A FEW 'SMALL' CHANGES MAY PRODUCE 'BIG' RESULTS: THE NDSU BIOTECHNOLOGY RESEARCH PROGRAM

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Man's a toolmaker. While evidence of this predilection to "improve the odds" ranges from stone hand axes to artificial satellites, the newest proof can be found in living cells.

In the last decade, researchers in the life sciences have developed a new set of chemical and biological tools and methods.

These tools and methods, collectively known as "biotechnology," may profoundly affect both laboratory and commerce.

According to North Dakota State University professors Gary Secor and Arland Oleson, biotechnology represents a new and unique practical approach to generating improved lines of useful plants, animals and microbes for society.

Secor, an associate professor of plant pathology, and Oleson, a professor of biochemistry, point out the biotechnology approach to plant improvement differs substantially from that of standard plant breeding methods. Unlike standard plant breeding, where improved varieties are the result of the sexual interactions of intact plants in field plots or greenhouses, plant biotechnology involves the genetic manipulation of populations of individual plant cells in a laboratory.

There are two approaches to genetically engineering plant cells: cellular and molecular. Because of the interrelationships between the two approaches, research programs using each are under way at the university.

In the cellular approach, altered cell forms are generated by fusing two different plant cell types or by the responses of a single type of plant cell to culture conditions.

Using newly developed techniques, these individual cells may be regenerated into intact plants with altered genetic compositions.

The NDSU cellular program involves faculty from the plant pathology, agronomy and botany departments. It's primarily directed at improving methods of establishing individual cell cultures from North Dakota crop plants and developing procedures to regenerate these cells back into intact plants.

Dry edible beans, potatoes and wheat are being tested as part of the cellular program.

Secor and Jim Venette, another associate professor of plant pathology, are working to improve the quality of dry edible beans. If their project succeeds, they say they should be able to engineer a bean plant, screen it for disease resistance, select a resistant one and regenerate it back into a plant. Initial steps–engineering the plants and screening them for disease resisitance–have gone well, according to Secor.

Initially, dry beans were put into callus cultures and maintained. (A callus is an undifferentiated mass of cells.) The maintained calli have been used to determine if the various bean selections tested are disease-resistant.

Using calli, Secor and Venette have screened 12 bean cultivars for resistance to halo blight *(Pseudomonas phaseolicola)*. The calli reactions have then been compared to those of plants innoculated with the blight bacteria.

Secor says comparisons have shown an excellent correlation between the way the callus reacts to the way a plant reacts in the field. Once the system's fully developed, which means trying it with other bacterial pathogens, both present and future bean varieties can be accurately screened for disease resistance.

"What we eventually want," Secor says, "is to be able to characterize currently grown varieties and take a look at upcoming germplasm or possible varieties, so we can tell the farmer which ones have resistance to what diseases. And, we want to be able to do it very quickly and accurately each time."

While the initial steps in the process have progressed smoothly, including the ability to test possible bean varieties accurately, the final step–regenerating the calli back into complete plants–has met with what Secor calls "semi-success."

What he calls an extremely difficult process that's never been done with dry beans, regeneration has been accomplished once this past spring. He says efforts to successfully replicate the first regeneration are still continuing.

"We've been able to do it once and we're going to continue this thrust. We need some more work done just to make this regeneration more consistent and reliable."

Secor calls the complete sequence of engineering, screening, selection and regeneration the "bottom line" of all the work done in the bean projects.

"We can take a series of calli from beans and screen them for disease resistance right now. If we find one that's resistant, and if we can regenerate it, we'll have our resistant plant on the spot-providing resistance mechanisms are expressed in whole plants."

"But," he adds, " if we can't regenerate, we can't do anything with the callus."

While Secor and Venette are studying variation in single bean cells, Glen Weiser is researching the next step: whole bean plants.

Weiser, an assistant professor in agronomy, is trying to gain a better understanding of the mechanisms involved in the variability that's found in tissue cultures.

As part of his project, he's studying chromosomes in callus cultures.

"Chromosomes aren't usually very stable," he explains. "Traditionally, when you're growing plant cells in culture, they gain some chromosomes, lose some or develop ones with abnormalities. This is an area I'm studying."

Weiser sees his work as the middle ground between Secor and Venette's research on single cells and plant breeder Ken Grafton's applied breeding of dry edible beans to produce a new variety.

"If Gary (Secor) can regenerate beans at a reliable rate, my work with whole plant genetics will come into play," he says. "I'll be able to take varients derived from culture and study them at the whole plant level."

