zinc (Lucca et al. 2001, Zimmermann and Hurrell 2002, Ghandilyan et al. 2006, Lucca et al. 2006), carotenoids including provitamin A (Botella-Pavia and Rodriguez-Concepcion 2006, Lucca et al., 2006), vitamin C (Agius et al. 2003, Ishikawa et al. 2006), vitamin E (Shintani and DellaPenna 1998, Van Eenennaam et al. 2003, Dellapenna and Last 2006), folates (Rebeille et al. 2006), and pantothenate (vitamin B_5) (Chakauya et al. 2006). A good example is the fortification of pro-vitamin A (β-carotene) in rice through transgenic technology. Ye et al. (2000) genetically engineered the first generation of pro-vitamin enriched rice, named *Golden Rice 1* (*GR1*), by transferring and expressing the *psy* gene, encoding

phytoene synthase from daffodil (*Narcissus pseudonarcissus*), and the *crtI* gene, encoding phytoene desaturase from the bacterium *Erwinia uredovora,* in rice endosperm to achieve a yield of 1.6 μg pro-vitamin A/g in the endosperm. Efforts were made to further increase the content of pro-vitamin A in *Golden Rice*. In 2005, Paine et al. developed the second generation *Golden Rice*, *GR2*, by replacing the phytoene synthase coding sequence of daffodil in *GR1* with that of maize to achieve a yield of 31 μg pro-vitamin A/g in the endosperm, a 20-fold enhancement. With this improvement, the daily vitamin A allowance of a 1–3 year-old child could be provided by 72 g of *GR2*, which is within the range of 100–200 g of rice consumed per child per day in the target countries (www.goldenrice.org).

Four international consortia, with the support from the Bill & Melinda Gates Foundation, under the Grand Challenges in Global Health (GCGH) Initiative (www.gcgh.org), are undertaking research projects to engineer nutrient-rich staple crops, namely banana, cassava, rice, and sorghum, through a combination of transgenic, genomic, molecular marker-assisted (and conventional) technologies. The common goal is to develop new varieties of staple crops with high β-carotene, vitamin E, protein, and enhanced bioavailability of iron and zinc, for populations in the developing countries who rely on these crops as major staple foods, especially those in the tropical Africa and Southern Asia regions (www.gcgh.org/projects/improveNutrition/NutrientRichPlants/default.htm); www.goldenrice.org/Content5-GCGH/GCGH1.html).

The *Golden Rice* and GCGH projects serve as examples that transgenic and associated molecular technologies do offer new possibilities, in addition to the existing breeding and selection methods, for improving tropical crop plants. Since no variety of rice has ever been found to contain β-carotene in its endosperm, the β-carotene biosynthesis pathway can be introduced for synthesizing pro-vitamin A in the new rice product, only through transgenic technology.

Plants as Bioreactors

Transgenic plants have emerged as an attractive bioreactor platform for large-scale production of industrial enzymes, pharmaceutical proteins, and other biomolecules (Goddijin and Pen 1995, Daniell et al. 2001, Sparrow et al. 2007). When compared to bioreactors based on other systems (such as bacteria, yeast, transfected animal cell lines, or transgenic animals) the bioreactors based on plants hold several advantages, including: low capital and operating costs, easy to scale up, eukaryote post-translational modifications, low risk of human and animal pathogen contaminations, and a relatively high protein yield (Fischer et al. 1999, Fischer and Emans 2000, Fischer et al. 2004). Proof-of-concept production of diverse biomolecules in plants, including carbohydrates, lipids, and proteins (such as high-value pharmaceutical polypeptides and industrial enzymes) has been demonstrated (Goddijn and Pen 1995, Hood and Jilka 1999, Mercernier et al. 2001). The first commercialization of plant-produced recombinant proteins was egg white avidin from maize (Hood et al. 1997) that is marketed by Sigma Chemical Company. Another example commercialized plant-derived recombinant protein is hirudin,

