15 Application of Technology of Gene Expression in Response to Drought Stress and Elimination of Preharvest Aflatoxin Contamination


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15.1 INTRODUCTION

A major milestone in biological science was the sequencing of the human genome which provided fundamentally new ways of studying the human body. Likewise, with regard to the complexity of factors involved in preharvest aflatoxin contamination of corn and peanut crops, genomics could tremendously impact our understanding of host resistance mechanisms, genetic improvement of resistance to insects, invasion by Aspergillus spp., and improvement in drought tolerance. The complete decoding of the 3-billion-letter human genetic codes marked an important milestone in biomedical research, suggesting that the human genome may contain fewer than the expected 50,000 to 100,000 genes. No matter how many genes are encoded in the human genome, only a fraction of them is expressed at any given time in any given cell within the human body. This is also true in the plant genome. To better understand plant response to stress, more information is needed on the dynamics of gene expression in plants and how their expression is controlled in the context of a cell as a function of time and space.

Corn and peanut become contaminated with aflatoxins when subjected to prolonged periods of heat and drought stress. To meet the challenge of preventing preharvest aflatoxin contamination, more detailed understanding of the expression and function of the genetic material of corn and peanut in response to biotic/abiotic stresses will be needed. Moreover, the genes that control functions leading to plant reactions to environmental stress and fungal infection must be identified. In this paper, we will discuss drought stress, aflatoxin contamination, molecular tools used to study the genetic response to drought stress, and genetic engineering approaches to control aflatoxin contamination. Research objectives include "prospecting" for useful plant genes that can be characterized and transferred into plants. Genomic research will help identify and understand the function and control of genes to improve the desired traits. Identifying and characterizing those genes that control significant biological processes and agronomic performance are crucial in the development of genetic approaches for control of preharvest aflatoxin contamination.

15.2 AFLATOXIN CONTAMINATION AND ENVIRONMENTAL FACTORS

Aspergillus flavus and A. parasiticus can colonize seed of several agricultural crops including corn and peanut. This can result in the contamination of the seed with aflatoxins, which are toxic fungal metabolites. These fungi are ubiquitous, being found virtually everywhere in the world. They are soil borne, but prefer to grow on high nutrient media (e.g., seeds). A. flavus appears to be the primary aflatoxin-producing fungus on these commodities, although A. parasiticus also occurs frequently on peanut. Both fungi produce a family of related aflatoxins; the ones most commonly produced by A. flavus are B1 and B2, while A. parasiticus produces two additional aflatoxins, G1 and G2. Damage due to insects or environmental stress (drought) can enable the fungi to invade seeds where they thrive at high temperatures and extremely dry conditions, such as those frequently experienced in the southern United States during the aflatoxin contamination we wide problem for human a

Plant stresses, dependin factors affecting plant survi mendsous efforts have been of the environmental facto heharvest corn is affected l ity, planting date, irrigation. Among these factors, resist. and drought are genetic pr to decipher the genetic mec fungal invasion, and toleran level of aflatoxin contami aflatoxin biosynthesis has al or eliminate aflatoxin conta to a greater resistance to ins made, the problem is fa

15.3 DROUGHT STRESS AND AFLATOXIN

Several agronomic practices include the use of pesticide: use of resistant varieties. A long-term research projects aflatoxin contamination. He technique to directly mea peanut. This technique us extended period of drou initial field tests conducted peanut plants died and their s could occur. The use of a s viability during the drought nation. Sanders et al. also peanuts in the pod zone were plants nonstressed by provid Drought tolerance is a c: selection tool for resistance t evaluted the resistance to pi that had been documented determined the correlation o tamination. Drought tolerance in Tifton, GA, and significan
United States during the summer. The development of crop lines with reduced aflatoxin contamination would be a valuable development in alleviating this worldwide problem for human and animal health.