Secor agrees with Weiser's appraisal of a team approach to discovering-and implementing-something useful in the course of the bean project.

"I'll be taking some cells, make them into callus and screen them," he explains. "If I find something that's got good resistance-and if I can regenerate it-I'll give the regenerated plant to Glen."

Weiser, he continues, will look at the regenerated plant, study its chromosomes and genetics, and find out what makes it different.

"If he finds out," Secor says, "he'll pass it on to Ken Grafton, who'll apply it in the field."

Grafton will develop the new genes for incorporation into a new variety by cross-breeding. The traditional sexual crossing program will save the germplasm and keep it in a bean variety.

"A lot of times we envision what we're doing as introducing some new variability we'll use in a crossing program," adds Weiser. "That's going to happen a lot more often than getting out a new variety."

"One of the ultimate aims of the program is to take a plant that's really perfect in every way, except for one small flaw," agrees Secor. "If we can simply adjust or eliminate that little flaw by tissue culture, gene splicing or recombinant DNA, that would be really great."

While the bean program is currently attempting to replicate the regeneration stage, Secor points out this ability to make a "new" plant has been accomplished in the course of the university's work with potatoes.

When working with potatoes, Secor and his colleagues have used protoplasts (individual cells) rather than calli. The protoplasts are collected from potatoes grown under certain conditions in growth chambers. Three to four million individual cells are collected each time. The protoplasts are plated out onto artificial culture media and regenerated. Each cell can be regenerated back into a complete plant.

In the initial testing with Russet Burbanks, Secor says the regenerated clones (both plants and tubers) showed much variation induced by the cellular engineering process.

The variations include plant height, flower color, maturity, tuber shape and degree of russeting.

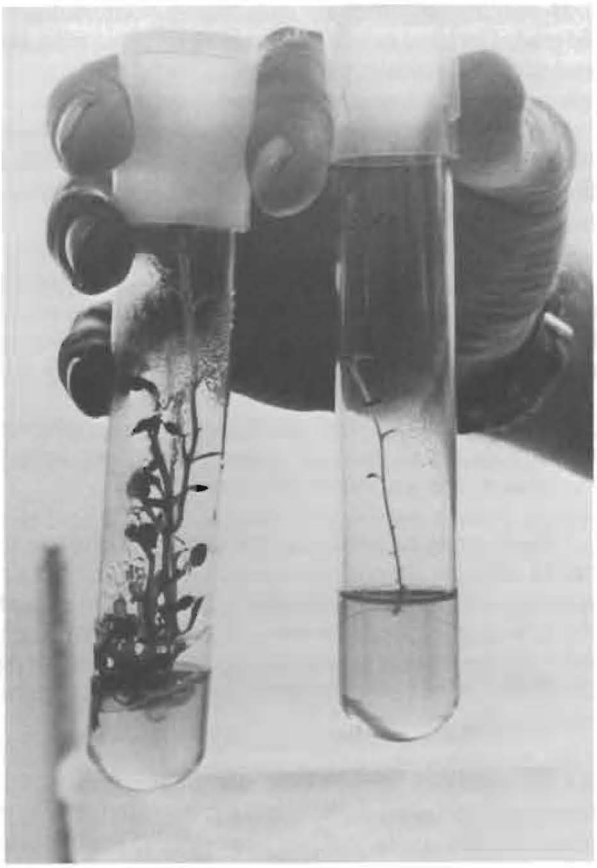
Tubers of some of the more promising regenerated plants have been propagated in the university greenhouse and subjected to field trials. Some, according to Secor, may be superior to the original Russet Burbank "parent."

Of the 5,000 original clones in the field, Secor says five were saved. "One, Number 5788, matures earlier, is smoother and blockier and looks like it'll produce higher yields and gravity than the parent Russet Burbank."

The fact the clone grows smooth in the heavy soils of the Red River Valley may assure it popularity with area growers, he adds.

The clone was planted in six field locations this year, he continues, and the research team is waiting to collect the planting data.

"This clone looks like it has promise as a future varient or selection of Russet Burbank that would grow better in this area."



As testing of the regenerated Russet Burbank continues, he says, Crystal and Norgold Russet varieties are also being regenerated.

