Plant Biotechnology

Ed Deckard and **Gary Secor**

Exciting discoveries during the past 20 years in the biological sciences provide for important applications in agriculture. These discoveries have come to be called biotechnology. Biotechnology is defined by the USDA as the modern technology of using living organisms, cells, and subcellular organelles, as well as molecules, to understand and solve economically and socially important problems. Biotechnology provides new opportunities to study, understand, and modify the complex life processs.

To date, most of the publicity and applications of biotechnology have centered around genetic manipulation of the less complex life forms, the microorganisms. It is relatively easy to insert foreign genes into common bacteria. The pharmaceutical industry has been a leader in this area and the genes for many valuable medical compounds can be produced in large quantities at relatively low prices. Examples of such products are insulin, interferon, peptide biotherapeutics, blood clotting factors, and growth hormones.

Strains of bacteria also have been specifically selected or engineered to degrade pesticide spills in the soil or dissolve oil slicks, which was the first patent of a living organism. Other bacteria have been engineered to make fruit trees, strawberries, and potatoes more resistant to frost (the commercial product 'Frostban').

The gene for the active component of rennin, chymosin, which is used in cheese production, has been transferred to bacteria, and the gene product under the name Chy-Max is the first food ingredient produced through biotechnology to be approved by the Food and Drug Administration. Bacteria also have been modified to produce ethanol from cellulose, the world's most abundant organic compound. The food and industrial applications using microbes modified by biotechnology are virtually limitless, and undoubtedly many new products will be developed in this decade.

In addition to the biotechnology successes with microorganisms, many universities and commercial firms now are using biotechnology to increase our knowledge base and solve problems related to plants and animals. The main areas of animal biotechnology research include nuclear transfer, reproductive physiology, in vitro fertilization, disease diagnosis/treatment, growth hormones and gene transfer. Animal biotechnology was discussed in the previous issue of *North Dakota Farm Research*.

Greenhouse and field trials are necessary to evaluate the acceptability of plants modified by biotechnology. These potato plants have been regenerated from single cell culture, screened for disease resistance, and are undergoing evaluation in the greenhouse.

Deckard is professor, Department of Crop and Weed Sciences, and Secor is professor, Department of Plant Pathology.

Microbial, medical, and animal biotechnology applications will play an important role in shaping our future. However, as only a few species of plants stand between a healthy civilization and a starving, malnourished civilization, plant biotechnology is just as important as medical biotechnology for the future prosperity of our civilization.

Although biotechnology and genetic engineering frequently are used interchangeably, the term genetic engineering has a narrower meaning — the manipulation of genetic material by cellular or molecular techniques to modify organisms for specific uses and adaptations. The broader term, biotechnology, will be used throughout this article.

PLANT BIOTECHNOLOGY AND THE FUTURE OF AGRICULTURE

Understanding and improving plants for agricultural purposes began when plants growing in their natural habitat were selected for domestication. This early selection occurred long before the science of genetics was developed. The 19th century paved the way for a new era in plant improvement when the first basic laws of genetics were discovered by Gregor Mendel. The 20th century brought the development of statistical probabilities and additional genetic principles. For the first time, breeding methods could be developed that were based on these genetic principles and our understanding of statistics. That is, the **science** of plant breeding was added to the **art** of plant breeding.