Plant stresses, depending on the type of stress and the type of plant, may include factors affecting plant survival, growth, and development of seed for harvest. Tremendous efforts have been made by scientists worldwide to study the mechanisms of the environmental factors that affect crop yield. Aflatoxin contamination in preharvest corn is affected by many factors, including drought, temperature, humidity, planting date, irrigation, tillage, insect damage, resistance, or susceptibility. Among these factors, resistance to insects, fungal infection and aflatoxin formation, and drought are genetic properties of the crop variety. We have spent years trying to decipher the genetic mechanisms of resistance or susceptibility to insect damage, fungal invasion, and tolerance to adverse environmental conditions in relation to the level of aflatoxin contamination in preharvest corn. Likewise, the action of aflatoxin biosynthesis has also been investigated extensively. Our goal is to reduce or eliminate aflatoxin contamination by screening for genetic traits that contribute to a greater resistance to insect damage and to *A. flavus* infection as well as tolerance to environmental stresses, such as drought. Although significant progress has been made, the problem is far from solved.

### 15.3 Drought Stress/Tolerance and Aflatoxin Contamination

Several agronomic practices can reduce preharvest aflatoxin contamination. These include the use of pesticides, altered cultural practices (such as irrigation), and the use of resistant varieties. Although resistant varieties are not currently available, long-term research projects are ongoing to develop varieties that resist preharvest aflatoxin contamination. Holbrook et al. developed a large-scale field screening technique to directly measure field resistance to preharvest aflatoxin contamination in peanut. This technique uses subsurface irrigation in a desert environment to allow an extended period of drought stress in the pod zone while keeping the plant alive. In initial field tests conducted in the desert environment without subsurface irrigation, peanut plants died and their seeds rapidly dehydrated in the soil before contamination could occur. The use of a small amount of subsurface irrigation, to prolong plant viability during the drought stress, resulted in higher and more consistent contamination. Sanders et al. also observed high levels of aflatoxin contamination when peanuts in the pod zone were artificially stressed with heat and drought while keeping plants nonstressed by providing root zone irrigation.

Drought tolerance is a characteristic that has the potential to serve as an indirect selection tool for resistance to preharvest aflatoxin contamination. Holbrook et al. evaluated the resistance to preharvest aflatoxin contamination in a set of genotypes that had been documented as having varying levels of drought tolerance and determined the correlation of drought tolerance characteristics with aflatoxin contamination. Drought tolerance was very effective in reducing aflatoxin contamination in Tifton, GA, and significant positive correlations were observed between aflatoxin
Aflatoxin and Food Safety

A significant negative correlation was also observed between aflatoxin contamination and visual stress ratings. A similar relationship between drought tolerance and reduced aflatoxin contamination has been observed in a drought-tolerant peanut cultivar in Australia. The cultivar, "Streeton," has up to 40% lower aflatoxin contamination during years of high aflatoxin incidence in comparison to other commercial cultivars. Physiological studies have shown that the lower aflatoxin incident is associated with better root water uptake, resulting in better maintenance of plant water status during severe end-of-season drought.

In corn, research has been conducted to evaluate drought tolerance of corn germplasm in rain-out shelters for 3 years. In rain-out shelter screening for drought-tolerant germplasm, we identified and selected several corn lines with excellent drought tolerance based on a "stay-green" character. We also evaluated lines selected from the GT-MAS:gk population that have drought tolerance. Multiple-location field evaluation of commercial hybrids for drought tolerance and aflatoxin production demonstrated that drought-tolerant commercial lines, in general, had lower aflatoxin contamination under drought conditions. This positive association of drought tolerance with lower aflatoxin production is encouraging, and hybrids made from drought-tolerant lines will be tested further to evaluate drought tolerance, yield, and aflatoxin contamination.

15.4 GENETIC ENGINEERING AND PREVENTION OF AFLATOXIN FORMATION

Genetic engineering approaches to control aflatoxin contamination in corn and peanut have focused on three main areas: resistance to the fungus, inhibition of aflatoxin production, and resistance to insects. The focus on resistance to insects is a result of the intimate relation between insect damage and aflatoxin contamination. Aspergillus flavus and A. parasiticus are able to survive and out-compete other fungi under hot, dry conditions. These conditions are also conducive to the development of outbreak populations of certain insects, such as the lesser cornstalk borer, Elasmopalpus lignosellus (Zeller), in the United States, and termites (Odontotermes spp. and Microtermes spp.) in Africa that feed on peanut pods/kernels. Other insects such as the corn earworm, Helicoverpa zea (Boddie), and the fall armyworm, Spodoptera frugiperda (Smith), damage kernels as they feed in an ear of corn, which provides a direct avenue for infection by A. flavus and A. parasiticus, exacerbating the infection and contamination of ears with A. flavus and aflatoxin. The highest levels of aflatoxin contamination in both corn and peanut are usually associated with insect damage. Indeed, aflatoxin contamination in peanuts from insect-damaged pods is 30 to 60 times greater than that in undamaged pods. Thus, one approach to reduce aflatoxin contamination is to reduce insect damage.

