

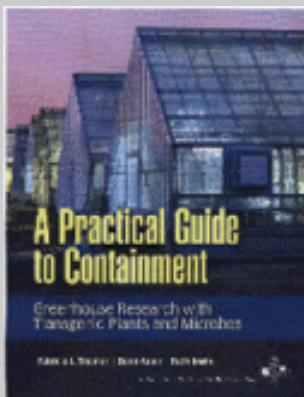
ISB NEWS REPORT

COVERING AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY DEVELOPMENTS

AUGUST 2001

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NEWS AND NOTES

ISB HIRES PROJECT MANAGER

ISB is pleased to announce that LaReesa Wolfenbarger has joined our staff. Dr. Wolfenbarger is an evolutionary ecologist. She has conducted field research on the ecology, evolution, and behavior of plant and animal populations and in particular has focused on understanding ecological factors that affect survival and reproduction. She also has experience in molecular genetics and completed a quantitative trait loci (QTL) mapping study.

In her position at ISB, she will have responsibilities for organizing special projects that address timely scientific issues associated with regulating agricultural biotechnology. She is organizing a workshop to discuss the environmental effects of engineering traits into plants. These workshops will bring together diverse groups of scientists and regulators to evaluate existing data and to identify the types of data and experiments that will provide key information for regulators.

Dr. Wolfenbarger obtained a B.Sc. in biology from the University of California, Los Angeles and a Ph.D. from Cornell University. She has taught Introductory Biology courses as an instructor at the University of Maryland. Prior to coming to ISB, she worked at the US EPA's National Center for Environmental Assessment. She is also an Adjunct Associate Professor at the University of Nebraska at Omaha and manages a 160-acre tall grass prairie preserve outside of Omaha, Nebraska, where she resides. She can be reached at lwolfenb@vt.edu.

NEW RELEASE: A PRACTICAL GUIDE TO CONTAINMENT

Greenhouse Research with Transgenic Plants and Animals

Patricia L. Traynor – Dann Adair – Ruth Irwin

Information Systems for Biotechnology

July 2001

ISB announces the publication of our manual for managing research with transgenic organisms in greenhouses. This 60-page Guide is intended as a simple and convenient reference on appropriate biosafety and containment levels for GMO research conducted in greenhouses. Information about handling transgenic plants in greenhouses is relatively sparse. Appendix P of the NIH Guidelines specifies

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ISB welcomes your comments and encourages article submissions. If you have a suitable article relevant to our coverage of the agricultural and environmental applications of genetic engineering, please email it to the Editor for consideration.

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facilities and practices for meeting containment standards appropriate for each of four biosafety levels, but there presently is no single source of practical guidance on managing greenhouses containing GMOs, nor on the requirements for building or renovating plant growth facilities to make them suitable for containing transgenic plants and associated organisms.

Printed copies of the Guide are available by completing the Request Form on ISB's Publications Page <http://www.isb.vt.edu/isb_publications.cfm>. Check the box titled "Greenhouse Manual." The manual is free of charge.

TECHNOLOGY NEWS



EMERGING PLANT BIOTECHNOLOGIES: NEW WAYS TO FIND NEEDLES IN HAYSTACKS

The biotechnology industry has a clear need and a natural responsibility to address the concerns of its customers and those of the public at large if it is to contribute meaningfully to the current debate over its future. The strongest voices raised against the use of new genetic technologies in agriculture are those of activists whose ethos is based on objections to contamination, usually manifested as environmental pollution. These organizations also tend to oppose perceived political, cultural, and commercial colonialism, and often fail to disentangle these opinions from those that are based on an ecological stance. Emerging technologies can deal with arguments against genetic modification (GM) based on legitimate scientific standpoints, and thereby impact upon the political debate.

Runaway genes

The major stated concern of many opponents of GM is the movement of genes from modified organisms into other organisms in the natural environment, a process termed 'gene escape' in scientific discourse and 'genetic pollution' in activism pamphlets. One brand of anti-GM zealotry anticipates apocalyptic consequences in the event of any gene derived from a GM event moving out into the biosphere, but research does not support this.¹ For most people who express an opinion, the creation of antibiotic-resistant pathogens ('superbugs') or herbicide-resistant uncultivated plant species ('superweeds') are seen as the greatest potential dangers of gene escape, and regulatory approval around the world is increasingly being withheld for GM plants with such genes. Except in cases in which engineered resistance is the desired outcome (e.g., Roundup Ready maize), such genes are unnecessary beyond the laboratory-based phase of a biotechnology program, and the failure to remove them or avoid their use entirely implies that the industry is woefully or willfully ignorant of



one of the public's major concerns. Fishermen cannot leave their hooks in the catch and expect their customers to return. Techniques exist for the removal of such genes before field release², but these delay the development of products and introduce additional complexity and unpredictability.

