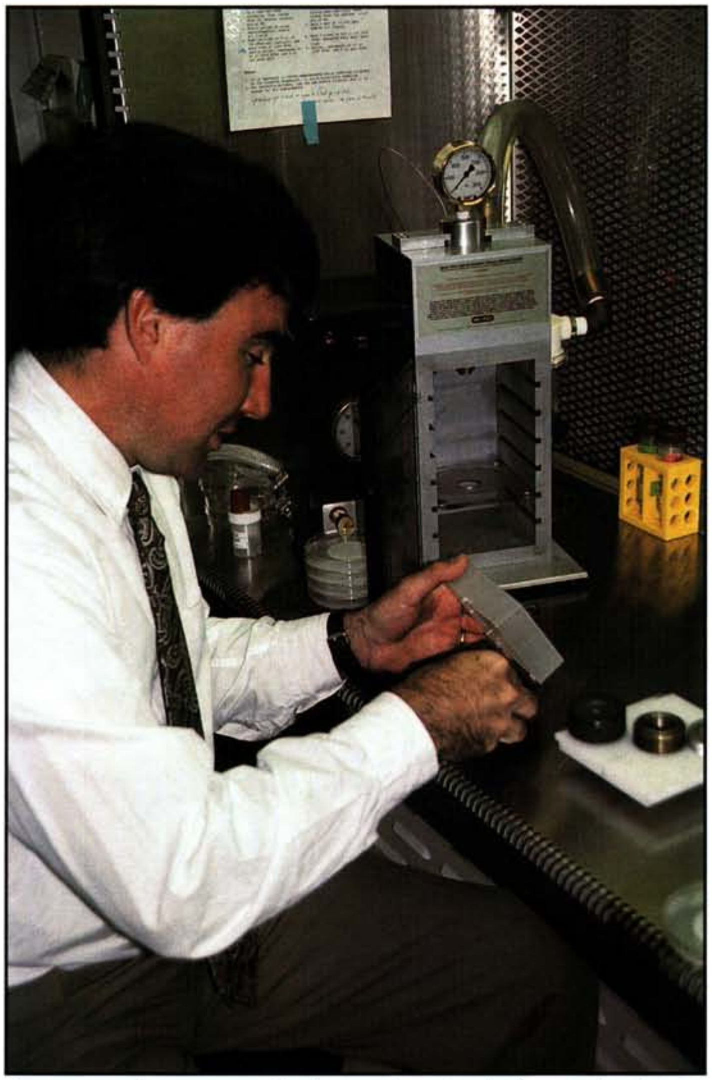
TRANSGENIC TURFGRASSES

A unique tool to advancing the development of new turfgrass varieties.

by ARTHUR P. WEBER



A more modern means of microengineering turfgrass plants involves the use of a gene gun. Selected DNA is fired into living plant cells using an explosive charge to insert the DNA into the host chromosomes. The cells are regenerated into whole plants, hopefully carrying the new gene.

OUR fundamental understanding of the biological sciences stands ready to explode, and the benefits to be derived loom large. For golfers, biotechnology promises to accelerate the improvement of turfgrass species using genetic engineering techniques.[1] Genetic Modification (GM), i.e. the splicing of genes from one organism to another unrelated organism to combine traits that would otherwise be highly unlikely to occur together, is a natural succession to the earlier realization and success of the USGA Green Section Research Program, a goal of which is to "develop turfgrasses with enhanced stress tolerance and reduced supplemental water requirements, pesticide use and costs."

Among the most desirable characteristics of such GM turfgrasses would be:[2]

1. Ability to survive high and low temperature extremes.
2. Reduced need for pesticides by increasing resistance to disease, insects, nematode, and weed encroachments.
3. Tolerance of intensive traffic.
4. Reduced requirements for mowing, irrigation, and fertilization.
5. Tolerance of non-potable water.
6. Stability of inherited characteristics.
7. Tolerance of acid, alkaline, or saline soils.
8. Tolerance of smog and other pollutants.
9. Increased shade tolerance.

Transgenic biotechnology, although still in its infancy, has already become a significant commercial reality. Genes taken from other organisms can be spliced into food plant DNA, e.g. corn, soybeans, and canola. Herbicide tolerance to products such as Roundup (glyphosate) has been conferred using genetic engineering so weeds can be controlled without harming the crops. Corn seed, by carrying a gene derived from the bacterium *Bacillus thurnigiensis* (Bt) produces the Bt toxin that will kill cornborer pests that try to eat the plant.