15.4.1 INSECT RESISTANCE

The bacterium Bacillus thuringiensis produces a protein (termed Cry) toxic to certain insects. Over 30 strains have been sequenced. Each of these strains has Cry proteins for insect control. Since the mid-1990s, in 20 million acres worldwide, 88.2 million acres worldwide, 

Gene Expression, Drought

Registration of Bt crop var...

The primary target of Bt corn is...
15.4.1 Insect Resistance in Transgenic Crops

The bacterium *Bacillus thuringiensis* (Bt) is ubiquitous and unique in that it produces a protein (termed Cry proteins because of their crystalline nature) that is toxic to certain insects. Over 240 insecticidal Cry proteins have been identified and sequenced. Each of these proteins is encoded by a single gene. Corn, cotton, potato, and other crops have been genetically engineered to express one of these proteins for insect control. Transgenic Bt crops have been commercially available since the mid-1990s. In 2001, genetically engineered crops were grown on 130 million acres worldwide, up 19%, or almost 20 million acres, from 2000. Of this total, 88.2 million acres were planted in transgenic crops in the United States in 2001 and included soybean, cotton, corn, and potato. Herbicide resistance accounted for 77% of the total acreage planted to transgenic crops, Bt crops accounted for 15%, and stacked genes for herbicide and insect resistance accounted for 8%. Registration of Bt crop varieties was recently renewed for another 7 years.

The primary target of Bt transgenic corn is the European corn borer, *Ostrinia nubilalis* (Hübner). This insect not only reduces the yield of corn grown in the Midwest by an estimated $1 billion annually but is also associated with ear infections with *Fusarium* spp. and *A. flavus*. Field studies in the Midwest with transgenic corn have consistently shown that hybrids that express the BT11 and MON810 events have a significantly lower incidence and severity of *Fusarium* ear rot and significantly lower concentrations of fumonisins than isogenic corn lines without the Bt gene. These events produce the Cry1Ab toxin in all parts of the corn plant including silks and kernels. Events that do not express the Bt toxin in the kernels are less effective in reducing European corn borer damage and *Fusarium* ear rot.

Although present, the European corn borer is not the major pest of corn in the South and Southeast, and the relationships between reduced insect damage in transgenic corn and aflatoxin in southern grown corn is not as clear as that for fumonisins in midwestern grown corn. There are two reasons for this difference. First, the corn earworm and fall armyworm are the major pests of corn in the South, and commercially available Bt corn hybrids are not as effective against these insects as they are against the European corn borer. Second, the environmental conditions conducive to the infection of corn with *Aspergillus flavus* and formation of aflatoxin are also much more severe, on average, in the South and Southeast than they are in the Midwest. In the South and Southeast, the corn earworm, fall armyworm, and southwestern corn borer (*Diatraea grandiosella* Dyar) are the major lepidopterous pests of corn. Ear damage by the corn earworm and fall armyworm can be quite extensive and lead to increased levels of aflatoxin contamination under appropriate environmental conditions. In the Midwest, Bt corn reduced kernel infection by *A. flavus* and lowered aflatoxin concentrations in BT11 and MOB810 hybrids, however, in the Southeast, no such relationship between insect resistance in Bt corn (YieldGard, BT11, MON810) and aflatoxin concentration could be established. Although YieldGard corn did reduce the percentage of infested ears and the number of larvae in the ears, slower larval development did occur in ears of the resistant plants. Under heavy fall armyworm infestations, YieldGard corn did not reduce the percentage of infested ears but did reduce the rate of larval development and the amount of kernel.
damage. Sims et al. reported reduced corn earworm feeding damage and reduced larval development on 8 of 12 independently transformed lines of corn containing a Cry IAb gene. Williams et al. reported significantly less fall armyworm leaf feeding damage, reduced survival, and slower larval growth on Bt corn (BT11 event) and near immunity to feeding by the southwestern corn borer. These differences in the effect of Bt transgenic plants on corn insects is directly related to the susceptibility of the insects to the Cry IAb protein; LC50 values ranged from 2.22 to 7.89 ng/cm² for the European corn borer, considerably lower than the 70.3 to 221.3 ng/cm² for the corn earworm and lower than the 0.36 to 10.22 µg/cm² for the fall armyworm. Windham et al. reported that corn hybrid N6800Bt had a lower southwestern corn borer damage rating and about a 50% reduction in aflatoxin concentration than N6800 when they were artificially infested with A. flavus spores and southwestern corn borer larvae. Research conducted in South Texas with Cry2Ab, Cry1Ab, and non-Bt isolines showed a positive correlation between the number of fall armyworm larvae per ear with ear insect injury rating at harvest and with aflatoxin content.