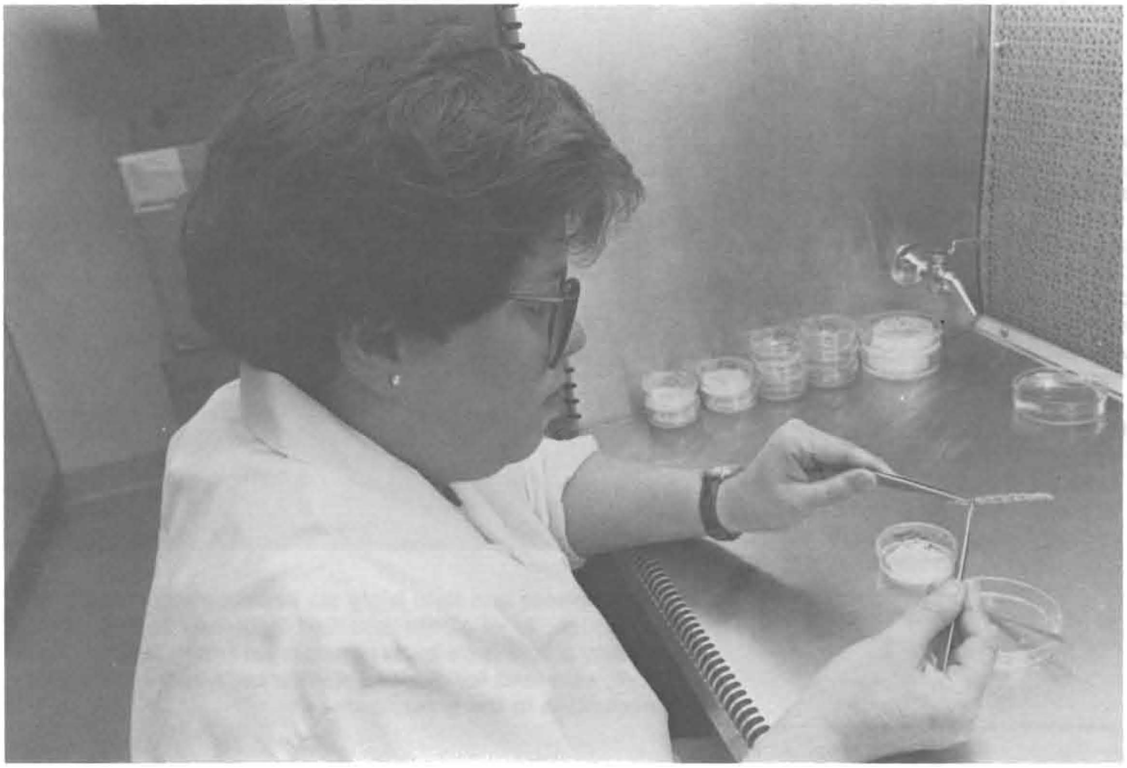
With these scientific breeding methods, breeders have made steady progress in plant improvement. The development of plant biotechnology during the latter years of the 20th century will usher in a new era of plant improvement. These new technologies, although not a replacement for the scientific breeding methods, will markedly improve the science of plant breeding and will become an important component of the plant breeder's toolbox.

The traditional breeding methods utilize many plant crosses, which mix the thousands of genes from each parent. Large numbers of subsequent progeny are grown for identifying the particular plant providing the desirable gene combinations. It becomes a procedure of numbers. Biotechnology procedures will reduce the need to mix the thousands of genes from the diverse parents.

In addition, for a gene to be available to a plant breeder, that gene must be in an organism that has the potential for hybridizing with the crop of interest. For instance, one wheat cultivar can be crossed with another wheat cultivar, but wheat cannot be crossed with barley or soybean. Therefore, barley or soybean genes are not available to the wheat breeder. Successful biotechnology procedures allow the genes from any organism to be available for crop improvement, as well as allowing for the modification of existing genes.

But, plant biotechnology is more to agriculture than a tool to improve the science of plant breeding. This application to plant breeding is just one of the many important examples where plant biotechnology can assist the agriculture industry in meeting the demands and needs of crop producers, processors, and consumers.

Biotechnology is being intensely investigated and utilized throughout the United States as well as overseas. Commercial companies involved in biotechnology are far too numerous to list, but include Monsanto, Dupont, Calgene, Genetech, Cetus, and Mycogen. Most pharmaceutical and oil companies have a significant biotechonology compo-



Regenerated plants from cultured haploid anther cells will increase selection efficiency and reduce the time required to develop varieties. Marilyn Schroeder of the crop and weed sciences department researchers the conditions necessary for inducing individual haploid wheat cells to develop into whole plants.

nent. Scientific societies such as the American Society of Agronomy, Crop Science Society of America, American Society of Plant Physiologists, American Association for the Advancement of Science, and the American Phytopathological Society are intensely involved in many ways. Many federal agencies including the Office of Technology Assessment, United States Department of Agriculture, Environmental Protection Agency, National Institute of Health, and the National Science Foundation are involved with regulatory or financial commitments. This broad business, governmental and international commitment to biotechnology gives it legitimacy and shows the enormous diverse potential of these new and exciting technologies.