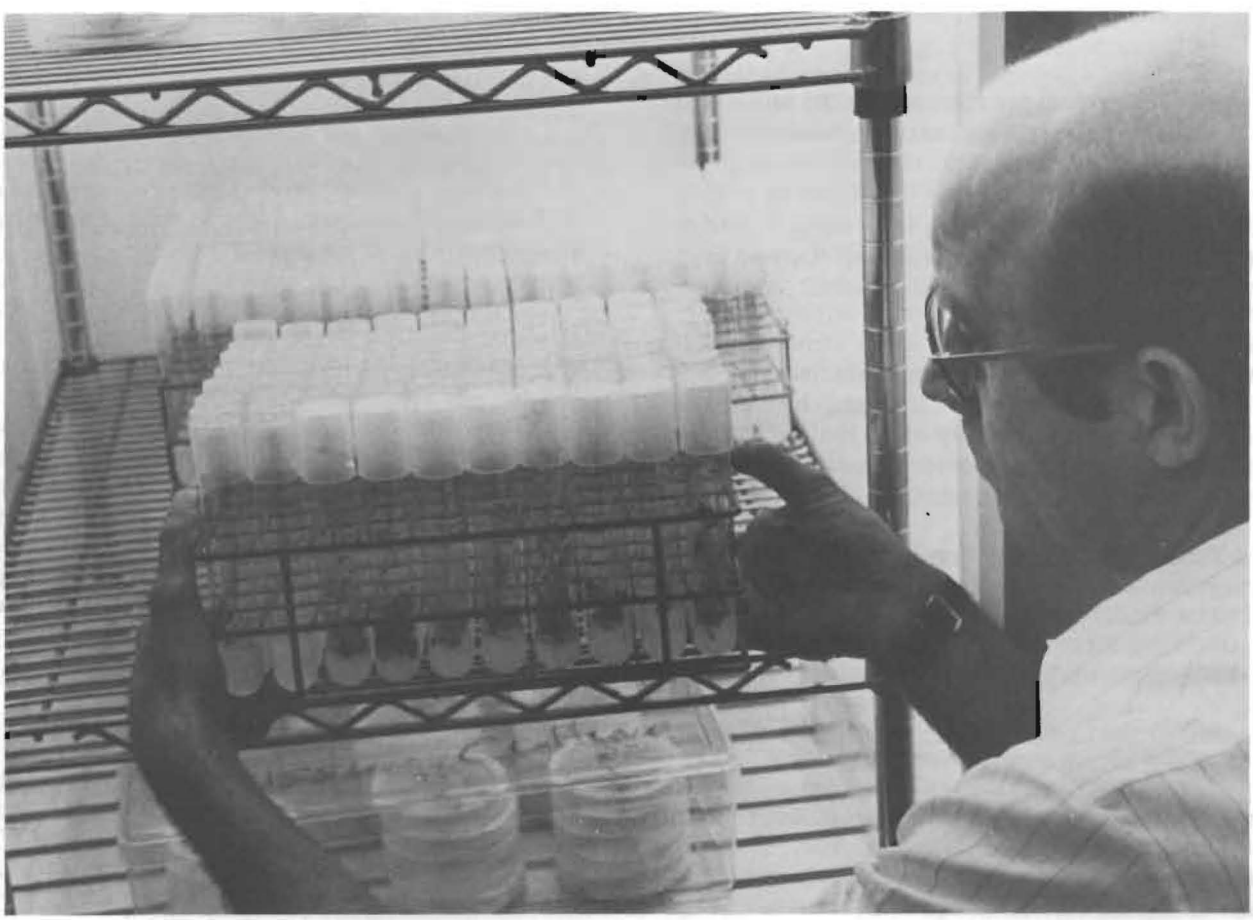
One aspect of the new cycle of regeneration will be the search for disease-resistant specimens of the two varieties. As part of the study, Secor says, disease toxins and pathogens will be added to the protoplast cultures.

"On a plate of millions of protoplasts, almost all will die when we add the toxin or pathogen," he says. "However, those that survive may be resistant. We can regenerate those immediately and have a resistant variety."

Crystal is being studied because of its susceptibility to Blackleg and soft rot, Secor says.

Currently, 100- to 200-cell calli of Crystal are being innoculated with bacteria. While most die immediately or within seven days, a few remain green for periods up to 16 or 20 days.

"This looks very promising," he says. "We're following this up to see if we can induce resistance in Crystal, since there's no known resistance to *Erwina* in it or most other varieties."



Dr. Gary Secor, plant pathologist, with young potato plants (close-up) regenerated from protoplasts. Callus, below in growth chamber petri dishes, starts the process.