Peanut has also been genetically engineered to contain the Cry1Ac gene, which confers resistance to feeding by the lesser cornstalk borer (LCB). This gene also confers resistance to the corn earworm and velvebean caterpillar but not to the fall armyworm (Lynch, unpublished data). The transgenic peanut is primarily aimed at control of the LCB because this insect is intimately associated with aflatoxin contamination. Only external scarification of peanut pods by LCB is needed to enhance infection of peanut kernels with Aspergillus flavus. Field tests have been conducted to evaluate the efficacy of the Bt peanut in reducing lesser damage and aflatoxin contamination under drought stress. In 2000, no difference was observed in the percentage of pods showing scarification due to LCB feeding on transgenic vs. nontransgenic peanut pods. The aflatoxin concentration in scarified, transgenic peanut pods was significantly lower than in scarified, nontransgenic pods. The experiment was repeated in 2001, but aflatoxin analyses have not yet been reported.

**15.4.2 Fungal Resistance and Inhibition of Toxin Production in Transgenic Crops**

Progress has been made in the development of crop resistance to aflatoxin through genetic engineering. Research in Peggy Ozias-Akins’ lab in Tifton, GA, on aflatoxin reduction is using a three-tiered approach: (1) resistance to insect damage using a Bt gene, (2) resistance to fungal growth using the tomato anionic peroxidase gene (tapi) or an antifungal peptide D4E1, and (3) inhibition of the aflatoxin biosynthetic pathway using the lipoygenase gene loxI.

Art Weissinger, at North Carolina State University, is testing transgenic peanut containing synthetic Peptidyl Membrane Interactive Molecules (Peptidyl MIMs™) developed by Demegen, Inc. His group developed transgenic peanut encoding DSC, an α-helical peptide that is highly active against Aspergillus flavus. In a test of 15 lines that carried the DSC transgene, none contained DSC mRNA, and the peptide was not detectable using western blots. Furthermore, DSC transgenic peanut plants produced significantly fewer pods than control plants. Subsequent tests indicated that DSC was phytotoxic.

**15.5 MOLECULAR STUDY GENES**

Plants tolerate environmental stresses which have been attenuated. Arabidopsis thaliana, a model organism, has been used to study the role of the hormone abscisic acid (ABA) and the phospholipase D (PLD) in drought tolerance. Drought tolerance, for example, involves a number of plant factors, including leaf structure, photosynthesis, and the number of stomata. These factors interact with each other, and the genetic control of drought tolerance is complex. It is now possible to develop transgenic plants that are more tolerant to drought and other environmental stresses.

**15.5.1 EXpressed (Genes)**

Liang and Pardee developed a temporally and spatially regulated gene expression system. It is now possible to develop transgenic plants that are more tolerant to environmental stresses.
that D5C was phytotoxic to peanut at levels required to kill *A. flavus*. Demegen, Inc., has also developed other antimicrobial peptides that may warrant testing for *A. flavus* inhibition in transgenic plants. D4EI has emerged as one of the most active peptides against several species of bacteria and fungi. Activity against *A. flavus* is also present but at a lower level than that for other pathogens. Research is either planned or underway to integrate this gene in several crop species.

Charles Woloshuk and colleagues at Purdue University are investigating the possibility that transgenic corn containing an α-amylase inhibitor will inhibit *Aspergillus flavus* infection. Their previous research had indicated that α-amylase produced by *A. flavus* may facilitate colonization and aflatoxin production in corn kernels. They also found that the α-amylase inhibitor from *Lablab purpureus* inhibits α-amylase production in several fungi but not those from animals or plants. It also inhibits conidia germination and hypha growth of *A. flavus*.