All methods for inserting DNA into plants are inefficient and result in large numbers of plants of which only a tiny proportion are transformed. Resistance to an antibiotic or herbicide 'marks' a transformed organism as carrying the modified DNA. Marker genes are therefore essential in laboratory research, being the equivalent of using an X-ray machine in the proverbial search for a needle in a haystack. Unpredictability of transformation outcome further contributes to the need for markers; many transgenic plants must be manufactured via transformation, identified by the presence of markers, and assessed for useful traits before one with the optimum balance of characteristics can be found. To extend the metaphor, a large number of needles must be found, as some needles are better than others.

Markers are therefore a prerequisite for all current methods of plant transformation. Unfortunately, the most widely used marker genes are precisely those that raise the most public concern by conferring abilities that are advantageous beyond the laboratory, for example, the capacity to degrade a potent herbicide before the plant succumbs to its effects. This is an example of 'negative selection'—the entire population is subjected to a negative (toxic) selection pressure that only transformants can bear. The main advantage of negative selection is that it negates some of the inefficiencies of transformation; untransformed plants are culled by the antibiotic or herbicide, leaving a population enriched with transformants. One disadvantage of negative selection systems, in addition to concerns over gene escape, is that they cause destruction of untransformed tissues, which then release toxic, inhibitory, or suicide-signalling compounds to the detriment of the transformants.

A different kind of marker gene exists that can signal its presence but does not provide a fitness advantage, obviating the improbable apocalyptic scenarios peddled by some anti-GM activists and the practical problem of toxin release during the experiment. The first such gene was isolated from the laboratory workhorse bacterium *E.coli* and encodes the β -glucuronidase (GUS) protein; this confers upon transformed tissues the ability to break down a synthetic chemical added to their growth medium into a fluorescent product.³ 'Neutral' markers of this kind are screenable rather than selectable, meaning that the identification of transformants involves more labor and time. There is a clear need for a marker technology that poses no

realistic or hypothetical danger of superbug or superweed formation, yet is as efficient as the negative selection systems currently favored.

Building a bigger biotechnology toolkit

While the development of entirely novel selectable or screenable marker technologies is desirable, it is also important that the range of options within the current paradigms is expanded. The tools available for plant modification at the genetic level are limited to a handful of well-understood regulatory or targeting elements and a few options for selection and transformation systems. There is only limited knowledge about the characteristics and potential uses of the many other genome elements such as introns, terminators, enhancers, and repressors.

The torrent of information from the large-scale automation of biological research embodied in genomics and proteomics must be trawled for new tools for the genetic engineer. Exigent additions to the toolkit are non-viral promoters (another public concern) and new marker systems to permit greater control and precision in genetic modification. A recent paper by Gough and co-workers⁴ describes the development of a new negative selection system for the toolkit in which, rather than detoxifying a herbicide, the transformed plant possesses a backup metabolic step to replace the essential one attacked by the herbicide. By the introduction of an altered cyanobacterial enzyme called glutamate-1-semialdehyde aminotransferase, which, unlike the plant's own copy, is not susceptible to the effects of the toxin gabaculine, negative selection is achieved. This is analogous to the use of the glyphosate resistance gene, a mutant and immune form of the glyphosate target enzyme.

A positive selection system is one that enhances the performance of transformants over that of normal plants, and could be a viable replacement for negative selection systems, yet offers advantages over neutral screenable systems. In this type of marking, a fitness advantage is conferred in an artificial situation that is harmless to other plants. This is the most active and interesting area of current research in marker development and (to extend the haystacks metaphor yet further) allows the seeker to use magnetism in the hunt for needles. The GUS neutral marker gene can be adapted for use in a positive selectable marker system.⁵ In this system, one of the required growth hormones of plant culture medium is supplied in a form activated only by the GUS protein, so only transformants can grow. The widespread use of GUS in plant GM could permit existing research programs to switch painlessly from screening to applying selection.