an anticoagulant used to treat thrombosis, produced in transgenic oilseed rape (Boothe et al. 1997). More recently, bovine trypsin was produced at commercial levels in transgenic maize, with functional equivalence to native bovine pancreatic trypsin (Woodard et al. 2003) and a recombinant antibody against hepatitis B was commercially produced in tobacco plants in Cuba (Pujol et al. 2005). Other products including oligopeptides, sugar oligomers, starch, fatty acids, oils, secondary compounds, and degradable polymers have been demonstrated as feasible to manufacture in transgenic plants, while the composition and property of oil, starch, and protein can be modified in the production (Willmitzer and Topfer 1992, Galun and Breiman 1997, Broun et al. 1999, Herbers and Sonnewald 1999, Slattery et al. 2000, Napier et al. 2006). As reviewed above, literature indicates that plant transgenic technology can certainly be applied to develop tropical plants as bioreactors for production of naturally occurring, or logically designed high value bio-products.

3.2.2.2 Tropical Germplasm as Source of Transgenes

Tropical soil, water, and climate sustain a vast assemblage of plants, wild and cultivated, with wide range of genetic variability. These plants provide us foods and materials for construction, clothing, medicine, and industry uses. Tropical plants also offer invaluable biological systems for studying plant biology and evolution under unique tropical environments, and they are a rich source of genes and their regulatory elements/ mechanisms for crop improvement. A few examples are given to illustrate the exploitation of tropical plants for genes with agronomic importance. Sugarcane, a tropical crop known for its highest productivity in the world, was the plant system that contributed to our understanding of C4 photosynthesis. Through transgenic approaches, the phosphoenolpyruvate carboxylase (PEPC) genes of C4 photosynthesis was cloned from C4 maize and transferred into C3 rice. Results revealed that transgenic rice plants with high level of expression of the maize PEPC enzyme exhibited reduced sensitivity of photosynthesis to O_2inhibition (Ku et al. 1999). The gene encoding the methionine-rich 2S albumin protein in the seeds of tropical Brazil nut tree (*Bertholletia excelsa* H.K.B.) was cloned (Altenback et al. 1987), transferred, and expressed in tobacco (Altenback et al., 1989). Results demonstrated that it is feasible to enhance the essential amino acid methionine (by 30%) in the tobacco seeds, representing the first successful transgenic approach to significantly increase the essential amino acid methionine content of seeds (Willmitzer and Topfer 1992). In our more recent search for genes encoding high lysine protein for nutritional improvement, a gene encoding a 18-kDa protein with 10.8 mol % lysine was identified in winged bean, an edible tropical legume of the tropics, and the lysine-rich protein gene was cloned (Sun et al. 1993) and expressed in rice, resulting in 20% increase in lysine content (Liu 2002). A seed albumin, AmA1, with nutritionally balanced amino acid composition was identified and cloned from a tropical grain amaranths (*Amaranthus hypochondriacus*) and

expressed in potato, resulting in a significant increase in most of the essential amino acids and total tuber protein (Chakraborty et al. 2000).

3.3 Future Prospects

3.3.1 Transgenics for Crop Improvement

Transgenic approaches and technologies have produced a generation of crops with improved input and output traits that are beneficial to farmers, consumers, and the environment. These modified crops are already on global markets and have been widely adopted globally for more than 10 years. Many other crops are under development with new phenotypes with improved biotic and abiotic stress tolerance (especially drought resistance in view of concerns over future water supplies), agronomic properties, product quality, and therapeutic and industrial bioreactor products. These advances strongly demonstrate that transgenic technology offers innovations and new possibilities that conventional breeding and selection methods can not achieve, so they compliment conventional methods for crop improvement. Recent rapid progress in genomics including complete sequence information, genetic maps, arrays of molecular markers, ESTs, and bacterial artificial chromosome libraries, first available for model plants such as *Arabidopsis* and rice, are becoming available for many crops, including those of the tropics (see crop examples of this book). With these biological data, resources, and genetic materials, and the application of knowledge from synteny, functional genomics, and bioinformatics, tens of thousands of plant genes/alleles and their regulatory elements/mechanisms will be discovered. Through transgenic technology and approaches, these plant genes and their regulator elements, either in native or modified form, can be used to produce improved and novel products, from single or stacked traits (a current and future trend in plant transgenics), for improved crop function. The potential and future prospects of producing improved and new crop products through transgenic technology are very promising indeed.

