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This publication includes lecture notes of papers presented at the 2000 New Jersey Turfgrass Expo. Publication of these lectures provides a readily available source of information covering a wide range of topics and includes technical and popular presentations of importance to the turfgrass industry.

This proceedings also includes research papers that contain original research findings and reviews of selected subjects in turfgrass science. These papers are presented primarily to facilitate the timely dissemination of original turfgrass research for use by the turfgrass industry.

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Dr. Ann B. Gould, Editor
Dr. Bruce B. Clarke, Coordinator

THE ROLE OF BIOTECHNOLOGY IN IMPROVING TURFGRASS PERFORMANCE

Barbara A. Zilinskas[1]

OVERVIEW

Turfgrasses and turfgrass breeding are of significant economic importance worldwide. In the United States, the annual sale of turfgrass seed is second only to hybrid corn (Kidd, 1993). It is therefore somewhat surprising that the application of biotechnological advances to turfgrass improvement has been slow to catch on relative to other agronomic crops. However, research in several laboratories in both the public and private sector will soon lead to the commercialization of genetically modified turfgrass cultivars. Whether these engineered varieties will be adopted by turf professionals depends on many factors, not the least of which is knowledge about the benefits of biotechnology and awareness of possible risks that are part and parcel of any new technology.

My intention is to present an overview of advances in turfgrass biotechnology with the aim of setting the stage for what I predict will dramatically affect the turfgrass industry in the not too distant future. The focus of this paper will be on genetic transformation of turfgrass, although it must be mentioned that there are other biotechnological approaches to turfgrass improvement. These include use of molecular markers in assisting conventional breeding programs and in protecting breeder's rights; somatic hybridization; and selection of somaclonal variants, produced in tissue culture, which may have improved qualities. Readers are referred to three comprehensive reviews on these subjects (Chai and Sticklen, 1998; Lee, 1996; Spangenberg et al., 1998).

TURFGRASS TRANSFORMATION

Traditionally, turfgrass improvement has depended on conventional breeding programs which have been very successful. Developments in biotechnology are now permitting those involved in turfgrass improvement to use genetic material from any living organism, rather than from only those species with which they are sexually compatible. Access to this so-called 'world gene pool' offers the opportunity to transfer genes into turfgrasses that would otherwise be impossible using conventional breeding. Thus, if there is no known source of germplasm for resistance to a certain pathogen to be incorporated into breeding programs, new technologies are now available to introduce genes for disease resistance that may be found in unrelated species. Thus, biotechnology can complement and augment breeding programs, and it is important that breeders and biotechnologists work closely together to achieve the most from each approach to turfgrass improvement.

In addition to the biotechnological advantage of elimination of species boundaries, valuable traits can be transferred into commercial crops within an economically viable timeframe. Such is possible because there is targeted transfer of single genes, or at most several genes. This

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[1] Professor, Department of Plant Science, Foran Hall, 59 Dudley Road, New Jersey Agricultural Experiment Station, Cook College, Rutgers, The State University of New Jersey, New Brunswick, NJ 08901-8520.
zilinskas@aesop.rutgers.edu

eliminates years of backcrossing to remove undesirable genes as is necessary with conventional breeding.

HOW ARE TURFGRASSES TRANSFORMED?

There are three major issues that must be addressed to produce stable transgenic plants. These include: 1) a DNA delivery method; 2) integration of DNA into germ-line cells such that the transgene will be inherited by the progeny; and 3) a means to regenerate fertile plants from explants, callus, or cells into which the foreign DNA was introduced.

The commonly used targets for heritable transformation of turfgrasses are protoplasts (cells stripped of their cell walls), embryogenic callus (dedifferientiated cells with the potential to produce somatic embryos), and suspension cell cultures (which are usually derived from embryogenic callus). Tissue culture is as much an art as science. Appropriate concentrations and chemical forms of the auxins and cytokinins which are added to the tissue culture medium are empirically determined to effect the proliferation of undifferentiated cells from meristematic cells of germinating seeds, stolon nodes, and immature embryos. Microscope-assisted selection of embryogenic callus improves the success rate in transformation and subsequent regeneration of healthy, fertile transgenic plants. It is also important to minimize the time that plant material is maintained in tissue culture in order to decrease the chances of introduction of somaclonal mutations.

