# *Manipulating* N Uptake

## *Corn Of The Future Will Yield With Less Nitrogen Inputs*

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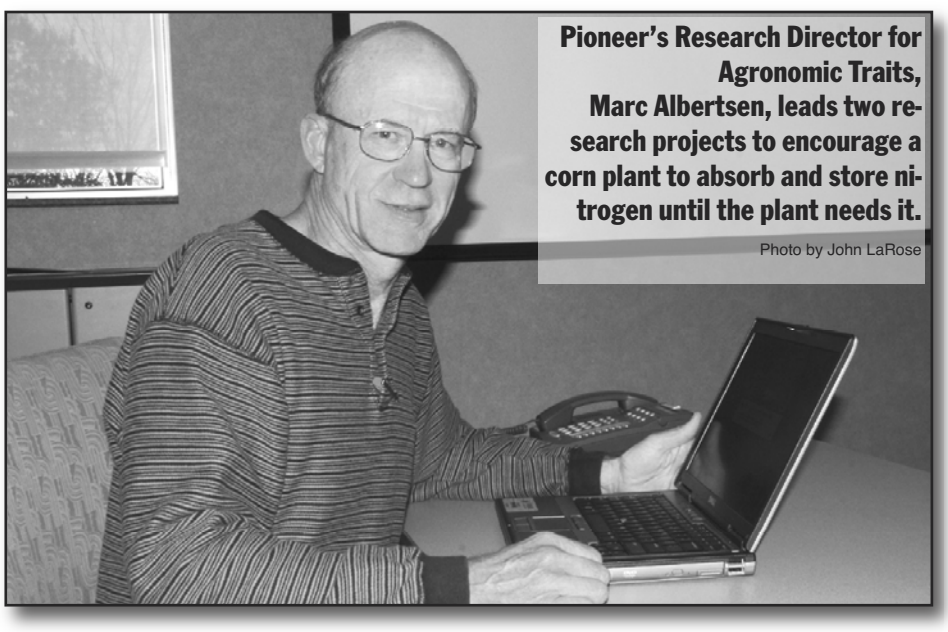
Work to encourage a corn plant to absorb more nitrogen when it is available and to store it within the plant until the plant needs it are two of the projects underway by a group of researchers led by Marc Albertsen, Pioneer's Research Director for Agronomic Traits.

"We want each plant to be as effective as possible in taking nitrogen out of the ground as it grows," he said.

Albertsen showed pictures of plants that were able to extract every bit of nitrogen that they could in order to produce higher yields. A transgene had been inserted into these plants to enhance their nitrogen uptake capability.



**Pioneer's Research Director for Agronomic Traits, Marc Albertsen, leads two research projects to encourage a corn plant to absorb and store nitrogen until the plant needs it.**

Photo by John LaRose

In trials the past year and in work that has been underway for about five years, Pioneer researchers have been able to identify genes that influence a plant's ability to absorb and to store nitrogen. These genes have been inserted into hybrids as part of the Nitrogen Use Efficiency (NUE) Discovery pipeline. The past year, trials were conducted to compare these transgenic hybrids with their non-transgenic hybrid control lines. These trials were conducted in fields that had been deliberately depleted of nitrogen. Only 40 pounds of nitrogen per acre were applied to these fields. Hybrids transgenic for the NUE genes significantly out-yielded their non-transgenic counterparts. Products resulting from these studies are expected to be released in seven or eight years from now.

"The long development time is a consequence of the difficult nature of this work, along with the regulatory processes mandated by government agencies through which all transgenics must pass," Albertsen stated. "In the meantime, we are going to work to improve these lines to ensure they meet our expectations for improving nitrogen use efficiency in corn."

The results of these trials also are expected to have a positive impact on the environment. Developing more efficient nitrogen uptake and utilization may enable farmers to reduce the amount of nitrogen applied, resulting is less nitrogen runoff and keeping it from leaching into nearby streams and waterways.

Today, plant breeders primarily improve hybrids under conditions representative of the conditions that farmers currently grow corn. In general, this means relatively high levels of nitrogen.

"For the future, we want to give farmers hybrid options that will impact nitrogen application levels to have both a positive effect on a farmer's bottom line and to curb any adverse effect on the environment," he continued. "So we are working on several approaches to understand how a plant responds to different levels of nitrogen. For example, consider the nitrogen levels present during the seedling stage. The seedling is probably in the best environment for nitrogen that it will experience throughout its entire cycle. Yet, the plant does not have a good 'sink' to either store or utilize nitrogen. Eventually, the ear becomes a powerful 'sink' once grain filling commences. The irony is that nitrogen levels at the grain filling stage can be limiting, right when the plant needs it the most."

Researchers have looked at various things they can do at an early growth stage that would result in more nitrogen uptake when nitrogen is plentiful. They have looked at modifying the plant with transgenes so it can store some of the nitrogen for use later on. That approach is still in its infancy, although candidate genes have been identified.

"It sounds like a great idea, but a lot of times what seems like a great idea doesn't always work out as simply as we would like," Albertsen said. Still they are optimistic.

Another approach taken is to improve the biochemical pathways that are involved in nitrogen uptake, nitrogen transport, and final nitrogen utilization. This research approach seeks to find the way nitrogen is converted into harvestable yield.