### 15.5 Molecular Tools to Study Gene Expression

Plants tolerate environmental stress because of numerous physiological adaptations, which have been attributed to the function of various genes. For example, in *Arabidopsis thaliana*, transcription of *RD* (responsive to dehydration) and *COR* (cold responsive) genes are activated by hyperosmotic or cold stress. The plant hormone abscisic acid (ABA) activates transcription of some *RD* and *COR* genes, while *PLD* (phospholipase D) gene transcription is activated by drought stress.

The genetic control of these traits for tolerance to abiotic stresses is complex. Drought tolerance, for example, may be determined by many genetic factors. Quite a number of plant features contribute to drought tolerance and include both physiological and biological elements such as waxy skin layer on plant surfaces, size and number of stomata, extensiveness of root system, respiration rate, and nutritional status. These factors are genetically controlled. The isolation of one of these genes for a specific function is not easy, and the determination of all of these genes is almost impossible using traditional genetics and cloning techniques. The "one-gene-at-a-time" approach for analyzing gene expression is wholly inadequate. The development of differential-display, reverse-transcription PCR (DD-RT-PCR) and expressed sequence tag (EST) methods provided new tools for isolating more genes. It is now possible to locate multiple genes that enable plants to withstand biotic and abiotic stresses. Several major tools are used for gene expression analysis, four of which are discussed briefly here: DD-RT-PCR, EST/microarray, proteomics, and transgenes/genetic transformation.

#### 15.5.1 Expressed Gene Differential Display

Liang and Pardee first described DD-RT-PCR, which is a powerful and cost-effective method to detect variations in mRNA expression. DD-RT-PCR technology was developed to identify and isolate selectively those genes that are expressed in a temporally and spatially regulated manner in different tissues and organs. The technique uses a limited number of short arbitrary primers in combination with the
anchored oligo-dT primers to systematically amplify and visualize a certain proportion of the expressed genes (mRNA) in an organism or tissue. In cowpea, sunflower, and tomato, cDNA libraries constructed from drought-induced mRNA have been used to characterize genes associated with drought response. Differential display fragments can then be used as probes to screen the cDNA library to get the full-length drought-inducible genes. Using DD-RT-PCR, we have identify mRNA transcripts that are up- or downregulated due to drought stress. In addition, differences in the composition of selected metabolite levels between the drought-tolerant and -susceptible genotypes following drought stress may be determined.

To investigate gene expression patterns in response to induced drought stress in plants we have used DD-RT-PCR to differentiate gene expression in drought-susceptible and drought-tolerant corn and peanut. Polymorphic mRNA transcripts have been identified. Some cDNA fragments that were up- or downregulated by induced drought stress have been cloned and sequenced. Using this method, we identified a novel PLD gene, which encodes a putative phospholipase D, a primary enzyme responsible for the drought-induced degradation of membrane phospholipids in plants. The PLD gene expression under drought stress has been studied in the greenhouse using two peanut lines, Tifton 8 (drought tolerant) and Georgia Green (drought sensitive). Northern analyses showed that the PLD gene expression was induced sooner by drought stress in Georgia Green than in Tifton 8. After the PLD gene in peanut is completely characterized, we will attempt gene silencing using genetic transformation to suppress PLD gene expression and induce drought tolerance. The limitation of DD-RT-PCR is that it can be used to identify only those genes that are amplified by a few arbitrary primers within hundreds and perhaps thousands of expressed genes. In order to identify and isolate all of the expressed genes, expressed sequence tag (EST) offers the best solution.

15.5.2 EXPRESSED SEQUENCE TAG AND MACRO/MICROARRAY

Expressed sequence tag (EST) is used to sequence cDNA (DNA copies of RNAs) clones in an expressed cDNA library and identify all of the unique sequences (genes) to study their functions. Generating sequences from cDNA fragments can be used to discover new genes and to assess their expression levels in the representative tissue. The level of an mRNA species in a tissue is reflected by the frequency of occurrence of its corresponding EST in a cDNA library. EST technologies are attractive because they do not rely on established sequence data from the organism under study, and they also fit well with labs already equipped to carry out high-throughput DNA sequencing. Auxiliary techniques to reduce the amount of sequencing include subtraction hybridization, representational difference analysis (RDA), and suppression subtractive hybridization (SSH). The identified cDNA sequences, either fragments or homologous oligos, can be used to fabricate a DNA microarray for functional study.