Similarly, the use of the phosphomannose isomerase gene isolated from *E.coli* in a positive selection marker system has recently been demonstrated in some commercially important crop plants.⁶ Transformed plant tissues expressing this gene can grow on culture medium containing the sugar mannose as the only source of carbon, while untransformed tissues can maintain their size but, lacking utilizable carbon, do not grow further. The gene confers no advantage in the natural environment where plants are self-sufficient in carbon derived from the atmosphere using photosynthesis, and so cannot contribute to the generation of a superweed. Other carbohydrates that do not support plant growth can be used similarly when there exists a non-plant enzyme that can convert them into a form usable by the plant, e.g., xylose and the xylose isomerase gene isolated from *Thermoanaerobacterium thermosulfurogenes*.⁷

Broader use of the better toolkit

The most controlled method of gene delivery into plants is via an intermediate modification of the plant pathogen *Agrobacterium*. This method introduces into the plant only the genetic material contained within defined boundaries on a large loop of DNA. Other methods of transformation, the most popular of which is microprojectile bombardment ('biolistics'), often introduce the rest of the DNA loop. This extraneous genetic material is only necessary during the construction of the transforming DNA and commonly contains antibiotic resistance genes as components of negative selection systems used in *E.coli*. Unfortunately, most commercially important agricultural plants are not amenable to *Agrobacterium*-mediated transformation, and so biolistic methods are employed without removing these undesired elements; this is one route by which antibiotic-resistance genes arrive in the genomes of modified plants.

LaFayette and Parrott⁸ have developed a positive selection marker system for *E.coli* that avoids the use of formerly ubiquitous antibiotic resistance markers. In their system, the presence of the *rtl* gene permits the growth of transformed bacteria on culture medium containing the sugar alcohol ribitol as the sole carbon source; untransformed bacteria lack the gene and therefore cannot multiply. DNA that has been built in bacteria using this system can be transferred to a plant since the bacterial marker gene is incapable of contributing to superweed creation. Such plants must still harbor a second selection system to identify transformed plants, such as those described above, and these plants would then address scientifically founded concerns over gene escape.

The expansion of the range of available markers and the applications of positive selection systems promise to

increase the abilities and precision of plant genetic engineering, and consequently raise the confidence of the public in the biotechnology industry. Several prominent scientific concerns over the current applications of gene technologies are addressed by the emerging technologies in this field. Until an unlikely and unpredictable quantum leap in the efficiency of transformation technology takes place (allowing us to prevent the formation of metaphorical haystacks entirely), the development of new marker technologies is a keystone of advancing biotechnology research and development.

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PLANT RESEARCH

TRANSGENICALLY ENHANCED COTTON FIBER STRENGTH EXHIBITS VANISHING ACT

Increased cotton fiber strength has long been a goal of cotton improvement programs and is especially appropriate today as yarn manufacturing technologies evolve to produce more yarn at less cost in response to global economic competition. Rotor and air-jet yarn manufacturing technologies are increasingly being adopted at the expense of established ring spinning in order for domestic manufacturers to compete in world markets. Though more productive than ring spinning, rotor and air-jet yarn manufacture require a higher input fiber strength to produce a yarn of comparable strength. Although conventional breeding has been very successful at enhancing fiber properties to meet the needs of fiber consumers, the speed of evolution in yarn manufacturing equipment has nearly surpassed the ability of traditional crop improvement strategies to keep pace with technological enhancements in fiber processing. Thus, transgenic approaches to enhance fiber properties, including strength, offer the possibility of quicker response.

It is helpful to know some background information on the origin of the transgenic cotton used in this study. The Agracetus unit of Monsanto conducted an active cotton output trait development program through the mid-1990s, including enhancing basic properties of cotton fiber such as strength and composition.¹ Agracetus had developed a biolistic-based, genotype-independent cotton transformation system that circumvented the slow *Agrobacterium*-mediated technique (proceeding from co-cultivation with *Agrobacterium* through tissue culture to plants expressing the desired trait can take over a year). Most cotton genotypes except the cultivar Coker 312 and a few close relatives are recalcitrant to regeneration of plants from the tissue culture phase of *Agrobacterium* mediated transformation protocols. The Agracetus genotype-independent, biolistic-based transformation method allows for direct transformation of differentiated tissue (typically hypocotyls) capable of growing into a plant of nearly any cotton genotype, albeit germline transformants still occur at low frequency.³

Middleton, Wisconsin based Agracetus cannot conduct field trials with their transgenic cotton germplasm because of the northerly clime. Thus, we (O.L. May and M.E. John) partnered under the terms of a cooperative research and development agreement to assess expression of the

enhanced fiber strength under field conditions in the US cotton belt and conducted breeding efforts. Agracetus supplied R4 seed from R3 plants expressing high fiber strength under greenhouse conditions. The popular cultivar Deltapine 50 with low fiber strength had been transformed with a proprietary gene, increasing strength by some 50% under greenhouse conditions. The objective was to assess expression and inheritance of the enhanced fiber strength under field conditions.