BENEFITS, RISKS, AND PUBLIC CONCERNS

Biotechnology already has delivered many benefits to society such as new drugs, vaccines, diagnostic tools, and exciting research techniques to fight human diseases and genetic disorders. Similar biotechnology techniques with plants will benefit society in many ways, including providing a better understanding of plant growth and development, a more stable production of food and fiber, a reduction in crop production inputs, greater profits for farmers, greater international competitiveness, and a higher quality end product.

The benefits from this powerful technology will be enormous. However, powerful technologies also provide risks. Risks or perceived risks of biotechnology include diverting financial resources from other needs (biotechnology is expensive), increasing pesticide usage, reducing the labor force in agriculture, and manipulating the genetics of nature. In addition, growing concerns over food safety and environmental quality may become attached to biotechnology.

Scientists believe that their research will benefit society. However, some segments of society accuse scientists of disregarding animal rights, polluting the environment, tampering with the genetics of nature, and benefiting larger industries and corporations. No scientist fully understands all the diverse ramifications that a popular biotechnology application may have on society. However, the types of effects will be similar to those resulting from conventional agricultural technologies. These effects include costs of production, profits, food prices, food quality, food availability, and environmental quality. Predicting the socioeconomic impacts of specific plant biotechnology applications is not an easy task but is helped by the fact that we have comparable experiences.

Scientific research projects that we undertake need direction and endorsement from diverse groups, including the general public. Certain societal groups, such as the no-progress group, will make their accusations; but discussions involving diverse public groups, industrial representatives, and scientists from several disciplines will have a positive effect on the direction of biotechnology and the satisfaction level of society. These discussions need to begin before the research is initiated and must be maintained throughout the research and development process. However, if public groups are to be an important part of the discussions, they have to invest in the research, especially in that necessary portion of the research not carried out or financed by agricultural industry.

The segments of society that have concerns regarding biotechnology generally are most concerned about the genetic engineering aspects of biotechnology. Perhaps this is the most controversial aspect because it is new, complex, and viewed as altering nature. In general, the public is mostly opposed to genetic engineering of animals or microorganisms, frequently considering this to be morally wrong. Engineering of plants to provide tolerance to environmental extremes, diseases, or insects, a smaller fertilizer requirement, or a better nutrition generally has little opposition. In fact, these genetic engineered changes are frequently considered to be desirable. Therefore, of the various types of biotechnology, plant biotechnology provides the least public concern. However, plant biotechnology is not without controversy.

One such controversial area is the biotechnology effort to improve herbicide resistance in plants. Certain groups believe this research is backed by profit-oriented chemical companies and leads to increased herbicide use and environmental problems. Scientists conducting the research believe that there will be a reduced use of herbicides with inherent safety and environmental problems as they engineer tolerance to an environmentally friendly compound that can be used in relatively small quantities. In addition, scientists believe that research to improve herbicide resistance frequently produces spin-off benefits such as genetic variants that can be used as genetic markers or novel producers of specific plant products. This is one research area that is neither all good nor all bad and would benefit with inputs from diverse groups. This research area also would benefit from public investment, assuring that plant biotechnology for improved herbicide resistance is not limited to those projects with immediate high payoff to industry.

