ISB NEWS REPORT

COVERING AGRICULTURAL AND ENVIRONMENTAL BIOTECHNOLOGY DEVELOPMENTS

JUNE 2001

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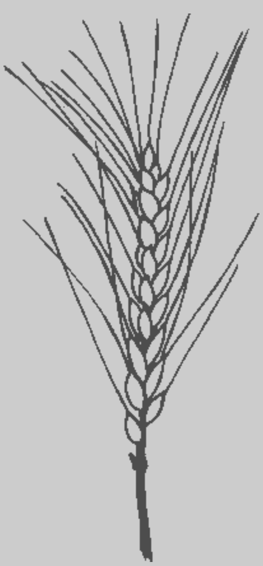

NEWS AND NOTES

BIOTECHNOLOGY: PRESENT POSITION AND FUTURE DEVELOPMENT

The term ‘biotechnology’ is used nearly on a daily basis in the mass media in referring to technologies employed on organisms ranging from microbes to humans. However, as those working in the area know, to be able to put your hand on a text describing the range of technologies in a concise scientific manner has been nearly impossible—until now. Irish born Dr. Martina Newell McGloughlin (Director, UC Davis Biotechnology Program <http://www.biotech.ucdavis.edu>) and Irish-based Dr. James Burke (Chief Crop Scientist at the Teagasc Crop Research Centre, Oak Park, Carlow, Ireland <http://www.teagasc.ie>) have skillfully managed to create a concise but informative overview of the numerous current and next generation biotechnology applications. This book highlights the broad range and scope that modern biotechnology will take and also shows how it will touch most corners of human society. It covers the main areas of crop production, animal biotechnology, the environment, industrial biotechnology, and human health. The authors structured the book into nine chapters:

1. Overarching Platform Technologies
This opening chapter reviews the backbone technologies on which modern biotechnology relies, including descriptive outlines of genomics, nucleic acid technology, gene expression, microarrays, and imaging, just to name a few. The chapter ends with informative insights into future technologies such as biosensors, bioelectronics, and bionetworks.

2. Crop Biotechnology
Areas dealt with in this chapter include plant transformation, direct DNA delivery methods, transformation constructs, T-DNA mediated site specific recombination, biological containment and high expression, progress to date in crop agriculture, secondary plant metabolites, nutraceuticals, plant architecture, the industrial scene, issues and concerns, and priorities for Ireland.

3. Animal Biotechnology
The new retroviral transformation systems, progress to date in animal biotechnology, choosing sex, enhancing reproductive potentials, and the future of this technology are some of the subjects explored in this chapter.

4. Biotechnology and the Environment
A near global insight into the biotechnological applications of bioremediation, use of rhizobia, alternates to chemical applications in agriculture, no-till systems,

THE ISB NEWS REPORT

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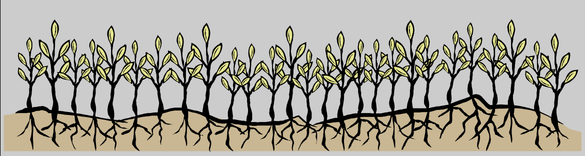

biomass conversion, and the use of biotechnology in the mining and metals recovery industry are presented. The issues surrounding environmental safety concerns are also discussed.

5. Industrial Biotechnology
The advantages of genetic engineering for industrial biotechnology are discussed and outlined. There are descriptions of how biotechnology (in particular enzymes) is used in the production of starch and sugars, and oils and fats, as well as its utility in the cleaning, textile, pulp, and paper industries. Biotechnology's importance in the animal feed and food diagnostics fields is discussed. Excellent examples are provided.

6. Animal and Human Health
How biotechnology is impacting on the fields of therapeutics and diagnostics is explored. Other topics such as DNA vaccines, EST database mining, 2D gel electrophoresis, disease targeting, and gene targeting are described. Sensitive areas such as gene therapy, the human genome project, embryonic stem cell use, and cloning are also discussed.

7. Regulations
This chapter gives an overview of the regulation pertaining to modern biotechnology in the US and the EU. There is a focus on Ireland from a historic point of view in the development of regulations.

8. Intellectual Property Issues
The important and often controversial topic of patenting genes is explored in this chapter. Topics such as the technology transfer, genetic resources, and the positions of the EU and OECD on patenting are presented.

9. End Overview
This brief last chapter gives policy makers something to chew on in relation to concepts and issues to be dealt with.

The five appendices not only serve as an excellent reference to the preceding text, but they also serve as a quick reference guide. They outline everything from the types and number of biotech enzymes on the market and recent agbiotech merger deals, to agbiotechnology products on the market and what is expected to be approved for commercial release within the next six years. The book is well researched and written; however, a greater use of graphics and color would be an added benefit to enhance the lay reader's experience.

The book often tends to take an Irish perspective on biotechnology in several of its chapters, which offers North Americans a unique view of the priorities and directions currently being taken by a smaller European country like Ireland.

As the technology and its impact on our lives rapidly unfold, it is certain that a second edition within the next two years will be a must for McGloughlin and Burke. Several areas have already changed since the book's publication last September. These include developments in human and plant genomics and changes in the agbiotechnology regulatory systems in several countries.