About 50-100 R4 seeds from the R3 greenhouse plants were planted in field trials at Florence, South Carolina in 1996. Standard cotton cultural practices were adopted. Non-transformed cultivar Deltapine 50 served as the fiber strength control. The R4 plants were manually self-pollinated and allowed to set normal open pollinated bolls for fiber analysis at a commercial testing laboratory. Though cotton is mostly bred with procedures appropriate for a self-pollinated plant, out-crossing can occur through insect vectors, necessitating manual self-pollination to maintain genetic purity at the transgenic loci. The GUS (β -glucuronidase) gene served as a selectable marker during regeneration, and in field trials its expression was amenable to visualization under ambient temperatures. GUS expression was determined as follows. Leaf punches were immersed in GUS-buffered substrate in microcentrifuge tubes attached to plants, and degree of blue staining was read the next day. GUS-expressive plants were assumed to also carry the fiber strength gene, although the size of this experiment precluded DNA analysis to confirm its presence.

Interestingly, the R4 progeny rows exhibited wide segregation for fiber strength. Thus, high-strength R3 greenhouse plants produced R4 plants with a range of fiber strengths.² For example, there was over an 80 kN m kg⁻¹ range in fiber strength among 22 GUS-expressive plants derived from a single R3 parent (standard deviation for fiber strength among plants was 3.3 kN m kg⁻¹). We can only speculate about reasons for the apparent disappearance of the fiber strength between parents and progenies, as this study was not designed to answer this question. Obvious possibilities include gene silencing and intragenic recombination. GUS expression in pollen suggested heterozygosity at the fiber strength locus/loci was not the reason for the segregation in fiber strength. After evaluating several hundred parent-offspring (R3:R4, R4:R5, and R5:R6) relationships over three years we were unable to identify a plant that transmitted the enhanced fiber strength to its progeny.

We next attempted to salvage the enhanced fiber strength trait through exploitation of transgene x genetic background

interactions. Such interactions have been demonstrated to affect expression of other transgenic traits such as Bt protein production.⁴ In this experiment, high-strength GUS expressive plants in the 1996 and 1997 field trials were used as females in crosses with upland cultivars and germplasm lines. Crosses were made the same season prior to measurement of fiber strength and F1 seed from only these types retained for next year field trial. Populations developed from these crosses included F1s, F2s, and F3s evaluated from 1997 – 1998 in field trials. Other than an expected amount of variation in segregating F2 and F3 populations, we observed a similar magnitude of variation for fiber strength as that found within the direct transformed DP 50 lines. A range of increased strength over the DP 50 control was observed, and even plant-to-plant variation in fiber strength within F1s was noted. Findings from this experiment and those from the direct transformed DP 50 material led to the conclusion that this transgenic event should be discarded.

Throughout these experiments we noted an unusually low frequency of GUS expressive plants compared with that expected for a single locus and simple-dominant gene action. For example, putative heterozygotes, determined through pollen staining, produced fewer than 50% GUS expressive progeny, prompting the hypothesis that the transgenic gametes had less fitness than their non-transgenic counterparts. Classical cotton genetics suggests that the male gamete can be recalcitrant at transmitting chromosomal abnormalities. The addition of chromatin in the form of a transgene could be considered a chromosome addition and might result in altered transmission through the male gamete. Alternatively, transgenic pollen might not germinate or grow through the style at the same rate as their non-transgenic counterparts, which could result in preferential fertilization of ovules by non-transgenic pollen.

Although a great technological feat, we concluded that the instability in expression of the enhanced fiber strength precluded further efforts at exploitation of this trait. More generally, transgenic events that impart unstable expression and/or inheritance should be discarded early in the breeding process to focus on more favorable transformation events. During the execution of these studies, Monsanto purchased the Agracetus Company (in 1997), and the cotton transgenic output trait program was de-emphasized to instead focus on insect- and herbicide-resistance input traits. No other private sector efforts have reached fruition in recent years, and public efforts to enhance fiber properties through transgenic approaches remain for the future. It remains to be seen whether sufficient value for enhancing cotton fiber properties can be captured in the marketing

and processing chain to warrant renewed efforts at expensive transgenic approaches. The current cotton marketing system offers no price incentives to spur transgenic or traditional breeding approaches to enhance fiber properties. The survival of the US textile industry is dependent, however, on producing high quality yarns at a cost that can compete amid global competition. Thus, fiber properties must keep pace with technological advances in processing, otherwise the history of the cotton industry teaches that cotton's share of the fiber market will decline.

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ENVIRONMENTAL INFLUENCES ON GENE SILENCING

Of late, gene silencing in transgenic plants has emerged as a topic of intense interest for both basic and applied researchers. Gene silencing may be important for inactivating deleterious foreign genes and for normal growth and development of plants. However, plant genetic engineers do not welcome the phenomenon, as it interferes with the expression of newly inserted genes.