"We try to exploit some of our general knowledge and say 'look, if we were going to modify some of these pathways, which ones and where would we make those modifications?'" he said. "Some of these ideas have resulted in specific genes being cloned and have resulted in transgenic plants expressing these genes. Our testing will determine whether these genes are the right ones to impact the nitrogen-related pathways."

Pioneer researchers also work with model plant systems in their quest for genes impacting nitrogen use efficiency. Model systems often are advantageous in that they have shorter life cycles and/or require less growing space than does corn. This enables researchers to challenge specially-designed collections of model system plants with stresses to determine how the plants respond. For example, populations of model plants may be challenged with low nitrogen in one experiment and with high nitrogen in another. While it seems logical to challenge plants with low nitrogen to identify genes that might enable a plant to more efficiently utilize the nitrogen it is presented with, it may be less intuitive why Albertsen and his team would use a high nitrogen challenge.

"Sometimes under plentiful nitrogen, roots don't develop as much as they could," he said. "It is as if the plant becomes 'lazy' since it doesn't have to search too hard to obtain the nitrogen it needs. We want those roots to develop as much as possible. We want those plants to take up as much nitrogen as they can at every stage of the plant's life cycle. We want that plant to develop a robust root structure to ensure the plant is able to uptake adequate water and nutrients, as well as contribute to overall standability of the plant at harvest. Asking a plant to develop robust roots under plentiful nitrogen is against what a plant is normally programmed to do."

Albertsen further explained the model systems used to look for genes that might offer improvements to the efficient use of nitrogen by corn. He said that genes identified from these model systems can be isolated, cloned, and inserted into corn for testing.

"At Pioneer we have several different types of testing environments, one of which we call our managed stress environment," he said. "For our nitrogen work, these are fields that we have purposely depleted of nitrogen.

"Sometimes, we have had to grow multiple crops without intervening fertilizer application to obtain the desired level of nitrogen depletion," Albertsen explained. "We maintain the fertility of these fields at this level by applying low levels of nitrogen for each subsequent growing season. Using this low nitrogen testing environment, we are able to determine whether the transgenes we have put into the corn plant are leading to efficient use of whatever nitrogen is present."

They can see what the transgenic corn plants can do on a reduced nitrogen supply. They also can compare which of these genes do the best. Last year was the first year in running replicated yield trials for nitrogen use efficiency. These trials were conducted both at a site in California and at a site here in the Midwest, actually right across the street from Pioneer headquarters. There's also a replicated trial in another location in northern Iowa near Cedar Falls.

"We conducted replicated yield trials for the first time and we identified several interesting combinations of genes that we will be testing more extensively next year," Albertsen said.

He showed photos of the test plots and the comparisons of the different transgenes. One photo showed leaves from two rows of plants. One of the rows was of a promising transgenic, while the other row was of its non-transgenic control.

"You can see there is much more yellowing of the leaves in the non-transgenic row than in the transgenic row," he said.

The photos showed a clear variation, and he reported the transgenic plants did show significant yield increases. He stressed, however, that while these transgenics demonstrate the effect his group is looking for, there is still much more work to be done. The photographs plainly showed that the transgenes have an effect on the plant's performance.

Though the trials look promising, more testing will be required to understand all the benefits these transgenics might offer farmers. Although Albertsen recommends that farmers follow the fertilizer recommendations and practices for their particular area, he does believe that side-dressing nitrogen as late as possible without disturbing the root structure will be beneficial to final productivity.

"The key is to make sure nitrogen is available when the plant really needs it," he said.

Albertsen said that his research group has two goals in developing nitrogen use efficiency products. One is to enhance the yield with current levels of nitrogen. The other is to maintain current yields with reduced nitrogen inputs. With nitrogen costs increasing dramatically this year, and with prospects of the cost going even higher, more nitrogen-efficient corn could be a boon for farmers.

"If it is biologically possible, we are going to deliver on our goals," he said. "Remember though, at this point we have just completed our first year of yield trials. That means we still have a ways to go, but we are very encouraged and excited by our results."

Albertsen noted that researchers today have more techniques available than ever before to help them tease apart what can make a plant utilize nitrogen more efficiently.

"Scientists have a good understanding of some of the biochemical pathways involved in nitrogen utilization," he said. "Today, we have many of the genes responsible for these pathways cloned and ready for manipulation via genetic engineering. At Pioneer, we also have the gene shuffling technology available to us that we acquired about two years ago from a California biotech company. This technology enabled us to develop our GAT herbicide resistance gene. We hope to utilize this technology to take a gene critical to nitrogen utilization and to 'shuffle it' to make the gene perform more effectively."

Albertsen described another capability at Pioneer that gives their researchers an edge, which is the ability to test transgenes in a high thruput system in corn in the greenhouse.

"This system, called the 'fast' corn system, enables us to test transgenes in corn that were originally identified in model plants," Albertsen said. "We simply must find out as early as possible how these genes perform in corn. This system enables us to sort through transgenes quickly so that we are only taking transgenes to the field that have already proven their efficacy in corn. It allows us to 'hunt where the ducks are'," he concluded. Δ