This 316-page book,"Biotechnology: Present Position and Future Development" (ISBN 184170122X), written by Dr. Jim Burke and Dr. Martina Newell McGloughlin, is available for ordering at <http://www.teagasc.ie/publications/biotech-book.htm>.

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Plant Research

Transgenic Pollen Escape – Need for Consequence Assessment Instead of Containment

An increasing number of transgenic crops have been released into the environment, making the examination of environmental effects of these transgenic plants an important task of biosafety research. The establishment and spread of transgenic plants is one prerequisite condition for the occurrence of environmental interactions. Likewise, the potential impact of transgenic crops on community ecology will depend on the distribution and establishment of the new transgenic traits.

One of the most discussed environmental effects associated with the use of transgenic plants is the loss of control over the engineered genes. Flowering and pollen dispersal are important for outcrossing of the genetically engineered trait, and an escape of genes is likely by pollen since it is part of the reproductive system that is designed for gene movement. Pollen-mediated gene escape is difficult to control in mating plants. Wind-pollinators like sugar beet easily spread pollen over distances of more than 1000 m.

To investigate the efficiency of transgenic pollen movement under certain realistic environmental conditions, the use of bait plants might be very effective. To address this, our study identified two goals. The first was to test the suitability of so-called "cytoplasmic male sterile" (cms) mother plants for monitoring transgenic pollen flow. These plants are usually pollen acceptors used in the large-scale seed production of sugar beets. Cms is a maternally inherited trait characterised by the inability of the plant to produce functional pollen. Since cms plants are not able to self-pollinate, the male parent of the produced offspring must be a fertile plant. Analyses of the offspring should provide data on gene transfer into the investigated areas.

Our second goal was to test the effectiveness of containment strategies that aim to limit gene escape via pollen. The effectiveness of hemp (*Cannabis sativa*) stripe containment strategy was tested by measuring the frequency of pollinated cms bait plants placed at different distances and directions from a transgenic pollen source. Hemp is mostly effective against wind mediated pollen flow due to its 4 m height and sticky leaf surface. The hemp containment strategy was applied in the field release plot located at the Aachen University of Technology (RWTH) in Germany in order to limit the pollen escape from flowering transgenic sugar beet in large plots.

To investigate correlation between the wind direction and pollen spread, bait plants were placed in different positions around the pollen source. As a pollen source, transgenic sugar beets were used that expressed recombinant DNA for virus (BNYVV) resistance, and antibiotic (kanamycin) and herbicide (glufosinate) tolerance. Fifty-six cms bait plants were positioned around a field site containing 30 flowering transgenic plants as pollen donor source. Twelve bait plants were placed 9 m from the pollen source but inside a 5 m wide hemp containment stripe, 12 plants were placed just outside the hemp barrier, and the remaining bait plants were placed in eight different directions (north, northwest, west, southwest, south, southeast, east, and northeast) at distances of 50, 100, 200, and 300 m from the transgenic donor plants.

The frequency of gene flow from the transgenic plants was calculated as the percentage of individuals surviving herbicide-treatment in comparison to the total number of seedlings (originating from a single bait plant). The individual herbicide tolerance was confirmed at the DNA (PCR) and protein (ELISA) levels. Plants that survived the herbicide treatment were examined for the presence of transgenic DNA-sequences by PCR. ELISA was used to quantify expression of one transgenic protein as well.

Although not capable of self-fertilization, the bait plants produced a significant number of seed offspring. The mean number of seed produced was about 1300 per plant.

In the offspring, about 20% of the seedlings were tolerant to phosphinothricin application, indicating that they were potentially transgenic. This was confirmed by PCR analysis as well as by ELISA at the protein level. All plants of the transgenic progeny expressed the BNYVV coat protein gene at significant levels.

Analysis of the bait plant offspring demonstrated dispersal of transgenic pollen inside and outside the hemp containment stripe. Only 31% of the progeny from bait plants were not pollinated by any of the genetically modified pollen donors. As expected, the outcrossing was highest within the hemp containment area with rates of up to 80% per cms plant on the eastern side of the pollen source. These data correlated with the main wind direction from west to east. A significant outcrossing was observable outside the hemp isolation with rates between 0.5% on the west side and 40% on the east at a distance of 200 m from the transgenic pollen donor plants. However, seeds from plants within the hemp stripes were not completely transgenic, indicating that non-transgenic pollen from flowering plants located somewhere outside the boundary passed the hemp containment to the inner side and pollinated the bait plants.

The results clearly demonstrated the insufficient effectiveness of the hemp stripe containment strategy. Both physiological and molecular tests confirmed the escape and production of transgenic offspring more than 200 m behind the hemp containment. The use of cms bait plants proved to be a useful monitoring instrument for *in vivo* detection of gene escape via pollen. Another advantage of the bait plant system is the chance to characterize the resulting offspring. By using physical traps like sticky petri dishes or microscope slides instead of a bait plant, analysis of gene expression in the potential offspring is impossible.

It was clearly demonstrated that the transgenes were detectable at the DNA, protein, and physiological levels. Since the herbicide-tolerance test was much easier and cost-effective than the PCR and ELISA proof, this physiological screening offers advantages for large scale monitoring tasks. The genetic linkage of all three engineered genes in the genome of the progeny was confirmed.