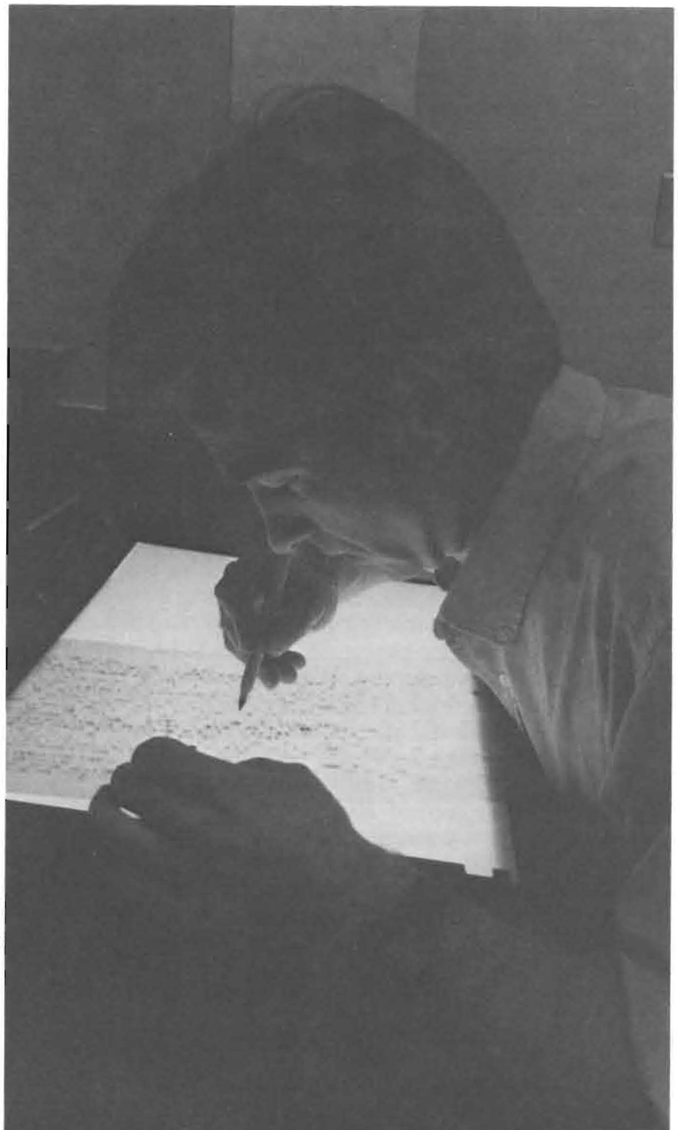
Another controversial area in plant biotechnology is field testing of genetically engineered plants, even though this activity is highly regulated by various levels of government. Field testing of engineered plants is not as controversial as the release of genetic engineered microorganisms, where microorganisms are often perceived to be associated with disease. But, public concern with moving genetically engineered plants out of the laboratory exists, and this concern is greatest if the public perception includes a potential risk to health or the environment. We agree with the International Food and Biotechnology Council position that "Biotechnology will not suddenly and dramatically alter our food supply and most biotechnologies will pose no different safety concerns than traditional methods." We also believe that biotechnology has moved ahead with unprecedented speed, too quickly for the many societal groups to analyze and understand. As a result, the present level of public concern is a natural outcome. Productive discussions among the various societal groups require concerned listening by all as well as the active sharing of biotechnology knowledge, the potential risks presented by the specific field test, and the historical safety record of altering genes by conventional means.

Biotechonology has and will continue to deliver many imporant benefits to society. The benefits promised from plant biotechnology are becoming available and these benefits will increase greatly during the next 10 to 15 years. The benefits will be felt by almost everyone, whereas many of the risks will be taken by a few, such as a specific agricultural industry. Scientists have expressed concern that this diffusion of benefits across society may leave plant biotechnology without a strong advocate, whereas the few perceiving a personal risk will be quick to strongly advocate their position against that plant biotechnology application. We believe that it is important for the general public to take an active role in plant biotechnology research to ensure that the desired research projects are done and the technologies of choice are applied appropriately to solve the increasingly complex agricultural problems.

PLANT BIOTECHNOLOGY AT THE NATIONAL LEVEL

Plant biotechnology in the United States is affiliated with universities, relatively new biotechnology companies, seed or chemical industries, or partnerships among these types of participants. The companies or industries have both U.S. and foreign ownership.