The two major ways that DNA can be successfully delivered into turfgrasses include direct DNA transfer and transformation mediated by *Agrobacterium tumefaciens*. Transformation of turf species was first achieved by direct DNA uptake by protoplasts (Horn et al., 1988). Cell walls are removed with cell wall degrading enzymes to produce protoplasts which are subsequently made competent to take up DNA by osmotic treatment or by subjecting the protoplasts to electric shock (electroporation). Several turf species have been transformed by direct DNA delivery into protoplasts; however, as it is difficult to regenerate fertile plants from protoplasts, this method is not commonly used today.

Biolistic delivery of DNA, modified after the invention described by Sanford et al. (1987), is now the preferred method of DNA transfer into turfgrasses and other plant species. Also known as particle bombardment, plant tissues or callus is bombarded with DNA-coated gold particles that are accelerated with considerable force by use of a ‘gene gun’ or, more commonly today, by a stream of compressed He gas. This method has been used successfully for several turfgrass species to produce fertile transgenic plants.

More recently, our laboratory at Rutgers University has succeeded in using *A. tumefaciens* to mediate DNA transfer into turfgrasses. *Agrobacterium*-mediated transformation offers several advantages over particle gun bombardment or other means of direct gene transfer. These include stable transgene integration without rearrangement of either host or transgene DNA; preferential integration of the transgene into transcriptionally active regions of the chromosome; ability to transfer large segments of DNA; and integration of low numbers of gene copies into plant nuclear DNA, which is particularly important to minimize possible co-suppression of the transgene in later generations.

Until recently, *Agrobacterium*-mediated transformation was thought to be limited to dicotyledonous plants. However, Hiei and coworkers (1994) described efficient transformation of rice by *Agrobacterium* and subsequently there have been convincing reports for maize, banana, barley, wheat, and sorghum. Numerous factors are of critical importance in *Agrobacterium*-mediated transformation of monocots including the type of tissue that is infected, the vector and bacterial strains used, plant genotype, tissue culture conditions, and the actual infection process. To date, we have used *Agrobacterium* to transform creeping and velvet bentgrass and tall fescue, and we continue to work on developing methods to use this ‘natural genetic engineer' to deliver DNA into other turfgrass species.

WHAT GENES ARE BEING INTRODUCED INTO TURGRASSES?

The first genes of commercial interest to be introduced into turfgrasses were genes conferring resistance to herbicides. It is likely that these herbicide-resistant turfgrass cultivars will be marketed in the not too distant future. In the pipeline are genetically modified turfgrass cultivars that are drought tolerant, salt tolerant, disease resistant, and insect resistant. Turfgrasses are also being engineered that will require less frequent mowing. Whether these genetically modified turf cultivars will be accepted by the turf industry remains to be determined. Recently in the United States, public concern over the safety of genetically modified foods has gained momentum. Genetically engineered turfgrasses will likely be perceived as providing less risk, and presumably will be more readily adopted.

ARE THERE RISKS THAT SHOULD BE CONSIDERED?

Questions that have been raised both in the turf industry and in university research laboratories have led to some controversy. Can the transgenic turfgrass outcross into weed grass species to, in effect, spread the transgene? Can the engineered turfgrass itself become a weed? Will resistance to pesticides develop in turf pests and pathogens? Few answers can be provided at this time to satisfy these concerns, although some research has been undertaken that begins to broach these questions (e.g., Wipff and Fricker, 2000).

As with most new technologies, potential risks must be weighed relative to expected benefits. These include lower costs and improved efficiency in operations and the environmental benefit forthcoming from use of smaller amounts of chemicals as pesticides, herbicides and possibly also fertilizers.

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