We can conclude that the bait plant method is an excellent method to monitor escape of transgenic pollen. Moreover, the results of the present study showed that the containment of flowering transgenic sugar beet within hemp stripes did not prevent the spread of transgenic pollen. Hemp containment is a suitable and well-established method of isolation for conventional seed production, but only to prevent the incross of foreign pollen at a reasonable threshold level.

Conventional plant breeding has a long history of experiences with unwanted effects by pollen escape. There is nothing new in this for transgenic plants—since absolute containment is unlikely to be effective, risk assessment should address the consequences of successful gene flow.

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Lethal Trichomes

From an insect's perspective, trichomes can impose significant impediments to blissfully dining on leaves. Trichomes, also known as leaf hairs, are specialized epidermal cells present in most plants that naturally excrete a host of lipid-based toxins which serve to discourage predation by a wide range of herbivores, including microbes, insects, and large grazing mammals. Trichome excretions from the mint family and other aromatic plants also serve as economically important sources of flavorings and fragrances. It is not surprising that investigators would ultimately look at bioengineering trichome constituents to improve the insect-repelling properties of plants. A project conducted by George Wagner's research team at the University of Kentucky used the intrinsic biochemical pathways of tobacco plants to metabolically engineer trichome gland exudates to be resistant to aphids.[1]

Metabolic engineering, which involves the overexpression or suppression of specific metabolic pathways, is a popular strategy for coaxing the fruits, leaves, or roots of plants to produce novel nutritional compounds, pesticidal chemicals, or agents that reduce environmental stress. Monoterpene biosynthetic pathways, which are linked to plants' cytochrome P450 system, are responsible for producing most of the trichome secretions. The genomics of these pathways are under investigation, including work that ultimately will lead to the development of cDNA libraries of pathway segments.[2, 3]

Wagner's research focused on the biochemistry of peltate (shield-like) trichome glands, which are either unicellular or aggregates of up to nine secretory cells attached to the leaf surface by a pendulous stalk. Multicellular trichomes may be arranged in a single row or in several layers of cells, and form a variety of shapes including branched tree-like patterns and shield-like structures. They are found in almost all plant families including many commercially important ones.

Cytochrome P450 proteins belong to a group of heme-thiolate proteins found in many prokaryotic and eukaryotic cells. These proteins, usually serving as terminal oxidases in electron transport chains, are involved in a profusion of metabolic pathways that produce biologically active intracellular compounds and secretions (refer to http://bmbsgi11.leeds.ac.uk/promise/P450.html). The cytochrome P450 system is the focus of numerous metabolic engineering studies because of its close association with various plant defense mechanisms.

In their most recent study, Wagner and colleagues were interested in increasing the potential pesticidal compounds in trichomes by suppressing a cytochrome P450 hydoxylase gene involved in terpene biosynthesis pathways. They focused on the inhibition of cembratriene-diol (CBT-diol), which is the predominant terpene exudate in trichomes. CBT-diol is biosynthesized from geranylgeranyl phosphate. A cytochrome P450 catalyzes the addition of the second hydroxyl to the cyclized diterpene ring. Wagner's team learned the suppression of CBT-diol results in the accumulation of its precursor molecules cembratriene-ol (CBT-ol). CBT-ol was shown in his study to combat the colonization of aphids on experimental tobacco plants.

Stage one of the study entailed isolating the cytochrome P450 hydroxylase gene responsible for CBT-diol synthesis from CBT-ol. Complementary DNA probes were made using PCR-based cDNA subtraction method. A partial 315 base pair cDNA attached to a CaMV (cauliflower mosaic virus) 35S promoter was then introduced into tobacco plants in an antisense position. A set of control plants was transformed with the cDNA placed in the sense direction. Transformation was accomplished using an *Agrobacterium* system using standard protocols.[1] Northern blotting for the reversed P450 gene mRNA confirmed selective gene expression in various plant tissues.

Trichome exudates were collected from leaf disks and measured using GC-MS. These quantitative values were later correlated to increased aphid toxicity and reduced aphid colonization inhibition. Final quantitation of CBT-diol and CBT-ol was accomplished by comparing their levels to leaf dry mass. Leaf and stem trichomes were studied separately to evaluate consistency of the metabolic engineering process throughout the plant. Plants with the partial antisense P450 cDNA showed a 41% decrease in CBT-diol production with a consequent 19-fold increase in CBT-ol accumulation in the trichomes. The experimental plants exhibited raised CBT-ol levels from the normal of 0.2% to 4.3% of leaf dry mass.

Successful resistance to red aphid (*Myzus nicotiana*) colonization was measured using standard tests for insect repulsion and toxicity.[1] Initial LC50 (lethal concentration 50%) studies were performed to confirm the aphicidal activity of the CBT-ols produced by the experimental and control plants. The LC50 for CBT-ol was 6.4 µg/aphid, showing better aphicidal activity than CBT-diol with an LC50 of 16 µg/aphid. Aphid colonization was assessed on intact greenhouse plants by monitoring the population of the insects on the infected plants. The experimental plants with the antisense cDNA had significantly fewer aphids than plants producing normal levels of CBT-diol. Survivorship curves calculated for the aphid populations confirmed that higher levels of CBT-ol were responsible for the aphid decline.