Secor believes the research done on Russet Burbanks has indicated the direction work on the other two varieties will take.

"What we're looking for is new genes by using this massive screening system of individual cells," he says. "Based on the fact each cell can be different-at least because of the cloning experiment-we think we can, by some unknown mechanism, induce some disease resistance not now found."

What Secor and Venette are doing with beans and potatoes, Agronomy Professor Edward Deckard and Botany Professor Murray Duysen are attempting with small grains.

Working with wheat, the pair are culturing microspores in nutrient media to form calli or, in some cases, embryoids. Duysen describes the research as an attempt to get the microspores to divide and produce a callus-type growth that will regenerate back into an intact plant.

"We're working with microspores because they're haploid; that is, they have only one-half the normal chromosome level," he explains.

"If we can make this procedure a routine, successful operation, plant breeders can use our information to get homozygous plant lines-lines with the same type of parent chromosomes-in their breeding programs with little trouble."

While the researchers are regenerating the calli or embryoids back into plants, they're trying to increase their level of success.

"Right now, we're getting calli from about 5 percent of the pollen we're putting on the plates," Duysen says. "However, if we go into the fields, success rates have been up to 20 or 25 percent."

"We're moving the haploid cell right into the embryoid stage and on to the plantlet stage," he explains. "But, we want to get our ability to do that up from its present level. At a 60- or 70-percent success rate, we'll have something the plant breeders could work with."

Like Venette, Secor and Weiser, Duysen and Deckard are using tissure cultures as a means of improving plant stock. The basic steps-engineering undifferentiated cells, screening them, selecting the desired ones and regenerating them back into intact plants-are the same.

The aims of the teams-developing methods of rapid, accurate plant improvement-also match.

While Deckard and Duysen admit they haven't found any useful wheat varieties thus far, they maintain specimens resistant to disease and stress will turn up in the course of their research.

If just the number of cells being examined is considered, Deckard explains, the odds of producing useful varieties are actually quite good.

"The callus is probably made up of something like 10,000,000 cells," he says. "The longer we maintain these cells in liquid suspension, the more chromosome mix occurs."

"The potential is just tremendous. I'm sure we'll throw away 99 percent of the possible things we find, but of that remaining 1 percent, we're still going to be able to get some pretty good parents for gene pools."

Both researchers feel the successful completion of their project will prove a means of getting improved varieties to the grower in less time.

"Right now, to release a new variety, a plant breeder has to take the gene of one particular plant and put it in another, retaining the quality of the parent accepting the new gene," say Deckard. "It takes about five or six years to back-cross or inbreed to achieve a homozygous condition where the desired gene is retained in the backcrossed material."

Genetically engineering a desired change in an undifferentiated cell and regenerating it back into a complete plant, he continues, can cut that time span drastically.

"If you go our way, it should take two to three years to do this. The time needed to release new varieties could be chopped by one-third."

Deckard points out that their research project isn't just aiming to improve plant stock by increasing variability at the cell level. Like researchers working with beans and potatoes, they also want to know how to stop the process at the right moment.

"When you're at the point of using recombinant DNA or changing DNA, you don't want any random changes during the regeneration process," he explains.

"So, what we're looking at is the nature of the variation-what's causing it and how to control it-so we can either increase it from a breeding standpoint or decrease it for any of these other reasons."

Frequently, Deckard explains, breeders attempt to improve something like yield. However, once they've achieved thier objective, they're not sure how they did it.

"I guess you could say we're trying to make a road map, so people will know where they're going," he says. "And, once they've arrived at their destination, they'll know why they got what they did."

The researchers see their work providing plant breeders with another tool-not supplanting them.

"I don't think this research is a way of replacing the conventional plant breeder," Deckard says. "I think it's merely another beneficial tool."

"The breeder's always going to be the most valuable part of these improvement programs. After all, while we may be able to select out something like a stress-hardy or heat-resistant variety, they'll still be out there to make sure the quality exists in the completed project."

In the molecular approach to the genetic engineering of a plant cell, a desired gene is isolated and chemically coupled to a carrier to produce a molecular form known as recombinant DNA.

This material is introduced into bacteria to produce many copies (clones) of the desired gene. The cloned gene is then administered to plant cells to produce an altered cell form that can be regenerated into a genetically modified plant.

This type of genetic engineering results in the specific introduction of a desired gene into a particular plant.

