A Ray of Sunshine for Sunflower Breeding

How a government agency, an agricultural trade group, and a biotech company came together to bring sunflower breeding into the genomics era.

Introduction

The sunflower, *Helianthus annuus*, is a large annual crop with a strong global market for a variety of domestic and industrial uses. Aside from tasty sunflower seeds and healthy vegetable oil, sunflower products are used as bird feed, livestock feed, cooking oils, biodiesel fuel, and latex production. Last year, United States sunflower farms harvested 1.5 million acres valued at nearly 500 million US dollars.[1] However, in recent years sunflower acreage in the United States has been declining and farmers are looking for ways to improve resistance to diseases such as sunflower rust and Sclerotinia stalk rot.[2]

iCommunity spoke with Denise Thiede, PhD, Vice President of BioDiagnostics (BDI), and Farhad Ghavami, PhD, Manager of Molecular Breeding and Genomics Technology at BDI, to discuss how the company participated in modernizing breeding programs in the sunflower industry.

Q: What is BDI and how is it involved in genomic breeding?
Denise Thiede (DT): BioDiagnostics is a seed testing company that offers a full range of genomics tests to large and small seed companies. It was founded in 1996 by Quentin Schultz, our current president, who wanted to bring modern approaches to quality assurance testing. Our business has invested significantly in genetic analysis platforms. We've been building and developing technologies that will have a significant impact on advances in agriculture.

Q: What are some of the challenges faced by the US sunflower industry?
DT: Fifteen years ago, large seed companies in the US, such as corn and soybean companies, began focusing their research on transgenic products and genetic breeding programs. They built a large research infrastructure that identified markers for native traits and transgenic traits. This drove the development of many genomic breeding programs, and ultimately led to improvements in the quality of their seed products.

However, the sunflower industry rejected transgenics and as a result, none of the modern genetic tools were developed for sunflower breeding. Over time, the National Sunflower Association (NSA) began to see that significant improvements were being made in corn and soybeans by using advanced genomic breeding techniques. While improvements in drought tolerance and disease resistance were expanding the acreage of corn and soy, the acreage of sunflowers was decreasing. The sunflower industry and the NSA realized they needed these genomic tools to protect the future of sunflower farming.

Sunflower fields in North Dakota. The sunflower industry realized they needed genomic tools to protect the future of sunflower farming.

Denise Thiede, PhD, Vice President of BioDiagnostics and Farhad Ghavami, PhD, Manager of Molecular Breeding and Genomics Technology at BDI.

Q: What role did BDI play in addressing these challenges?
DT: Historically, agricultural research has been funded through the United States Department of Agriculture (USDA), which has research stations around the US. North Dakota is the heart of sunflower country, so the USDA research unit in Fargo was focusing on sunflowers as part of their effort to serve local industries. The NSA started talking to the USDA researchers in Fargo to see how they could facilitate and speed up their genetic discovery process. The USDA researchers knew that whole-genome, high-density marker technologies were needed to associate markers with the phenotypic traits they had identified in the field. However, the USDA in Fargo did not have the infrastructure to develop a high-density array for sunflower.

Farhad Ghavami (FG): They were using markers like simple sequence repeats (SSRs) and restriction fragment length polymorphisms (RFLPs)—marker information that did not provide a dense and robust set of markers for modern breeding needs.

DT: At the same time, BDI was also serving the sunflower industry. We were doing a lot of quality assurance testing—germination testing, genetic purity testing. Through discussions at an annual NSA Research Conference in January 2010, a trifecta came together: the USDA had the critical phenotypic data and research infrastructure, the NSA had the funding, and BDI had the genomic technology. Through this collaboration, BDI helped the US sunflower industry adopt the modern genomic tools that would enable them to be successful in their breeding programs and maintain production here in the US and abroad.

Q: How did BDI develop genomic tools for the sunflower industry?
FG: The first step was to sequence the sunflower genome. Crops such as corn and rice had full reference sequences, but the sunflower did not. This made finding relevant markers and single nucleotide polymorphisms (SNPs) difficult. Also, the sunflower genome is large and complex; it's ~3.5 Gb, which is larger than the human genome. Because of its size and complexity, we decided to use restriction site associated DNA sequencing (RAD-Seq). RAD-Seq is based on identifying polymorphic variants next to restriction enzyme digestion sites. It can reduce the complexity of the genome by eliminating duplicates and repetitive sequences.

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DT: The sequencing project was done in collaboration with Floragenex, which has expertise in RAD-Seq. We provided them with 6 sunflower lines, including oil and confection lines, for the sunflower sequencing project.[3]

Q: What platform was used to sequence the sunflower genome?
FG: We used paired-end Illumina sequencing. We started with an early Illumina sequencing platform, but now RAD-Seq is performed on the HiSeq® or MiSeq® Systems. Floragenex also did the bioinformatics work to find and refine a SNP set for a whole-genome, high-density SNP array. A final set of 16,467 SNPs was used to create the sunflower array with the Illumina Infinium® iSelect® platform.