DNA microarrays or a gene chip typically consist of thousands of immobilized DNA sequences present on a miniaturized surface the size of a microscope slide. Arrays are used to analyze a sample for the presence of gene variations or mutations (genotyping) or for pattern recognition in that the thousands of DNA sequences are tagged with a radioactive DNA will bind to a cDNA probe immobilized DNA no hybridization occurs. The array where binding occurs is listed by hybridization even if DNA sequences that are turned on or off.

The mode of action extensively studied in the control and regulation of aflatoxin production in response to temperature, fungal E, at the USDA-ARS South USDA-ARS Labs at Tifton has been identified from the identified A. flavus genes and to identify factors involved in aflatoxin formation by the and peanut have been initiated. ESTs show that some cysteine-rich antifungal like protein, glutathione S-ESTs has been released from these ESTs and array EST-derived SSR markers over 20% SSR produced types.

15.5.3 PROTEOMICS

Proteomics is the identification and type and an organism. The genome, thus proteomics is the determination of their the proteins directly and to expression analysis. Identifying potential and practical applications, approximately 30,000 genes define to 1 million proteins who...
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Microarrays are distinguished from macroarrays in that the DNA spot size is smaller, allowing for the presence of thousands of DNA sequences instead of the hundreds present on macroarrays. The samples of cDNAs are then prepared for expression analysis. The DNA samples are tagged with a radioactive or fluorescent label and applied to the array. Single-strand DNA will bind to a complementary strand of DNA. At positions on the array where the immobilized DNA recognizes a complementary DNA in the sample, binding or hybridization occurs. The labeled sample DNA marks the exact positions on the array where binding occurs, allowing automatic detection. The output consists of a list of hybridization events reflecting the presence or relative abundance of specific DNA sequences that are present in the sample, thus indicating how much a gene is turned on or off.

The mode of action, metabolism, and biosynthesis of aflatoxins have been extensively studied in the last decade.17,69-75 For a better understanding of the genetic control and regulation of toxin production by Aspergillus flavus and the mechanism of toxin production in response to environmental conditions such as drought stress and temperature, fungal EST and macro/microarray programs are being carried out at the USDA–ARS Southern Regional Research Center in New Orleans, LA,75,76 and USDA–ARS Labs at Tifton, GA. Currently, about 8000 expressed unique genes have been identified from the A. flavus EST programs. A microarray containing these identified A. flavus genes will be produced to study gene expression and regulation and to identify factors involved in the plant–microbe interaction. The A. flavus EST program will help to identify genes that could be used to inhibit fungal growth or aflatoxin formation by the fungi. The EST and macro/microarray projects in corn and peanut have been initiated in Tifton, GA, to study the gene expression profile of drought response based on suppression subtractive hybridization.77 The preliminary ESTs show that some plant defense genes have been identified, such as a small cysteine-rich antifungal protein, Ca2+/H+-exchanging protein, peroxidase, 14-3-3-like protein, glutathione S-transferase, and trypsin inhibitor. The first batch of 1345 ESTs has been released to GenBank. Four hundred unigenes have been selected from these ESTs and arrayed on glass slides for gene expression analysis, and 44 EST-derived SSR markers have been characterized for cultivated peanut, in which over 20% SSR produced polymorphic markers among 24 cultivated peanut genotypes.78,79

15.5.3 **PROTEOMICS**

Proteomics is the identification and examination of the proteins produced by a cell type and an organism.80 The term proteome refers to all the proteins expressed by a genome, thus proteomics involves the identification of proteins in the organism and the determination of their role in physiological and biochemical functions. To study the proteins directly and to identify their genes is another effective method for gene expression analysis. Identifying drug receptors and inhibitory factors has tremendous potential and practical applications for the pharmaceutical industry. The approximately 30,000 genes defined by the Human Genome Project translate into 300,000 to 1 million proteins when alternate splicing and posttranslation modifications are
considered. Although a genome remains unchanged to a large extent, the proteins in any particular tissue change dramatically as genes are turned on and off in response to its environment. As sequencing of the entire genomes of many prokaryotes and eukaryotes has been completed, the technology of proteomics is necessary to separate proteins from each other and to study proteins. The main way this has been achieved is through one- or two-dimensional polyacrylamide gel electrophoresis (2-D PAGE). Using 2-D PAGE, the separation of several thousands of different proteins can be achieved in one gel.