And, according to professors Oleson and Secor, since the cloned gene can originate from a different life form–anything from another plant species to a microbe or animal–the approach is much less restrictive than standard breeding methods.

The molecular research program at NDSU involves faculty from the biochemistry, bacteriology and agronomy departments. In tests with wheat, corn and barley, the project focuses on analyzing the structure and function of three types of plant genes.

In one project, say Secor and Oleson, an enhanced ability of plants to tolerate such stress factors as limited nutrient supplies, adverse climatic conditions and plant pathogens is being studied.

Another gene under investigation is important in protein formation, a process required for growth in all living systems. At this time, the gene has been cloned in bacteria and its structure is being analyzed.

A third system currently being examined is a group of genes involved in generating the energy needed for plant growth and development. This group of genes has been isolated and genetic engineering tools are being used to study it.

David Berryhill, an associate professor of bacteriology, is using recombinant DNA techniques (gene cloning) to locate nitrogen-fixation genes in bacteria.

"Crop production is largely dependent on the availablility of nitrogen," he says, "and the industrial synthesis of nitrogen fertilizer currently consumes nearly one-third of the fossil energy used for agriculture."

As natural gas supplies shrink, he continues, fertilizer's going to get increasingly expensive. Moreover,

Dr. Arland Oleson, professor of biochemistry, demonstrates the separation and identification of gene fragments as technician Donald Stallman observes.

he doubts industry will keep up with the doubled demand for fertilizer predicted for early in the next century. However, industrial synthesis isn't the sole source of nitrogen. Some bacteria convert it our of the air into a useable form.

Rhizobium bacteria, he points out, convert atmospheric nitrogen into a form useable by plants (fixed nitrogen) while living in root nodules. This symbiotic nitrogen fixation is primarily limited to such leguminous crops as peas, soybeans, clover, beans and alfalfa. In the United States, rhizobia provide over 40 million tons of fixed nitrogen to legumes annually, he says.

Berryhill's goal is to genetically engineer superior nitrogen-fixation bacterial strains. If he succeeds, he feels the benefits to world agriculture will be enormous and myriad.

Currently, he considers his research very basic: learning to understand the process sufficiently to make informed efficient changes to improve it. His work centers on *Rhizobium phaseoli*, the nitrogen-fixing bacterium that associates with dry edible beans.

"Symbiotic nitrogen fixation is a highly complex process that's poorly understood," he explains. "Before rhizobia can be genetically manipulated, more basic information on *Rhizobium* genetics is needed."

"Presently, we're using gene cloning to locate nitrogen-fixation genes in *R. phaseloi*."

Genes can be cloned by cutting DNA at specific points with enzymes. The fragments are attached to vector DNA molecules in a test tube to form recombinant DNA molecules, which are inserted into bacteria to multiply.

If Berryhill can engineer bacterial strains with greater nitrogen-fixing ability, he sees the products having a major impact on the world's agricultural economy.

While bacteria currently fix atmospheric nitrogen, he explains, their contribution to agricultural production is far less than its potential.

"It's currently estimated that when the traditional *Rhizobium-legume symbiosis* is working, it's on the average about 15 percent efficient from what its potential might be."

If the existing process is merely improved 1 percent, he points out, it could be worth millions of dollars in reduced production costs or increased yields.

In addition, a long-term benefit of improving the process is the possible expansion of the crops that can benefit from symbiotic nitrogen fixation.

"Currently, plants like wheat and corn can't benefit because they're not legumes," he says. "But the potential may exist for doing manipulations genetically to make it a possibility."

"Ultimately, people are looking at taking the genetic information contained in the bacteria and incorporating it into the genetic information of such plants as corn and wheat."

Once that stage is achieved, he says, the original bacteria will no longer be a concern.

"Basically, when the genetically altered seeds are planted and germinate, they'll have their own capacity to convert atmospheric nitrogen into a form they can use."

If the ability to convert nitrogen in the air can be successfully grafted to the makeup of a wider range of plantlife, Berryhill adds, it could mean saving billions of dollars in lowered production costs.

While plant biotechnology seems well-established at NDSU, with a number of research projects under way, William Slanger, an associate professor of animal science, is working on a plan to initiate a similar program with livestock.

Just as plant scientists are using biotechnology to improve existing varieties, he says, animal scientists are looking at ways of using it to improve breeds.

Someday it may be possible to get any number of animals genetically identical to a dairy cow that produces 45,000 pounds of milk a year, he says. Or obtain thousands of offspring from such a cow, rather than the normal five or seven.