Wagner's favorable preliminary results warrant additional investigation for the utility of this technique on other plants and pests. The pesticidal properties of CBT-ols in various crop plants will have to be determined for targeted pest insects. However, further testing needs to be done to ensure that the elevated levels of CBT-ols achieved in Wagner's study are safe for animal and human consumption. In addition, the potential toxicity of CBT-ols on biological control organisms, such as parasitic wasps, and pollinators, including lepidopterans and hymenopterans, needs to be evaluated. This is a promising preliminary strategy for reducing the need for pesticide applications to crops.

The research projects of Wagner[1] and Croteau[2] demonstrate similar promising lines of research with disparate commercial outcomes. The Croteau team is interested in increasing essential oil biosynthesis from mint trichomes. Investigators wishing to produce plants with trichomes that are more effective in combating a variety of plant pests will find equal value in both studies. This is also true for researchers desiring plants capable of making large amounts of flavorings and fragrances. The possibility of producing plants with dual commercial value is feasible with this and related metabolic engineering methods.[3] Root crops could reasonably be produced with leaves that simultaneously provide marketable oils. These studies may also benefit researchers studying the metabolic engineering and regulation of terpenes or other compounds.

Wagner sees future value for trichome terpenes as potential tumor suppressing drugs, anti-inflammatory compounds, and immunotherapeutic agents. Concentrated exudates may also be useful as biological control agents for domesticated plants and animals. The type of metabolic engineering accomplished by Wagner's team may provide the means to produce plants with predictable alternate physiologies.

Altering a cell's metabolism to redirect it for the accumulation of desired biochemicals is proving to be a practical way to exploit natural compounds synthesized by plants.

Sources

1. Wang E, et al. 2001. Suppression of a P450 hydroxylase gene in plant trichome glands enhances natural-product-based aphid resistance. *Nature Biotechnology* 19 (4): 371-374.

2. Lange MB, et al. 2000. Probing essential oil biosynthesis and secretion by functional evaluation of expressed sequence tags from mint glandular trichomes. *PNAS* 97(6): 2934-2939.

3. Chapple C and Carpita N. 1998. Plant cell was as targets for biotechnology. *Plant Biology* 1: 179-185.

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Biotechnology in the Garden

A rose by any other color . . .

Blue roses and black orchids were among the first wonders promised by horticultural biotechnology. However, difficulty in achieving stable plant transformation and problems expressing heterologous genes have shown that genetic modification of ornamental crops is far more complicated than originally anticipated. Despite biotechnology's tremendous success in improving traditional crop plants, genetically enhanced ornamental plants are still few and far between. Now, thanks to recent advances in plant transformation technology and a better understanding of plant metabolic pathways, horticultural biotechnology's time may have finally arrived.

At the vanguard into ornamental plant biotechnology was Australia's Florigene (at the time Calgene Pacific). In the early '90s, this company created a buzz with its well-publicized attempt to create a blue rose. Species such as rose, tulip, and carnation are not naturally blue as they lack the "enzymatic machinery" to synthesize blue colored pigments. Therefore, researchers at Florigene cloned two enzymes from petunia, flavonoid 3'5' hydroxylase (F3'5'H) and dihydroflavonol reductase (DFR), that are responsible for producing the blue pigment, delphinidin, found in the vacuole of petunia cells. In an effort to generate a blue rose, researchers at Florigene transferred these two metabolic genes into rose plants. Despite the difficulties often encountered with ornamental transformation, stable transgenic roses were successfully regenerated. However, when the blooms finally opened, they were anything but blue.

The problem is that the delphinidin pigment acts very much like litmus paper—in the alkaline vacuolar environment of the petunia, delphinidin is blue, but in the acidic environment of the rose vacuole, it is pink. Similar experiments performed by Florigene with carnation, which has a more alkaline vacuolar environment, were more successful and the company has since released two transgenic varieties, Moonglow and Moonshadow. Nevertheless, even these "blue" flowers appear more mauve than truly blue.

Now, a recent report by deVetten et al. published in the *Proceedings of the National Academy of Sciences* (*PNAS*) has reawakened interest in the possibility of creating a blue rose.[1] Production of blue pigments in flowers requires the 3', 5'-hydroxylation of the purple anthocyanin precursors. As mentioned above, two of the enzymes for "blue genes" required for this process, F3'5'H and DFR, have already been characterized. In the *PNAS* paper, deVetten and colleagues report the discovery of a third blue gene, *difF*, that is required for blue pigment synthesis in petunia. This novel gene is required for the formation of 3', 5' substituted anthocyanins. After cloning and sequencing the *difF* gene, they found that it encoded a b(5) cytochrome that was only expressed in flowers. By creating *difF* gene knockouts through transposon mutagenesis, they were able to demonstrate that floral tissues lacking *difF* activity displayed a 60% reduction in delphinidin accumulation compared to wild type. This was due to the fact that in *difF*-plants, F3'5'H activity in flowers is reduced by as much as 20-fold. These findings therefore demonstrate the importance of the *difF* gene product in the synthesis of blue flower pigments.