As a reflection of the dynamic nature of the proteome, some researchers prefer to use the term functional proteome to describe all the proteins produced by a specific cell in a single time frame. Riccardi et al. reported that protein profiles change in response to water deficits in corn. The induced changes of protein profile in leaf tissue of 3-week-old plants in response to drought or water deficit were studied by 2-D electrophoresis. Out of a total of 413 plants, 78 showed a significant quantitative variation (increase or decrease), and 38 of those exhibited a different expression in different genotypes. Eleven proteins increased by a factor of 1.3 to 5 in stressed plants, and 8 proteins were detected only in stressed plants. Some proteins are already known to be involved in the response to water stress (responsive to ABA). Most cellular processes are carried out by multiprotein complexes. Through proteomics, new plant resistance genes could be identified and DNA markers could be derived from these proteins that could be used as markers for breeding selection or genetic transformation, such as antifungal proteins identified from corn kernels.

15.5.4 Transgenes/Genetic Transformation

Molecular techniques allowed the identification, isolation, and characterization of genes that encode specific protein products controlling plant development (see above discussion on molecular tools). Genetic engineering is the next step to modify the genome of plants to contain and express foreign genes or modify native or endogenous genes to alter/enhance/suppress the traits of a plant in a specific manner (see earlier discussion on genetic engineering). Such foreign and modified genes are referred to as transgenes.

15.5.4.1 Expression Vector

Plant transformation involves the construction of an expression vector that will function in plant cells. Such a vector is comprised of DNA, including a gene under the control of or linked to a regulatory element, such as a promoter. Expression vectors include at least one genetic marker linked to a regulatory element (a promoter) that allows transformed cells containing the marker to be either recovered by negative selection, such as inhibiting growth of cells that do not contain the selectable marker gene, or by positive selection by screening for the product encoded by the genetic marker. One commonly used selectable marker gene for plant transformation is the neomycin phosphotransferase II (nptII) gene, which when placed under the control of plant regulatory signals confers resistance to kanamycin.

Another commonly used gene transferase gene, which confers resistance to kanamycin and bleomycin resistant herbicides such as glyphosate and streptomycin requires screening of pre selection of transformants and confers resistance to kanamycin.

15.5.4.2 Promoter

Genes included in expression vectors containing a regulatory element known in plant transcription in plant cells that preferentially initiates transcription. Such promoters such as the phaseolin gene is one that is under an inducible promoters contains an inducible promoter that allows RNA to be transcribed in plant cells. Such promoters such as the phaseolin gene is one that is under an inducible promoters contains an inducible promoter that allows RNA to be transcribed in plant cells.

15.5.4.3 Methods for Transformation

Numerous methods for transformation based on the natural transformation of A. rhizogenes are plant cells. The Ti and Ri plasmids carry genes responsible for transformation. The Ti plasmid is referred to as direct DNA transfer, wherein DNA is introduced into plant cells by the microprojectiles to produce transgenic plants. In peanut, the microprojectile bombard
Another commonly used selectable marker gene is the hygromycin phosphotransferase gene, which confers resistance to the antibiotic hygromycin. Other selectable marker genes that confer resistance to antibiotics include gentamycin acetyl transferase, streptomycin phosphotransferase, aminoglycoside-3'-adenyl transferase, and bleomycin resistance. Selectable marker genes may also confer resistance to herbicides such as glyphosate, glufosinate, or broxynil. GUS (β-glucuronidase) and luciferase represent another class of marker genes for plant transformation and require screening of presumptively transformed plant cells rather than direct genetic selection of transformed cells for resistance to an antibiotic. More recently, a gene encoding green fluorescent protein (GFP) has been utilized as a marker for gene expression.

15.5.4.2 Promoter

Genes included in expression vectors must be driven by a nucleotide sequence containing a regulatory element, a promoter. Several types of promoters are now well known in plant transformation. A plant promoter is capable of initiating transcription in plant cells. Promoters under developmental control include promoters that preferentially initiate transcription in certain tissues, such as leaves, roots, or seeds. Such promoters are referred to as being tissue preferred or tissue specific, such as the phaseolin gene and light-induced promoter. An inducible promoter is one that is under environmental control. Tissue-specific, tissue-preferred, and inducible promoters comprise the class of nonconstitutive promoters. A constitutive promoter is one that is active under most environmental conditions, such as the 35S promoter from CaMV, rice actin, or corn ubiquitin promoter. With an inducible promoter, the rate of transcription increases in response to an inducing agent. A constitutive promoter is linked to a gene for expression or to a nucleotide sequence encoding a signal sequence that is linked to a gene for expression.