Many factors affect the expression of gene silencing. Reports indicate that it can occur at either the transcriptional or posttranscriptional level. Silencing that occurs at the transcriptional level may be caused by direct physical association or pairing of alleles. Also, the ploidy of the plant may sometimes influence gene expression, since reduced transgene expression is observed in triploid, as compared to



diploid, or in homozygous, as compared to hemizygous transgenic *Arabidopsis* plants. Posttranscriptional transgene silencing is affected by both plant developmental stage and environmental factors. The experimental data accumulated to date do not fully explain the mechanism of gene silencing, nor do the suggested models accommodate all experimental data.

To investigate the incidence of gene silencing using different T-DNA configurations under a variety of environmental growth conditions, a team of researchers from the University of Oslo, Norway and University of Skovde, Sweden developed a collection of 111 independent transgenic *Arabidopsis thaliana* lines. Two vectors, pPCV002 35SGUS and pKOH110 35SGUS, containing *gus* and *nptII* reporter genes, were used for the transformation. The 111 lines were divided into six categories according to the number of loci and state of hemizyosity or homozygosity using segregation ratios for kanamycin resistant and kanamycin sensitive phenotypes in T₂ seeds. Segregation data were used to determine the extent that *nptII*-silencing in the different categories was different from the lines screened.

For each transgenic line, eleven four-week-old seedlings were selected and kept at normal growth conditions for three days. Then stress treatment, consisting of 30°C day and night temperatures, was given to seven plants, of which four were also sprayed with insecticide. The other four plants were given a 30°C stress treatment during the day and 4°C during the night. After two weeks, the plants were transferred to original growth conditions (22°C). *NptII*-silencing was scored by planting dormancy-broken surface-sterilized T₃ seeds on plates containing kanamycin. Determinations of T-DNA copy number and DNA methylation were made using Southern hybridization.

Sixty-seven and 44 transformants were independently generated for pPCV002 and pKOH110 vectors, respectively. *NptII*-silencing was scored for each line after germination on medium containing kanamycin. Three silencing phenotypes, I, II, and III, were identified. White or light green cotyledonous plants were designated as Type I; those with white, spotted, and deformed leaves were classified Type II; and Type III had large, green, spotted leaves. Type II and III phenotypes are never found in wild-type seedlings. It was assumed that the white parts of the seedlings were displayed where *nptII* was silenced, inhibiting normal seed development. Those seedlings displaying *nptII*-silencing from the stress-treated groups were identified and the overall silencing frequencies of the stress and control groups were calculated for each line. The extra stress treatments, 4°C and insecticide, did not result in additional effects.

NptII-silencing of T₃ plants was found in 56% of the 111 transgenic lines. In the majority of lines having a low to medium overall frequency of *nptII*-silencing (<10 – 50%), the frequency was higher in the stressed group than the control group; however, this was not seen for any lines displaying more than 50% silencing frequency. Differences in silencing frequency were not correlated to the T-DNA vector source.

Interestingly, the silencing frequency differed between siblings in all but two lines. In the majority of lines, the frequency of *nptII*-silencing was significantly different between the stressed group and the control. In several cases, the frequency of silencing in progeny of stress treated plants was higher than for the control group, while in other lines this trend was reversed.

The team investigated whether there was a correlation between homozygosity and silencing in progeny of hemi- and homozygous siblings of the T₃ generation and found that silencing could be attributed to homozygosity in only one line. Likewise, a correlation between kanamycin resistance and methylation of the *Sac* II site in the *pnos* promoter region, as has been reported in other studies, was found in multi-copy, but not single copy, lines. Additionally, the team found that the frequency of silencing was not correlated with rearranged transgenes.

Stress-induced susceptibility of plants to gene silencing is exhibited at three levels: (i) stress can change the proportion of sibling plants producing silenced progeny; (ii) individual plants may influence the number of seeds in which the transgene is silenced due to a change in the number of cells with silenced transgene(s); and (iii) silencing phenotypes can be altered. All transgene copies in all cells of Type I seedlings are likely to be silenced. However, in seedlings of Type II and III phenotypes, which contain seedlings that have developed beyond the cotyledon stage, transgenes are generally only partially silenced.

Meza et al. report that environmental stress can have either a positive or negative influence on the frequency of silencing. One transgenic line, which exhibited an *increase* in silencing after stress treatment, displayed both Type I and II phenotypes, while the control seedlings displayed the II and III types. Conversely, in another line in which stress treatment produced a *reduction* in *nptII*-silencing, the silenced progeny from the stressed group showed the Type III phenotype, and the control group displayed Types I and II.