The authors suggest that by introducing this new blue gene into roses and carnations, along with the two previously isolated blue genes, it may now be possible to create truly blue flowers, although this has yet to be substantially evaluated. Whereas it may now be possible to generate much higher levels of delphinidin in transgenic roses and carnations with this approach, the effect of low pH on delphinidin color still needs to be addressed. To accomplish this, it will be necessary to either modify vacuolar pH, which would be an extremely ambitious undertaking, possibly with many unforeseen side effects, or to introduce other enzymes to engineer blue pigments that are less pH sensitive. Either way, as promising as these results are, it is still not clear whether the production of a truly blue rose is any closer to reality.

Staying Power

A major target for horticultural biotechnology is an increased floral lifetime for cut flowers and bedding plants—flowers that bloom earlier and last longer are obviously desirable from a horticultural standpoint. Ethylene is known to play a role in floral senescence. To block the action of ethylene, many research groups have tried to engineer plants with decreased ethylene production, either by creating anti-sense plants or through gene silencing, both with the gene for the ethylene synthetic enzyme, ACC synthase, as the target. Another approach has been to create ethylene-insensitive plants through the introduction of the *etr1-1* gene from *Arabidopsis*. This gene acts in a dominant fashion to make plants largely ethylene insensitive. However, problems have been encountered with ornamental varieties engineered in this manner.

In a report published last year, researchers from the University of Florida found that transgenic ethylene-insensitive petunias, though flowering earlier and possessing delayed flower senescence, exhibited variable horticultural performance, which depended upon the plants' genetic background.[2] When examining two transgenic ethylene-insensitive varieties of petunia, the researchers found that although both lines possessed the desirable early flowering feature, one line required a reduced culture temperature to exhibit this phenotype. In addition, both lines exhibited a delay in flower senescence when compared to wild type, but the extent of the delay was genotype-specific. These findings present a potential problem, as such genotype-based variations will make it hard to predict how a particular plant variety will respond to an introduced transgene. More worrisome was the finding by the Florida group that both ethylene-insensitive lines exhibited a marked delay in fruit ripening and a significant reduction in the rooting of stem cuttings. These last results indicate that there may be a trade-off between increased floral life and a decrease in the efficiency of propagation in plants with altered ethylene metabolism.

Activating transformation

A major problem that has confronted horticultural biotechnology is the issue of successful, stable transformation. *Agrobacterium tumefaciens*-mediated transformation is the standard method of transforming most dicot food crop species, but many ornamental species, both monocot and dicot, have proved to be resistant to this method of transformation.

Microprojectile bombardment and direct gene transfer have been successful with some species, but the efficiency is often too low to be economically practical. Transformation via *Agrobacterium* offers additional benefits, such as fewer integration events per genome and the transfer of a defined segment of DNA, the T-DNA. Now, new methods developed for facilitating *Agrobacterium*-mediated transformation of monocot crop species, such as maize and rice, are having success with even the most recalcitrant ornamental varieties. In the past two years there have been reports of successful *Agrobacterium*-mediated transformation of such widely divergent species as iris and orchid. The key has been the use of a super-virulent strain of *Agrobacterium tumefaciens*, carrying the *vir B*, *vir C* and *vir G* genes. As described in a recent paper in *Plant Cell Reports*, successful transformation of suspension-cultured phalaenopsis orchid cells required the use of this super virulent strain, hyper-activated by the inclusion of the *vir* gene stimulator, acetosyringone.[3] Using this method, coupled with a novel regeneration protocol, the researchers were able to recover 10 – 24 hygromycin resistant plants per gram of suspension culture. The regenerated plants also stably expressed the (beta)-glucuronidase (GUS) gene that had been transferred along with the hygromycin selectable marker. Based on colorimetric GUS assays, none of the plants exhibited chimerism, a problem often seen in plants transformed by particle-bombardment methods.

Despite the problems that have plagued horticultural biotechnology, many agricultural biotechnology firms are still pursuing lines of research to modify and improve ornamental species. Such firms as Monsanto, Florigene, and Suntory have filed new applications for field trials of ornamental species. The cut-flower industry is expanding rapidly and there is increasing demand for flowers with longer vase life and novel aesthetic traits. Many companies, especially firms in Japan, view genetically enhanced ornamental species as being more commercially acceptable, since consumers do not ingest the products and there is less risk of outcrossing to wild relatives. With this support, the field of horticultural biotechnology will continue to advance, and maybe someday soon the mythical blue rose will become a reality.

Sources

1. deVetten N, terHorst J, vanSchaik HP, deBoer A, Mol J, and

Koes R A. 1999. A cytochrome b(5) is required for full activity of flavonoid 3',5'-hydroxylase, a cytochrome P450 involved in the formation of blue flower colors. *Proceedings of the National Academy of Sciences USA* 96: 778-783.

2. Gubrium EK, Clevenger DJ, Clark DG, Barrett JE, and Nell TA. 2000. Reproduction and horticultural performance of transgenic ethylene-insensitive petunias. *Journal of the American Society for Horticultural Science* 125: 277-281.