Q: Why did the NSA and BDI decide to use the Infinium iSelect platform to create the sunflower SNP array?
FG: At that time, we had experience with the Illumina GoldenGate® platform and really liked it because it could analyze 1536 markers at a time. Then higher-density Infinium arrays, like the MaizeSNP 50K BeadChip,entered the market with over 10,000 markers per array. Because GoldenGate had been so successful, it was not difficult to convince us to use the Infinium arrays. When it came to high-density arrays in 2005/2006, Nimblegen and Affymetrix arrays were available, but Illumina had the best arrays on the market and had proven to be a good platform.

> "A significant advantage of the iSelect array is that it's highly flexible in the number of samples that can be processed."

DT: We recommended the iSelect array and told the NSA and USDA that this is the technology that would be most useful to them.

FG: A significant advantage of the iSelect array is that it's highly flexible in the number of samples that can be processed. We ran 1100 samples at the beginning of the project, but later were running a few samples at a time. We were not stuck having to run 96 sample plates. Running iSelect arrays was easy and we liked it.

Q: What do you and your customers think of the sunflower array data quality and data analysis?
FG: For data analysis, Illumina is one of the best companies on the market. The genotyping error rates are low and the GenomeStudio® software is robust and easy to use.

Q: What information are you able to provide to your customers with the sunflower array?
DT: With the iSelect sunflower array, we perform whole-genome SNP genotyping and provide customers with the final genotype calls. Our customers get the final results and then make their breeding decisions based on the genotype data. Some of our customers are sophisticated users of that genomic data and some are at the early stages of the learning curve. If they are new users of genomic data, we provide basic consulting services to help them use the data appropriately.

> "Now we have customers using those markers to breed for disease resistance against key sunflower diseases."

Q: What discoveries have been made using the sunflower array?
DT: After we had the sunflower array, the next major step was to start performing large-scale genotyping for the NSA participants. This really helped them gain an understanding of the diversity in their crop lines as well as the extent to which their lines were related. It gave them important information, however they still didn't have good disease resistance markers. That's where the sunflower array in combination with the work being done at the USDA became extremely valuable.

FG: The USDA had a lot of good phenotypic data and we were able to provide the genotypic data. Using the sunflower array, we merged the phenotypic and genotypic data through association mapping and found many agronomic traits as well as many disease resistance genes at the same time.

DT: The sunflower array allowed the USDA group to narrow down and locate the genetic regions associated with disease resistance. Since then they've published many papers about those disease resistance genes. Now we have customers using those markers to breed for disease resistance against key sunflower diseases.

Q: What are some of the most destructive sunflower diseases?
FG: In North America, sunflower rust is a fungus that has caused economic losses in sunflower yield and seed quality. Dr. Lili Qi from the USDA-ARS recently discovered the R_{12} rust resistance gene and performed high-density SNP mapping of the R_{12} gene with the sunflower array.[4] We now use these markers every day for customers who want to perform marker selection for the R_{12} rust-resistance gene.

> "The iSelect sunflower array is very effective for these breeding programs because it can scan the whole genome and find markers much closer to the disease resistance genes."

Sclerotinia stalk rot is another fungus that is extremely destructive to sunflower crops worldwide. Under certain conditions, it can reduce crop yield by up to 20%. Dr. Brent Hulke at the USDA used the sunflower array to identify over 180 markers associated with Sclerotinia head rot, Sclerotinia stalk rot, and Phomopsis stalk canker. Dr. Hulke found that testing for these markers greatly improves crop selection and allows them to select sunflower varieties that show the greatest resistance to disease.

The iSelect sunflower array is very effective for these breeding programs because it can scan the whole genome and find markers that are much closer to the disease resistance genes. It's also easier to use and more cost-effective than the older SSR/RFLP markers.

Q: Have you seen any changes in the plant breeding industry as a result of modern genomic breeding techniques?
DT: There's a lot more complexity in plant breeding today. One of the challenges is that historically plant breeders are phenotypically organized, or if they use quantitative genetics, it's more of a statistical approach rather than a genomic approach. In many companies, there are traditional plant breeders who know they need to use the new genomic tools, but they don't necessarily know how to use them. We try to help them adopt the new tools. The companies that have the greatest success are the ones that hire new plant breeders who have the knowledge and experience to immediately adopt and use modern genomic tools. Plant breeding is not performed by a single individual anymore. It's a breeding team that includes people who can do the bioinformatics and people who can do the field work.

FG: People are shifting from traditional plant breeding to genomic-based breeding. We are in a state of transition. Some people have adopted this new scheme quickly and can use the new technology. Some people are waiting and watching to see how successful the new approaches are. These days, most people involved in plant breeding are using genomic tools to some degree.

Q: One final question: do you like sunflower seeds?
FG: I love them! Most sunflower seeds are roasted and salted, but I eat them raw, the way birds do.

NSA SNP Consortium

This project was conducted in accordance with the NSA SNP Consortium, a public-private partnership between the nonprofit National Sunflower Association, public researchers at USDA, and private seed companies. It was formed with the intent of having widespread, economically feasible application of SNP markers for the advancement of sunflower germplasm. In 2014 the sunflower SNPs were published in PLOS One.[5]

References

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