The cell culture portion of biotechnology has advanced to the point that we can culture plant tissues as small as a single cell and regenerate plants from the cultured cells. These regenerated plants may exhibit variability, and selected plants from this procedure have been used to improve sugar cane, tomato and potato. Cell cultures of certain plants, ie. celery and saffron, can be used to produce flavors and fragrances in batch culture. Cell cultures can be screened in culture to select cells resistant to a toxin, and resistant whole plants may then be regenerated.



Painstaking biochemical and molecular sleuthing is necessary for identifying and transferring plant genes. Alexander Borovkov, a visiting scientist from the Soviet Union, is shown assembling the molecular puzzle pieces that will reveal the sequence of a gene to be transferred into potato.

Individual cells from sexually incompatible plants (cannot be crossed by traditional methods) can be fused, and a plant may then be regenerated from the fused product. Wild potato has been fused in this manner with the domesticated potato to incorporate disease resistance to leafroll virus and **Erwinia** soft rot. Regenerating plants from individual haploid cells, ie. male or female gametes, allows the breeder or geneticist to achieve instant homozygosity (requires years with conventional means) and increase selection efficiencies. This procedure has been used to develop improved wheat varieties in France and China and is being used throughout the United States.

The gene transfer portion of biotechnology utilizes molecular biology techniques for gene identification, construction, cloning and transfer to plants. Genes, composed of DNA, can be transferred to plant tissues by direct uptake, electroporation, injection, **Agrobacterium,** or ballistic particles. Some genes can be removed and reinserted backwards; this is called anti-sense technology and can serve to interfere with certain genes, such as the gene for endopolygalacturonase which causes softening during fruit ripening. However, most of the effort involves transfering novel genes from one organism to a plant of interest. Plants containing foreign genes are called transgenic plants. Three examples of transgenic plants can serve to illustrate the kinds of applications possible in plant biotechnology.

Herbicide resistance. A gene was discovered in petunia that produces an enzyme that degrades the herbicide glyphosate (Roundup), thereby rendering the plant resistant to the herbicide. Using biotechnology, this gene was cloned and transferred to tobacco and tomato. These transgenic tobacco and tomato plants, and their progeny, are resistant to glyphosate.

Insect resistance. The bacterium *Bacillus thuringiensis* produces a protein toxic only to certain insects, including the corn borer. The gene that codes for this protein has been identified and transferred to bacteria that normally inhabit the surfaces of corn. When corn borer larvae eat even a small amount of corn tissue, they ingest the toxin and die before much damage can occur. The corn plant is thereby protected from damage by the corn borer.

Disease resistance. The plant virus called potato virus X (PVX) commonly infects potatoes and can cause yield losses. Resistance does not occur in commercial cultivars. The gene from the virus that produces the coat protein surrounding the virus has been cloned into potato plants. These transgenic plants are immune to infection by PVX.

Many other examples exist and the future possibilities are limitless. To date, the Animal and Plant Health Inspection Service of the USDA has issued 62 permits to field test transgenic plants. These permits involved field testing in 23 different states and generally involved genes intended to provide resistance to herbicides, insects, or diseases. The 62 permits involved 12 species of plants, although most were tomato or tobacco. Several permits were to field test important agronomic crops such as corn, potato, soybean, alfalfa, and cotton. Many additional permits are pending at this time.

PLANT BIOTECHNOLOGY AT NDSU

In the late 1970s, "biotechnology" was becoming an often heard word. However, the actual impacts that it would have on research activities and the economic/social aspects of North Dakota were unknown. Aware of the potential impacts, NDSU and the Agricultural Experiment Station quickly began to focus their efforts toward incorporating biotechnology into their educational and research program.

Biotechnology research activities were increased by redirecting a portion of the current research effort and equipment funds toward biotechnology research and the hiring of new scientists with additional expertise in areas of biotechnology.

NDSU prepared a request to the state of North Dakota for funding to begin the building process of a first-class interdisciplinary biotechnology program. The state responded with $500,000 for operating, technical support, and necessary equipment. This vote of confidence by the state was a major stepping stone toward the present biotechnology effort on the NDSU campus.