3. Belarmino MM and Mii M. 2000. Agrobacterium-mediated genetic transformation of a phalaenopsis orchid. *Plant Cell Reports* 19: 435-442.

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Animal Research

Environmentally Friendly Transgenics

Animal waste containing high levels of phosphorus is a serious problem due to its adverse effects on the environment. The high phosphorus content of manure is due to the inability of monogastric animals such as pigs and poultry to utilize the phosphorus that is stored in plants as the chemical compound phytate. Therefore, animal feed must be supplemented with inorganic phosphorus to achieve optimal growth. The resulting high phosphorus manure is typically disposed of as fertilizer applied to land. This can lead to phosphorus runoff into streams and lakes causing eutrophication and the resulting large algae blooms and death of fish.

One approach that has been investigated to reduce the requirement for inorganic phosphate supplementation is to include microbial phytase as a feed additive. Phytase hydrolyzes the inorganic phosphate from the phytate molecule, thus releasing the phosphorus. However, this approach is limited by the cost of the added phytase and the inactivation of phytase activity by the high temperatures required for pelleting feed. An alternative approach that has been proposed is to generate transgenic animals that synthesize and secrete their own phytase.

In the May 2001 issue of *Nature Biotechnology*, researchers at the University of Guelph reported the generation of transgenic mice that secreted a bacterial phytase in saliva. The *E. coli* phytase gene was modified such that it was under the control of two salivary gland-specific promoters: the inducible proline-rich protein (PRP) promoter and the constitutive parotid secretory protein (PSP) promoter. In transgenic mice, the *E. coli* phytase gene was strongly expressed in the parotid and submandibular glands. Phytase activity in saliva averaged 35 – 400 units/ml for transgenic mice containing the phytase gene under the control of the inducible PRP promoter and 24 units/ml for mice containing the PSP promoter-phytase gene construct.

Biological potency of the *E. coli* phytase was examined by measuring the phosphorus content in fecal samples. Transgenic mice were fed a diet containing 40% of total phosphorus as phytate. An 11% reduction in fecal phosphorus content was observed for transgenic mice expressing phytase under the control of both the inducible PRP promoter and the constitutive PSP promoter. These results demonstrate that secretion of bacterial phytase in the saliva of a monogastric animal may be a valid approach to reducing fecal phosphorus content and reducing the amount of supplemental inorganic phosphate added to feed.

The production of transgenic livestock secreting phytase is the next logical step. In fact, this is the same group that in 1999 reported the generation of a transgenic pig that produces phytase in its salivary gland and was named the "Enviropig." This press release generated much enthusiasm, however no tests to evaluate the effectiveness of the phytase gene in these pigs have been published. Nevertheless, the results from the transgenic mouse studies are certainly intriguing enough to warrant a more detailed investigation in livestock.

Source
Golovan SP, Hayes MA, Phillips JP, and Forsberg CW. 2001. Transgenic mice expressing bacterial phytase as a model for phosphorus pollution control. *Nature Biotechnology* 19: 429-433.

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Industry News

US, Canada Face Biotech Wheat Showdown

The United States and Canada appear to be headed toward a showdown in the biotechnology arena. With global wheat markets at stake, the decision by one of these trade competitors to adopt biotech wheat will be critical to the decision of the other. Both the US and Canada produce spring wheat and compete for the same markets.

Biotech wheat won't be commercially available in either country until around 2003, at the earliest, when Monsanto will have Roundup Ready wheat ready for release in both countries. The wheat will be genetically modified to be resistant to glyphosate, which kills both grass and broadleaf weeds. More than likely, the US will have the opportunity to decide before Canada whether to adopt biotech wheat. Whether that decision is the right one, however, will depend on what Canada will do.

Bill Wilson, Professor of Agricultural Economics at North Dakota State University (NDSU) in Fargo, has developed a model to evaluate the strategic moves of both countries in adopting biotech wheat. His conclusions:

- If neither country adopts biotech wheat when it becomes commercially available, neither will have a payoff or net benefit.
- If both countries adopt biotech wheat at the same time, both countries will likely gain by first-tier payoffs or benefits (such as higher grain yields, less herbicide use, and better crop management) and through prospective second-tier benefits, such as better milling wheat or better quality bakery products.
- If Canada adopts biotech wheat and the US doesn't, the US would likely gain marketshare.
- If the US adopts biotech wheat and Canada does not, then Canada would likely benefit.

"I suspect there's nothing that the Canadians would like more than for us to liberally adopt genetically modified wheats without the ability to segregate them in the marketplace," says Wilson. He suspects the Canadians would raise immediately the price of their non-biotech wheat to export to countries wary of biotechnology. Thus, if Canada chooses not to adopt biotech wheat, the best alternative for the US is not to adopt it either. But if Canada does adopt biotech wheat, the US is better off to follow suit.

The decision is pretty simple on the export side—it all depends on what Canada does. "There would be serious market implications if the US adopts [biotech wheat with] the current state of buyer views toward GM wheats, without a system to reliably segregate wheats. We're seeing this already in corn. Rival countries are now selling non-GM corn to Japan at fairly substantial premiums as a result of the problems in the US," says Wilson.

