Applications of Biotechnology to Shelterbelt Improvement

Gerald A. Tuskan

When European settlers arrived in North Dakota, they found a vast prairie with less than 2 percent of the territory covered in trees. Since 1880, roughly half of the original native forests have been lost due to over-cutting, inundation and disease. Despite these losses, North Dakota today has nearly the same acreage of trees as it had at the turn of the century, mainly due to the establishment of shelterbelts. North Dakota is the number one state in the nation in the use and establishment of shelterbelts, with over 55,000 miles of field and farmstead windbreaks. The public and private investment in shelterbelts represents a $1.1 billion resource.

The future value and use of shelterbelts will be enhanced through selection and breeding. Traditional genetic improvement of forest species, however, is a long-term investment. The reproductive cycles and the shear size of the organisms make it difficult to accelerate breeding cycles and selection. Alternatively, biotechnology, the use of plant tissue culture and molecular biology techniques, offers an opportunity in forest genetics to reduce the time required to obtain a genetically superior selection.

PLANT TISSUE CULTURE

Ponderosa pine *(Pinus ponderosa* var. *scopulorum)* occurs naturally in southwestern North Dakota and is one of the most frequently used conifers in shelterbelt plantings. A tissue culture protocol for the production of callus or shoots from cotyledons was developed in our laboratory for use in an *in vitro* screening system. The in vitro screening system involves inoculating ponderosa pine callus, regenerated shoots, excised embryos and seedlings with spores of *Peridermium harknessii*. Differential response of full-sub progeny from a tester mating scheme will be used to set the criteria for the selection of superior genotypes. Gerald Tuskan and John Lundquist, Department of Horticulture and Foresty, and Jim Walla, Department of Plant Pathology, are currently investigating the screening system. Future tissue culture projects with ponderosa pine will deal with improving shoot development and overcoming problems associated with using mature plant tissue.

Siberian elm *(Ulmus pumila)* was widely planted in shelterbelts in the 1950s throughout the northern Great Plains. Many of these plantings have reached biological maturity and are succumbing to herbicide damage, canker development *(Botryodiplodia hypodermia* and *Tubercularia ulmea)* and cankerworm defoliation *(Alsophila pometaria* and *Paleacrita vernate)*. A somatic embryogensis protocol is being developed for use in in vitro screening and genetic transformation. Ultimately, experiments will be conducted to determine if *Bacillus thuringiensis* toxin can be produced and expressed in Siberian elm as a means of conveying cankerworm resistance. This project is being conducted cooperatively by Tuskan, Phil McClean, Department of Crop and Weed Science and Rich Cunningham, USDA-ARS, Mandan, North Dakota.

Silver maple *(Acer saccharinum)* is planted widely across the state and is susceptible to iron chloresis due to alkaline soil conditions. A tissue culture protocol is being developed which will be used in an in vitro screening system, as well as for micropropagation of superior selections. To date, differential response among selected genotypes has not been detected at the morphological level. In the future, tests with silver maple will be conducted to determine the heritability of iron chlorosis resistance and to examine the in vitro response at the physio-chemical level.

ELECTROPHORESIS AND ISOZYME ANALYSIS

Western gall rust *(Peridermium harknessi)* occurs naturally in North Dakota, and throughout western and northern North America. Western gall rust infects 13 species of hard pines, including ponderosa pine. Variability in virulence of this pathogen is unknown, and as a result its use in an in vitro screening system would be limited. Isozyme analysis, using horizontal starch gel electrophoresis, was used to characterize the amount of variability present in *P. harknessi*. Based on 13 enzymes representing 15 putative loci, there were 25 biotypes present in 166 samples collected throughout North Dakota. For the in vitro screening mentioned above, two distinct biotypes will be used across all tested pine genotypes. Future work in characterizing variability in *P. harknessi* and ponderosa pine will use restriction fragment length polymorphisms (RFLP), a recombinant DNA technique, in an attempt to isolate virulence genes in the pathogen and resistance genes in the host. A gene-for-gene model will be used in a linkage analysis with RFLP data. This project is being conducted cooperatively by Tuskan and Chengguo Wang, Department of Horticulture and Forestry, and Jim Walla.

SUMMARY

Traditional breeding programs for the primary shelterbelt species in North Dakota have been initiated. The benefits from these programs, however, will not be realized for many years. The new discipline of biotechnology will supplement and enhance the traditional breeding programs by reducing selection cycles, increasing selection efficiency and increasing selection differential. Plant tissue culture, co-culturing techniques and recombinant DNA technologies are being applied to ponderosa pine, Siberian elm and silver maple in such a manner.

Tuskan is assistant professor, Department of Horticulture and Forestry.