The authors suggest that environmental stress produces changes in methylation patterns and/or chromatin confor-

mations. They theorize that those transgenes that integrate into genomic regions which are subject to epigenetic modification during stress treatment are susceptible to environmentally induced silencing. However, in some instances, chromatin configuration may have more of an influence than methylation—as with the *Sac II* site of the single copy line in which methylation was not detected.

Studies of the sequences flanking the T-DNA in one-locus lines, some of which contain a single copy, may help generate transgenic lines with stable expression patterns. In addition to questions concerning the mechanisms underlying gene silencing, there is interest in isolating genes whose products modify the timing of silencing. There is also a need to clarify the involvement of methylation in silencing and to minimize this effect by targeting transgenes into compatible isochores that include flanking scaffold attachment regions in the constructs.

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REGULATORY NEWS

**RELEASE OF BT COTTON IN
INDIA DELAYED**



Environmentalists and opponents of GM crops in India have successfully pressured the Government of India to postpone the commercial release of Bt cotton by at least one year. During the last week of June, the Genetic Engineering Approval Committee (GEAC), a governmental agency that regulates the commercial release of GM crops, ordered MAHYCO (Maharashtra Hybrid Seed Company, Jalna, India) to conduct further multi-locational trials on

their Bt cotton hybrid. GEAC indicated that the trials are needed to collect comprehensive data on the agronomic performance of MAHYCO's GM cotton and to assess its impact on non-target insects and soil microflora, including an evaluation of the possible emergence of bollworms resistant to the Cry1Ac protein.

MAHYCO is a leading private seed company in India and is involved in a joint venture with Monsanto to produce Bt cotton. New large-scale field trials, to be patterned after the advanced verity trials of the All India Coordinated Cotton Improvement Project, will be organized under direct supervision of the Indian Council of Agriculture Research (ICAR). The trials will be monitored by a committee set up by ICAR, which will include representatives from other related ministries and departments.

Bt cotton was first introduced to India in 1996. Starting with a few 25 m² plots in 1996 – 97, the limited field trials were expanded in 1998 to include 40 locations nationwide. However, when news of the “terminator technology” broke, anti-GM activists in the southern states of Andhra Pradesh and Karnataka burned and uprooted Bt cotton test plots. Subsequently, central and state governments issued assurances that no GM crops with terminator gene technology would be allowed into the country, and all GM crops must be in compliance with biosafety and regulatory criteria stipulated by the government before they would be permitted for commercial cultivation.

Based on the results from limited field trials, GEAC, in July 2000, permitted MAHYCO to conduct large-scale field trials in different agro-climatic regions of the country. The trials were to be located on a total of 85 hectares in five states (Maharashtra, Andhra Pradesh, Karnataka, Tamil Nadu, and Madhya Pradesh). The company obtained additional permission to produce GM cotton seed on about 150 ha, but was prohibited from using the seed for commercial use. During these trials, MAHYCO was instructed to collect data on the health and milk production of buffaloes and cows fed with transgenic seedcake, and to report on any toxicity observed with other animals, in addition to collecting data on agronomic performance and development of insect resistance, if any. Other requirements included providing information on gene stability, pollen transfer, and production economics.

Based on data obtained from these large-scale trials during the 2000 – 2001 season, MAHYCO approached GEAC for commercial release permission. After careful evaluation of the data, the approval committee observed that the “Bt cotton hybrids generally performed better” in terms of a reduction in the number of pesticide sprays required.



However, they noted that the pest load in the test plots could have been less than normal, as the plantings were done late in the season. Overall, the committee felt that the agronomic performance data collected from the limited and large-scale field trials were still not convincing enough to allow approval of Bt cotton for commercial release. Though there has been some demand by NGOs to publish the field trials data and to make the decision-making process more transparent, such information is not generally accessible to the public at large.

Meanwhile, NGOs and anti-GM activists continue to pressure the government to prohibit commercial cultivation of GM crops in the country until their advantages and safety are proved beyond doubt. Some New Delhi-based organizations, like the Global Biotechnology Forum, believe that the time is not right for the introduction of GM crops into India. They argue that the country is suffering from income inequalities and a poor food distribution system rather than any food shortage. There are also allegations that GM foods have already entered India unknowingly in the form of US relief aid to cyclone victims in Orissa in 2000. The Government of India has ordered an investigation into the allegations. A section of the industry also believes that developing countries like India have an advantage in remaining GM free, so that exports to countries that ban GM products, such as Japan and the EU, can get favorable treatment. India is a major exporter of de-oiled soya seedcake to some of these countries.