15.5.4.3 Methods for Transformation

Numerous methods for plant transformation have been developed, including biological and physical. One method for introducing an expression vector into plants is based on the natural transformation system of Agrobacterium. A. tumefaciens and A. rhizogenes are plant pathogenic soil bacteria that can genetically transform plant cells. The Ti and Ri plasmids of A. tumefaciens and A. rhizogenes, respectively, carry genes responsible for genetic transformation of the plant. Another method is referred to as direct gene transfer, microprojectile-mediated transformation wherein DNA is carried on the surface of microprojectiles. The expression vector is introduced into plant tissues with a biolistic device (gene gun) that accelerates the microprojectiles to penetrate plant cell walls and membranes. In maize, several target tissues can be bombarded with DNA-coated microprojectiles in order to produce transgenic plants, including callus, immature embryos, and meristematic tissue. In peanut, the most reliable method for the introduction of foreign DNA is microprojectile bombardment of embryogenic tissue cultures.
15.6 SUMMARY AND PROSPECTS

In traditional genetics, a trait of interest is targeted and then research to identify the gene that caused, or coded, for that trait is conducted for several years. In the new paradigm of genomics, however, we take the opposite approach in that we map out all of the genes of an organism first, and then work to determine their functions. The first step is known as structural genomics, and the second step is functional genomics.\textsuperscript{108-110} The information obtained from genomics can be applied to the development of commercial crops for high yield with no or low aflatoxin contamination through genetic engineering.\textsuperscript{111} Practical examples of genetic engineering are the genetically improved (Bt) corn that protects against insects and the genetically engineered cotton that protects against the bollworm.\textsuperscript{112} These innovative products not only increase crop yields but also dramatically reduce the cost for insecticides and the chance for environmental contamination.

Genes are key components in manipulating plants and animals for more desirable and economic and agronomic traits. To reduce yield losses and to study genetic factors involved with plant stresses, the National Science Foundation granted $8.4 million to the “Functional Genomics of Plant Stress Tolerance Project,” which is being conducted by scientists at Purdue University, the University of Arizona, and Oklahoma State University. The corn genomics project is expected to define and discover the full suite of genes in corn as a route to new fundamental discoveries in plant biology and to find immediate application in basic research for use in the commercial arena. Corn genomics promises development of new commercial corn varieties that are able to withstand environmental stresses such as drought and heat, as well as resistance to insects and plant pathogens. A peanut genomics project will be launched soon.

Expressed sequence tag/microarray technology can be used to detect an entire set of genes transcribed under specific conditions and to study the biological functions of genes of interest. EST and microarray technology provides a tool for rapid identification of genes of interest expressed by plants under fungal challenged or environmental stress conditions. They can help in our understanding of the biological functions, coordination of gene expression in response to internal and external factors, mechanisms of plant–fungal interaction, plant–environmental interaction, fungal pathogenicity antifungal properties, and the mechanism of genetic regulation in relation to plant tolerance to biotic and abiotic stresses. This technology allows us to study a complete set of genes simultaneously for screening and identifying the most important host-resistance and stress-tolerance genes among hundreds or even thousands of relevant genes that could be used in genetic engineering for developing commercial crops. Our \textit{Aspergillus flavus}/corn/peanut EST/microarray programs are expected to provide valuable information on the prevention and elimination of aflatoxin contamination in these crops.

In the effort to prevent preharvest aflatoxin contamination in corn and peanut, knowledge obtained from the \textit{Aspergillus flavus} EST/microarray program can be integrated into the corn and peanut genomics programs for identifying host-resistance and stress-tolerance genes, and, at the same time, identifying biological targets for antifungal growth or inhibition of toxin formation by fungi. Corn and peanut

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Genomics combined with the A. flavus EST/microarray project will give us more specific genetic information to target critical regulatory components and genes involved in aflatoxin biosynthesis. These genes can then be engineered into commercial crops to alleviate the problems of preharvest aflatoxin contamination of food and feed.

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