Mixed Market Signals

Biotech wheat faces different challenges than biotech corn or soybeans, says Wilson. For one, wheat is more dependent on exports. About half of the US wheat crop is exported each year, compared to about 20% of the corn crop and about 35% of the soybean crop. Wheat is also used more widely for human consumption and has more grain export competitors to contend with, including Canada.

"The US wheat industry is getting mixed messages about biotechnology," says Wilson, "from a domestic industry that is generally more receptive or not as averse and an export market that is mostly intolerant of it." The US uses about half of the wheat it produces each year and exports the rest. Unlike consumers in Europe, US consumers and food industry leaders are generally confident in the safety of biotechnology and the government's ability to regulate it. Wilson also points out that second-tier biotech products that benefit consumers may boost consumption of wheat-based products in the US, a market that on the whole has been flat in recent years. "If a food company can differentiate its products, it can increase demand," he says.

While biotech emphasis is initially concentrating on first-tier benefits to producers such as herbicide resistance, little attention has been paid to the tremendous advantages of second wave benefits of biotech wheat—stronger flour, enhanced nutrition, the ability to replace additives, improved product quality characteristics such as food taste and texture, production of industrial products, and increased storability. Wilson says one study points out that bread products with a longer shelf life could reduce bakery costs by 12%. "That's a huge number," he says.

A NDSU survey indicated that domestic millers and bakers are indifferent toward purchasing wheat that is genetically modified to enhance farm production. However, they would expect to pay less for biotech traits with only on-farm benefits, such as improved crop yields and herbicide resistance. Conversely, most are willing to pay more for attributes enhanced by biotechnology that would increase revenue or decrease their production costs, including functional traits, and enhanced processing and end-use factors.

While domestic wheat users are more accepting of biotechnology, overseas wheat users are not. Seven out of 10 of the leading US hard red spring (HRS) wheat importers in the 1998-99 marketing year are currently averse or opposed to genetically modified foods (see Figure 1). In total, about 85% of the global customer base for US HRS wheat now oppose the development of biotech wheat, compared to only 30% of Canada's overseas customers who oppose the technology, says Wilson. China is a key reason for the disparity in the opposition among the cus-