The derived benefits have not occurred without environmental concerns. In the wake of these genetic advances are reports that corn engineered to carry the Bt toxin might harm butterflies or other non-target insects and wildlife. Controversy relating to human and animal health over the long term derives from concerns that plants with genes from viral pathogens might combine with other viruses to create new viral strains. It is thought by some groups that the transgenic plants could create new allergies or exacerbate existing ones, such as the recent claims of increased allergic reactions to genetically altered soybeans.

Notwithstanding, breakthroughs in genetics have made it possible to improve crop plants and farm productivity in ways conventional breeders could only dream about. The possibilities are endless. With conventional breeding, it can take seven or more years to produce a new plant that may be only marginally superior to its predecessors. Genetic modification allows researchers to insert a wide array of new genes into a plant and make improvements more efficiently.

Two main methods prevail for genetic engineering in plants: The first and older of the technologies uses the bacterial species *Agrobacterium tumafaciens* to carry the gene of interest into the host plant. *Agrobacterium*, a microorganism that causes plant disease (i.e., galls) and has been known since the turn of the 20th century, possesses its own genetic engineering system. In nature, the bacterium sends its own genes into the infested host and inserts them into plant chromosomes. Researchers take advantage of this means of transforming plants by infecting them with laboratory-developed *Agrobacterium* mutants whose disease-carrying genes were replaced with specifically chosen DNA. In effect, the bacterium then acts as a microengineer, doing all the work.

The second, more modern means of microengineering uses a *gene gun* that was developed, in part, to allow for the

Traditional breeding methods can take ten years and beyond to successfully bring a new turfgrass variety to market. Genetic engineering techniques can produce significant improvements beyond a traditional breeding program.

transformation of plants that cannot be infected with *Agrobacterium*. In using the gene gun, selected DNA, coated onto gold or platinum microparticles, is fired into living plant cells, either cell cultures or embryos, using an explosive charge. The cells are punctured by the microbullets, and the DNA enters the nucleus and then inserts into the host chromosomes.

The cells, those infected by *Agrobacterium* or shot by the *biolistic* gun, are regenerated into whole plants, which then carry the new gene or genes of interest. These plants are tested, cloned, and ultimately can provide the seed for a new plant variety.

One of the new and exciting experimental approaches for discovering the function of genes is DNA microarrays. From them, extensive databases of quantitative information can be obtained about the degree to which genes respond to pathogens, pests, drought, cold, salt, growth regulators, herbicides, and other agricultural chemicals. These gene expression databases will provide novel insights into the genes that control complex responses, and they will create an opportunity to assign functional information to genes of otherwise unknown function. This information will ultimately help conventional plant breeding programs become more efficient in developing new varieties.

Toward these ends, the USGA Green Section Turfgrass and Environmental Research Program, among other conventional and biotechnological turfgrass breeding methods, has committed $835,000 over the next five years to the following university studies already underway:

"A Multigene-Transfer Strategy to Improve Disease and Environmental Stress Resistance in Creeping Bentgrass," Michigan State University, Mariam R. Sticklen, Start Date 1998, 3 years, total funding $75,000.

This study focuses molecular solutions to the biotic (i.e., pest) problems and abiotic (i.e., heat, humidity, etc.) problems associated in the management of creeping bentgrass turf. A series of available genes has been inserted into existing creeping bentgrass varieties to ascertain if resistance to various stresses can be improved. Early results indicate that various genes (e.g., elm chitinase, proteinase inhibitor, glufosinate resistance, and mannitol dehydrogenase) can be successfully inserted into the bentgrass genome. However, with the exception of the glufosinate resistance gene, improved stress tolerance has been somewhat limited. The genes can be found in the transformed bentgrass plants but do not significantly improve disease resistance or increase the amount of mannitol to help with drought and salt tolerance.

"Hybrid Bermudagrass Improvement by Genetic Transformation," North Carolina State University, Rongda Qu, Start Date 1998, 3 years, total funding $75,000.