Some of the developing biotechnology research with livestock includes gene transfer, embryo splitting and cloning by nuclear transplantation.

According to Slanger, embryo splitting involves mechanically separating the cells of embryos with eight or fewer cells.

While four genetically identical animals are the most that can be expected at the present time, he says, researchers are attempting to get eight animals by splitting one embryo.

While embryo splitting's success rate is still much less than 100 percent, researchers have produced genetically identical animals by cloning seven-day-old embryos through nuclear transplantation.

In this procedure, Slanger says, a nucleus from one cell of the embryo is place into an egg of the same species. The genetic material is removed from the egg before the nucleus is inserted.

The result is a new embryo, genetically identical to the original, he says, adding that the potential number of identical animals is much higher than with embryo splitting.

While some researchers are attempting to increase the number of offspring from one embryo, he ways, others

are attempting to increase the reproductive potential of female livestock.

One approach is egg development in the laboratory.

"A cow's ovary contains thousands of potential eggs," Slanger explains. "But, only a tiny fraction are fertilized."

The idea is to remove one ovary, mature and then freeze the many eggs. They could then be thawed and fertilized to produce progeny when and where desired.

Slanger would like to see a series of research projects dealing with some of these techniques under way at NDSU.

"I'm interested in biotechnology as it applies to animal science," he explains. "Specifically, as it involves embryo transfer, semen sexing and cloning by nuclear transplantation and embryo splitting."

"This area is exciting to animal breeders because there are about 50 million beef cattle born a year," he explains. "Some of them have to be what we call genetic outliers, ones that really contribute to increasing the efficiency of beef cattle."

"From my point of view, the idea of our NDSU research is to identify these individuals and use reproduction enhancing techniques to get those very rare individuals' genes into the general population."

As Slanger conceives it, animal biotechnology at NDSU would look at both growth and reproductive traits.

"Common sense, as well as research, indicates that reproductive efficiency is by far the trait with the highest economic value in beef cattle," he points out. "I'm looking for individuals that could increase the number of calves marketed per cow bred."

According to Slanger, animal science researchers at NDSU will look for animals possessing a high potential for returning profit (from a performance point of view) to North Dakota ranchers.

Once these animals are found and identified, biotech procedures like cloning, embryo transfer, invitro fertilization and sexing will be used to feed the superior genes into the mainstream of the population.

Increasing the number of animals with superior growth and reproductive potentials is, to Slanger, a major goal of the proposed NDSU livestock biotechnology projects. If the results of the projects are positive, he sees more superior livestock proliferating across the state; a profitable situation for ranchers and breeders.

"We would like the average profitablility potential of the animals in the state to be that of the top 500 animals in the state now. We are taking about increasing the profitability by one to two times. Biotechnology has opened up the door to this kind of thinking."

While on-going (and proposed) biotech projects at NDSU vary, all share some common goals.

Both plant and animal science researchers are looking for way to improve efficiency. Both seek to increase the profit margins of the state's agricultural community.

However, all university researchers also share a common concern: public misunderstanding of biotechnology.

Oleson feels many of the misconceptions surrounding the techniques are the result of a fear of the unknown.

"During the industrial revolution in England," he explains, "a group of people called Luddites used to go around to the factories that introduced mechanization and break all the machines they thought were taking away jobs from people."

To Oleson, many of the people who fear the consequences of biotechnology share the same fears as the Luddites.

"This is another negative view that says, 'Let's not move forward with science and apply it to human needs.'"

"The thing is that once you've got the knowledge, you can't ever put it back in the bottle or blank out everybody's mind. When the ability to do this kind of science has come forth, people are going to make use of it."

"Biotechnology is mostly a tool," Oleson points out. "It's not really a unique area of science to itself, but a tool to probe molecular and cellular biology to a greater degree in ways we weren't able to do before."

Oleson points out that there are obvious commercial applications to using the new techniques, adding that some of the present experiments could be done in older, classical ways, but at a much slower rate.

Duysen tends to speak for all the NDSU researchers when he places biotechnology in the general scheme of plant and animal research.

"Biotechnology is a very important tool," he agrees with Oleson. "It's not an area that's going to replace any of the present work we're doing, but like a microscope, it's a tool to be used.

"I think it's a tool that will be used in agronomy and plant pathology, and in range and animal sciences," he adds. "It's a tool for all disciplines and for the benefit of people in agriculture."