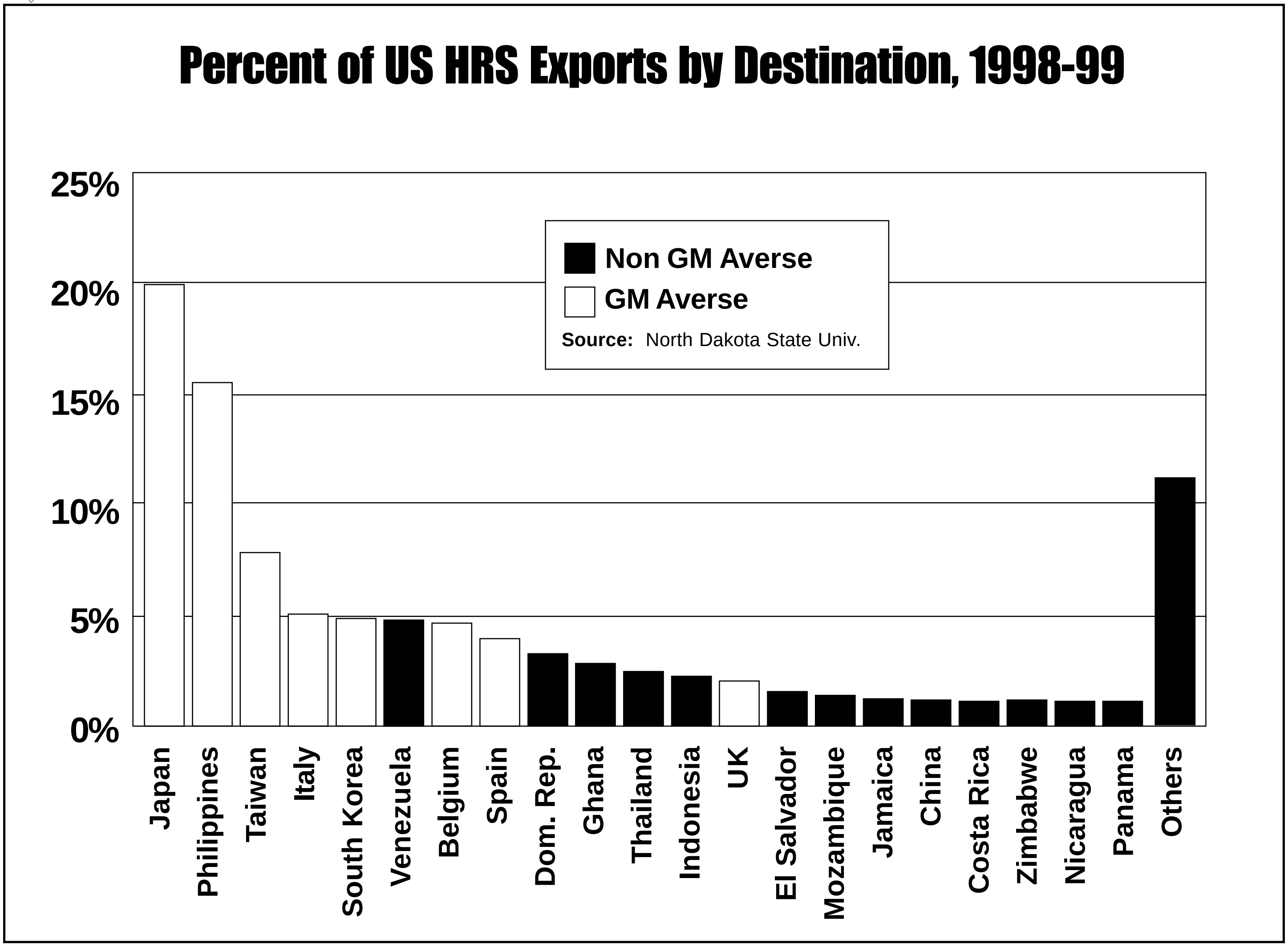

Figure 1: Seven out of 10 of the leading US hard red spring wheat importers in the 1998-99 marketing year are currently averse or opposed to genetically modified foods. In total, about 85% of the global customer base for US hard red spring wheat now opposes the development of biotech wheat.

tomer bases of the US and Canada, which compete aggressively for the world's spring wheat export market. China thus far has been neutral in its views toward biotech wheat, and while the Chinese have imported little to no HRS wheat from the US in recent years, China is Canada's largest customer for spring wheat.

Canada: Inherent Advantages

Canada has inherent quality control mechanisms to manage the adoption of biotechnology within its grain marketing system through the Canadian Grain Commission and the Canadian Wheat Board, which has the sole authority to market grain in Canada. The CWB has the authority to regulate wheat varieties—and deny release of varieties for marketing reasons—while no such authority exists in the US. Also, there are fewer spring wheat varieties released and grown in Canada compared to the US, and varietal quality performance is more uniform across growing regions in Canada compared to the US. Canadian varieties must also be visually distinguishable from varieties of a different class. Thus, Canadian wheat can be segregated more easily. According to Wilson, "It allows their market system to easily distinguish wheats that should be placed in different classifications. We don't have that."

Wilson says that it is quite possible Canada could create a separate classification for biotech wheat. "Of course they won't call it genetically modified. But when Prairie Spring and other wheat categories were developed, it was because of new production technologies. We don't do that and it's a dilemma we have."

Last year, Wilson conducted a survey of spring wheat users that estimated that the cost of segregating grain in the US may vary between $0.25 and $0.50 per bushel. Another survey of grain elevator managers earlier this year put the estimate at $0.15. It's not surprising that the estimated costs of segregating grain vary by each survey and study. "It's difficult to project, because you're asking somebody the cost to do something they've never done before," says Wilson.

It would not be unexpected to see political officials from Canada, the US, and other wheat export countries be passive promoters of biotech wheat, says Wilson. Otherwise, it could be damaging to market share in today's political climate to acknowledge supporting the development of biotech products when countries such as Japan oppose them. Then, if and when acceptance occurs, they'll move forward with the technology.

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UPCOMING MEETINGS

More meetings can be found at: http://www.isb.vt.edu

EUCARPIA ORNAMENTALS: 20TH INTERNATIONAL SYMPOSIUM

Strategies For New Ornamentals
July 3 – 6, 2001
Melle, Belgium

EUCARPIA (European Association for Research on Plant Breeding) is presenting this International Symposium on ornamentals. Five scientific program sessions will be offered on Breeding Techniques, Selection Criteria, Molecular Biotechnology, New Introductions And Use Of Genetic Resources, and New Releases: Protection, Legislation And Control. The session on Molecular Biotechnology will address molecular tools for modern ornamental plant breeding, and selection; anthocyanin modification in ornamental plants; genetical, biochemical, and molecular biological studies of flavone formation in Gerbera hybrids; modifying Lisianthus traits by genetic engineering; molecular characterization of flower color genes in azalea sports (*Rhododendron simsii* hybrids); and marker assisted selection for resistance to *Fusarium oxysporum* in the greenhouse carnation.

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TREE BIOTECHNOLOGY IN THE NEXT MILLENNIUM

July 22 – 27, 2001
Skamania Lodge, Stevenson, Washington

The week-long international meeting of the International Union of Forestry Research Organizations (IUFRO) unit on Molecular Biology of Forest Trees will share the results of studies on the molecular function, diversity, and modification of forest trees and will cover all research that uses molecular methods to study tree biology or biotechnology at the gene, genome, organism, population, ecological, and evolutionary levels.

The meeting will include a Symposium titled: "International Symposium on Ecological and Societal Aspects of Transgenic Forest Plantations," which will be held on July 22-24, 2001. A key goal of this symposium is to move past generalities and consider specific ecological benefits and safety concerns that apply to diverse kinds of genetic alterations and management regimes.The symposium will begin with consideration of societal and ethical context within which genetically modified trees are considered and employed. Ecological issues will comprise the majority of the symposium.

Contact:
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http://www.fsl.orst.edu/tgerc/iufro2001/index.htm

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