The research is developing a genetic engineering protocol for hybrid bermudagrass varieties. Bermudagrass has been more difficult to work with due to problems producing viable plant embryos, and eventually healthy plants, from tissue culture callus. Once a reproducible technique is developed, research efforts will focus on inserting genes that confer nematode resistance into the bermudagrass clones.

"Bermudagrass Cold Hardiness: Characterization of Plants for Freeze Tolerance and Character of Low-Temperature Induced Genes," Oklahoma State University, Charles M. Taliaferro, Start Date 1998, 5 years, total funding $125,000.

This research will reduce the risk of freeze injury to bermudagrass grown in temperate regions. The project is accurately assessing the freeze tolerance of bermudagrass cultivars, isolating genes responsible for enhanced freeze tolerance, and enhancing knowledge of the fundamental mechanisms associated with cold tolerance. Substantial progress toward isolating the characterizing cold regulated proteins responsible for improved freeze tolerance in bermudagrass was achieved. Measured gene activity increases of 75 to 100 percent in crown and root tissues occurred after 24 hours of exposure to cold temperatures.

"A Turfgrass Genome Project: Integration of Cynodon Chromosomes with Molecular Maps of Cereals," University of Georgia, Andrew H. Paterson, Start Date 1999, 5 years, total funding $125,000.

The research project is producing the first primary molecular map for the chromosomes of bermudagrass. This information will be compared with molecular maps of the major cereal crops in order to gain access to the wealth of genetic information produced by scientists around the world. The map will be useful for investigating many aspects of turfgrass population biology and genetics, and provide a molecular conduit for turf improvement. Significant progress on characterizing DNA from bermudagrass and developing molecular markers was accomplished during the last year. The focus in the future will turn to full-scale genetic mapping and identifying quantitative trait loci (QTLs) of important turfgrass characteristics.

"Development of Improved Bentgrass Cultivars with Herbicide Resistance, Enhanced Disease Resistance, and Abiotic Stress Tolerance through Biotechnology," Rutgers University/Cook College, Faith Belanger, Start Date 1998, 5 years, total funding $250,000.

This project will help conserve golf course natural resources while providing quality playing surfaces by improving creeping bentgrass through genetic transformation. The work has concentrated on important bentgrass varieties and selections developed for golf greens in the Northeast. New bentgrass cultivars with improved stress tolerance and disease resistance are under development through a combination of molecular and conventional plant breeding efforts. The effectiveness of genetically engineered herbicide resistance in creeping bentgrass was demonstrated in several field tests, and the trait is now incorporated into a new cultivar. There are 50 new transgenic lines of creeping bentgrass expressing one of five potential disease resistance genes.

"Transformation of Bermudagrass for Improved Fungal Resistance," Oklahoma State University, Michael P. Anderson, Start Date 1998, 5 years, total funding $125,000.

The long-term goal of this project is to improve bermudagrass resistance to spring dead spot using gene transformation. The disease is active in the fall and early spring when temperatures are cool and moisture is plentiful. A genetic transformation system was developed for a forage-type bermudgrass because it had previously demonstrated superior growth and plant regeneration potential in tissue culture. Efforts to identify an anti-fungal protein antagonistic to spring dead spot are making progress.

Despite some of the environmental and health concerns, research efforts should be supported to ascertain how turfgrass improvement efforts could use the new genetic engineering techniques. Propelled by the skillful application of genetic modification techniques, the dramatic improvement of golf course turfgrasses, as part of a comprehensive plant revolution, is now well underway. Ultimately, it will be a combination of new genetic modification tools with existing conventional plant improvement techniques that will provide turfgrass varieties with enhanced stress tolerance and reduced supplemental water requirements, pesticide use, and costs.

References

[1]Sticklen, M. B., & Kenna, M. P., 1998, Turfgrasses Biotechnology, Ann Arbor Press, Chelsea, Michigan.

[2]Kenna, M. P., & Snow, J. T., 1999, USGA Turfgrass & Environmental Research Program.

ARTHUR P. WEBER, *a semi-retired chemical and nuclear engineer, has been an active member of the USGA Green Section Committee since 1984. A longtime Green Committee chairman, he was the principal author behind the Old Westbury Golf and Country Club (NY) Code of Environmental Conduct, a leading set of principles for golf course maintenance.*

A gene resistant to the herbicide glyphosate was successfully inserted into the plant on the right. Plants without the inserted gene react with the results on the left when treated with glyphosate.