The farmers' organizations are a divided lot with some believing that they might save on pesticide costs and earn better profits by embracing GM technology, while others are worried about the high cost of GM seed. Some progressive farmers' groups, like the Federation of Andhra Pradesh Farmers' Organizations, argue that GM crops should be given a fair chance, and, if proved useful, farmers should not be denied their benefits. Ever since the terminator controversy became public, the governmental Department of Biotechnology and academics in universities and research institutes have made significant efforts to clarify many issues related to GM crops through seminars, popular writings, and television interviews. Today, in contrast to 1998, farmers and the public at large are beginning to take a more positive view of the subject.

GEAC's latest decision puts greater responsibility on the Ministry of Agriculture to become involved in regulating GM crops. ICAR, which is located within the Ministry, favors further testing for safety and agronomic performance before permitting GM crop release. The Department of Biotechnology, which so far has been at the forefront of those advocating the early release of GM

crops in India, believes that GEAC's decision may give the wrong impression to the world on the Indian attitude toward GM crops. This is because the questions raised by the committee pertaining to resistance markers, gene flow mediated by bees, and agronomic advantages are difficult to answer, even after two years or more of study. These sentiments are echoed by biotech experts like Gurumurthy Natarajan (Greenthumb, Chennai) who believe that GEAC's decision, made under pressure from NGOs like Greenpeace in India, is contrary to the interests of cotton farmers in India who are deprived of the benefits of these innovations. Nevertheless, the commercial release of Bt cotton in the country is now delayed by at least one year and possibly more. Though temporary, it is a win for those who advocate a more cautious and stringent evaluation before this new technology is adopted.

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INDUSTRY NEWS

HOW BIOTECH IS AFFECTING GRAIN R&D, MARKETS

Minnesota Ag Outlook Conference Insights

Insights on the changing face of crop R&D and the wide world of grain exports were discussed at a conference held earlier this summer involving leaders of the Minnesota Crop Improvement Association, Minnesota Wheat Research and Promotion Council, and the Minnesota Soybean Research and Promotion Council.

More Protection of Plant Genetics

Licensing and other means of commercial protection are increasingly being used to capture the value of the discovery of various plant development processes, including those developed with biotechnology. "Otherwise, you won't have companies take the risk to finish it," says John Gerard, former executive director of the Indiana Crop Improvement Association who founded J.G. Limited, a plant genetics marketing company. Gerard is spearheading the market development of "CystX," which is broad-based molecular technology that confers resistance to the soybean cyst nematode, a small, parasitic worm that attacks the roots of soybeans. The patented and trademarked plant technology is being licensed to soybean breeding programs worldwide.

The US has the best system in the world for protecting intellectual property rights and uses various means of protection. For example, utility patents allow for the patenting of live things, including the technology and methods to create new genes and plants. Utility patents also allow operational activity for licensees. There would be no biotechnology products without intellectual property rights, and properly enforced protection will encourage broad licensing, says Gerard. He notes that even in Europe, where biotechnology has considerable opposition, companies are coming to the US to learn how to protect genetically engineered products and processes.

Companies that license their commercial plant developments recoup their investments with royalties or "technology fees" attached to the cost of the seed, ultimately paid for by farmers. Gerard cautions that while the development of new plant technologies must be protected, commercial greed cannot overshadow market practicalities. "If the farmer dies, I die," he says.

Gerard says there is a common problem with licensing agreements—people don't read them, and if they do, they may not understand them. Those who sign such agreements—including farmers who contract to grow biotech crops—need to read the agreements. If they don't understand them, they should hire an attorney to review agreement terms with them.

The move to more privatization and protection of plant genetics doesn't come without potential downfalls. It means more involvement of lawyers, and some are uneasy about the notion that plant genetics (and thus food as well) may someday be owned and controlled by a handful of companies.

But there is opportunity as well, from farmers to consumers.

For consumers, it may mean food that is tastier, more nutritious, and keeps better. For US farmers, crops with improved or more specific attributes may mean better prices and markets for what they produce. Greater focus is already being placed on producing and delivering farm products that are identity preserved, with characteristics that can be traced from field to port.

IP Grain Certification Systems Being Put in Place

The Minnesota Crop Improvement Association (MCIA) has newly established an identity-preserved grain certification system and services. These will provide unbiased field inspections, record keeping, laboratory analysis, and official labeling of IP crops—whether they be biotech, non-biotech, or crops with special traits—to meet the needs of the product originator and end-user. The Association has also drafted a grain channeling system to help establish a process for handling specialized grain from farm to market.

Similar crop certification systems are being instituted in other states. The systems might be compared to paving streets and putting in waterlines before houses are built: IP certification systems may be little used now, but their use could become commonplace in the future. Indeed, some facets of the MCIA's new certification records are not being used now, "but the program will be there, when and if the market demands it," says Jim Boots, MCIA field service representative.