3.3.2 Untapped Food Sources and Novel Genes from Tropical Germplasm

With the ever growing world population and shrinking agricultural lands, future food security is a growing human concern. Plants of the tropics, with their great genetic diversity, offer potential underexploited, and unidentified food resources. Especially in need are plant foods rich in protein and micronutrients. With advances in plant transgenic technology and genomics of tropical crops (this book), we are now more ready than ever to identify and clone genes by tapping into tropical germplasm, especially that of unexploited and unidentified species, for improved and novel traits.

Then through transgenic and technology to generate new crop products for the world as well as the tropics.

3.3.3 Transgenics to Improve Tropical Crops

The new genomics and transgenic technologies applied to agriculture could bring great benefits to developing countries to fight hunger (Delmer 2005, Borlaug 2007). Many people in the tropics rely on starchy root and tuber crops as staple food supplies. These people frequently suffer from hunger and malnutrition since yields of these crops are vulnerable to unfavorable biotic and abiotic stresses that may be prevalent and unique to tropical environments, and whose food value are notably deficient in proteins, essential amino acids, vitamins, and minerals. Through molecular approach, genes target for overcoming these environmental stresses and nutritional deficiencies can be identified and transgenic technology can be applied for local crop improvement.

Plant bioreactors are a transgenic application with potential to produce high value pharmaceutical and industry products in large quantities at low costs. Tropical plants could be developed into highly efficient bioreactors. For example, tropical starchy roots and tubers, efficient in producing starch, would be good candidate bioreactors to produce starch with novel properties and functions. In addition, roots and tubers often grow robustly, even in poor soils, to produce high biomass and they are generally propagated asexually, thus can avoid issues of pollen transfer and seed spillage. These traits are all advantageous for their use as bioreactors, i.e. high production efficiency and fewer biosafety concerns (Sparrow et al. 2007). Likewise other tropical plants could be developed as bioreactors to produce high value products, for example, tropical oil plants (e.g. palms) for production of specialty oils; tropical legumes for production of therapeutic proteins and industrial enzymes; and tropical medicinal plants for production of bioactive compounds. It would be highly preferable that tropical plants selected for use as bioreactors be non-food crops, having high biomass, and are asexually reproduced or, if they are seed crop, they be self-pollinated.

An ability to transform the selected plant species is a requirement of transgenic technology. Many tropical crops are known as being recalcitrant in regeneration and transformation. Thus, it is important to develop and establish efficient transformation systems for the target crops.

During the 20 years since the first commercialization of biotech crops, only a few transgenic tropical crop products, among the 16,814 total permits approved, have been approved for field testing in the U.S. (Table 3.2). With the rapidly increasing interest and progress in tropical plant biology and genomics, there has been a growing effort to improve local tropical crops for food security and nutritional well-being of the people in the tropics. Applications of transgenic technology to further improve tropical crop productivity and the emergence of new crop products of tropical origin are expected to rise in the future.

Table 3.2 Number of Permits for Regulated Organism of Tropical Plant Approved for Field Testing

Tropical Plant	Number	Tropical Plant	Number
Banana	3	Ginger	0
Cavendish banana	1	Macadamia	0
Cacao	0	Papaya	28
Chickpea	1	Peanut	45
Citrus sinensis X Poncirus trifoliata	2	Pineapple	6
Coconut Palm	0	Rubber tree	0
Coffee	3	Sorghum	6
Eucalyptus grandis	32	Sugarcane	55
Eucalyptus hybrid	8	Yam	0
Eucalyptus camaldulensis	5	Tropical Maize	0
Eucalyptus urophylla	1		

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