Based on input from agricultural industry representatives, an undergraduate biotechnology program was developed by the biotechnology faculty at NDSU and approved by the Board of Higher Education in 1985. This has become a popular major for students in the College of Agriculture and the College of Science and Mathematics. Following the development of this undergraduate program, a Ph.D. program in cellular and molecular biology was developed and approved in 1988.

After several months of planning, an Agricultural Biotechnology Center (ABC) was formed in 1987. The goals of the ABC were to promote plant, animal and microbial biotechnology and to facilitate the use of biotechnology research in solving agricultural problems. This concept was expanded in 1989 and is now called the NDSU Biotechnology Institute.

Four research centers (Cell Biology, Biopolymers, Electron Microscopy, and Monoclonal Antibody) were developed in 1989. Additional equipment necessary for the operation of these research centers was provided predominantly by the Agricultural Experiment Station and a generous donation by the Dalrymple family of Casselton.

About four years ago, the Agricultural Experiment Station enlisted the support of U.S. Senator Quentin Burdick for federal funding of a new crop and weed sciences building. With his enthusiastic support, the funding was obtained. This building, scheduled for completion in spring of 1991, will provide a state-of-the-art facility for interdisciplinary plant research, and will complement plant research conducted in the Northern Crops Science Laboratories (NCSL) located at NDSU. One wing of the CWS building is dedicated to the Plant Biotechnology Program, which will include four research laboratories, a cell culture facility and the Cell Biology Center.

The Plant Biotechnology Program will bring together much of the basic plant biotechnology research activities, and the new knowledge and materials obtained in this group of laboratories will be utilized in the plant biotechnology application laboratories also located in this new building. In addition to providing an excellent research facility, these biotechnology laboratories will provide both undergraduate and graduate students excellent facilities and opportunities to learn the newest biotechnology techniques and applications.

Throughout the 1980s, the Agricultural Experiment Station and the College of Agriculture have provided financial support for faculty participation in biotechnology symposiums, conferences and workshops. This participation helps maintain the biotechnology research and teaching activities at the cutting edge.

The present research activities in plant biotechnology carried out by NDSU scientists and federal scientists at NCSL are diverse and include the following:

- Cell selection schemes for beneficial genetic changes in several crops, including wheat, potato, sugarbeet and sunflower.
- Identification of germplasm exhibiting tolerance to diseases or environmental stresses, e.g. sunflower, bean, sugarbeet and wheat.
- Anther culture and haploid plant production in wheat and barley.
- Molecular biology of photosynthesis in wheat.
- Molecular biology of barley — barley stripe mosaic virus interaction.
- RFLP analyses of nuclear genome of barley.
- Disease detection using complementary DNA hybridization, e.g. ring rot of potato.
- Improving quality of agricultural products, e.g. bruise resistance and sugar regulation in potato.
- Identification and selection of genes for environmental stress tolerance, e.g. drought and high temperature tolerance in wheat.
- Identification and transfer of genes for resistance to sugarbeet root maggot into commercial cultivars.
- In addition to these specific activities listed above, general research activities in progress include construction and cloning of desirable genes, control of gene regulation, development of gene transfer systems, and development of new agricultural products such as dyes or fuels.

For certain crops, notably potato, research teams at NDSU possess the capability to culture and select desirable cells; regenerate plants from individual cells; identify, synthesize, and construct desirable genes; and produce transgenic plants containing these desirable genes as part of their normal genome. That is, many key biotechnology techniques are available for application. Field evaluations of transgenic plants may begin as soon as 1991.

Current research activities at NDSU with many crops involve research teams dedicated toward developing the key biotechnology techniques and applying these techniques to study, understand, and modify the complex plant processes and the plant's interaction with the North Dakota growing environment.

MORE ABOUT PLANT BIOTECHNOLOGY

For additional information about plant biotechnology at NDSU and productive ways for you to participate, please contact the authors of this article or H. Roald Lund, Director of the Agricultural Experiment Station and the NDSU Biotechnology Institute, Morrill Hall, NDSU, Fargo, ND 58105.