Regulatory Approval, Grain Handling Keys to Roundup Ready Wheat

Kelly Clauss, industry relations consultant with Monsanto, says there are two critical factors that influence when the company will release Roundup Ready (RR) wheat: obtaining international regulatory approval of the technology, and establishing a system of handling the grain from farm to market. Monsanto made its first transformation of RR wheat in 1997, using Bobwhite, a winter wheat variety. The first commercially available RR wheat will be spring wheat varieties, however.

Focus group surveys have indicated that weed control is a major challenge that spring wheat producers face, says Clauss. RR wheat will help many of those producers meet that challenge, as one application of Roundup at a rate of 32 ounces/acre will provide season-long control of nearly all grass and broadleaf weeds, she says.

Field trial research indicates that improved weed control in RR wheat treated with Roundup Ultra translates into a per-acre yield advantage of 4.7 bushels or \$15, based on a wheat price of \$3.20 per bushel. Clauss says RR wheat



treated with Roundup Ultra yielded 45.1 bu/acre, compared with similar plots treated with other herbicides that yielded 40.4 bu/acre, and untreated plots that yielded 29.2 bu/acre.

Still, RR wheat may not be advantageous for all wheat producers, she acknowledges, because of their cropping systems. “I know there are growers who say they may not need Roundup Ready wheat, because they say their fields are so clean from Roundup Ready soybeans,” says Clauss. “And there are growers who use Roundup as a desiccant who will have to decide if they want to use Roundup Ready wheat.”

Monsanto is now seeking regulatory approval of RR wheat in countries around the world, including the US, Canada, Japan, and, by the end of this year, the European Union. However, regulatory approval will not necessarily be the key to launching the product, she points out. Concerns about weed resistance to Roundup, controlling volunteer RR wheat in subsequent crops, preserving RR seed at planting and at harvest, and consumer acceptance are all factors that must be addressed before RR wheat becomes commercially available, which will be two to five years at the earliest.

A committee representing a cross-section of the wheat industry has been established to advise Monsanto on these and other issues related to RR wheat. The group had its first meeting in May 2001. “We are working with food companies, especially those working with wheat products, to make sure they understand what we’re doing,” says Clauss. “The main thing we don’t want to do is make a move on Roundup Ready wheat that worries anyone.” Monsanto is closely monitoring consumer acceptance of biotechnology around the world, says Clauss. One thing that is consistent globally: greater knowledge of biotechnology is correlated with greater acceptance.

Europe Buying Beans Despite Biotech

Despite concerns over biotechnology in Europe, US soy exports to Europe have been increasing, in part due to demand for soybean meal to feed to livestock in place of bone meal and animal products, according to John Baize, a Virginia-based international grain marketing consultant. The largest supplier to the EU is Argentina—which plants about 90% biotech (RR) soybeans. Growers in Brazil are not legally allowed to grow biotech beans, but they are anyway, particularly in southern Brazil, according to Baize. “EU processors say they are receiving beans from Brazil with 1.5 to 60% biotech content, and it is illegal to grow,” he says. Don Nickel, a Mountain Lake, Minnesota soybean producer who also serves on the Minnesota Soybean Research and Promotion Council says Brazil does not

systematically test its soybeans for biotech content. This makes enforcing non-biotech production difficult and results in a product that is more uncertain for soybean importers.

Baize says the EU will soon have regulations on biotech labeling of feed ingredients and a system to trace feed and food ingredients “from the farm to the fork.” This will result in more hassles in shipping soybeans to Europe, but it also may create greater marketing opportunities for the US than South America, he points out, since the US is best equipped for grain testing and traceability.

There is some evidence that the anti-biotech sentiment in the EU may be waning, says Baize. “Nobody is dropping dead because of it. Greenpeace’s argument is not holding water,” he says. Nickel, who was in Europe meeting with prospective soybean buyers this spring, says biotech opposition in the EU is “completely media driven.” The European public and even the media there are more willing to accept the technology when they learn more about it. “All they have heard about biotech was from the Greenpeace side,” he says. Adds Baize: “Worrying about biotech is a rich man’s game. Poor people don’t worry about where their food is coming from.”

Nickel notes a conversation with an animal nutritionist in Italy who wished he could get 100% Bt corn for chickens produced there, since no other pesticide applications are needed, resulting in a healthier feed product. He believes that it’s just a matter of time before biotech beans are officially approved for production in Brazil; and further, that the Europeans will come to the conclusion that it won’t be easy to have non-biotech beans, and if they do insist on non-biotech beans, then they will cost much more.

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