Seeking Sustainable Protein Sources for Asia and Sub-Saharan Africa

ICRISAT researchers are using Illumina sequencing to develop disease-resistant pigeonpea varieties.

Recognizing that its technology could play a critical role in alleviating global hunger, malnutrition, and poverty, Illumina created the Agricultural Greater Good initiative. Each year, Illumina awards Greater Good grants to agricultural research organizations that are focused on identifying and breeding plants and animals that will increase the sustainability, productivity, and nutritional density of crop and livestock species. Under the grants, Illumina sequencing and genotyping reagents are provided free of charge.

ICRISAT
2013 Illumina Greater Good Initiative Award Winner

Introduction

Founded in 1972, the International Crops Research Institute for the Semi-Arid Tropics (ICRISAT)[1] is a non-profit, non-political organization conducting agricultural research for development in Asia and sub-Saharan Africa. It's an area of the world that is in desperate need of new farming tactics and tools, with more than 25% of its 2 billion people living in poverty and hunger.

According to Rajeev Varshney, Ph.D., Research Program Director-Grain Legumes and Director, Center of Excellence in Genomics at ICRISAT, one of the organization's objectives is to apply scientific innovations to dryland agriculture. This farming method is designed for regions of scant rainfall, focusing on water-retentive tillage methods and the selection of suitable crops, rather than relying on irrigation. "The objective of ICRISAT is to develop superior varieties of crops that can thrive in drought conditions," Dr. Varshney said. "There's not much variety within the few crops that are cultivated in Asia and Africa. While breeders have been successful in developing new, hardier crops using traditional approaches, it wasn't until 2005 that we really started to leverage genomics. Molecular breeding enables us to make strides in developing crops that are truly sustainable in this region."

The pigeonpea (*Cajanus cajan*) is a hardy drought-tolerant legume from India that is used for food, livestock feed, soil erosion amendment, and as a wind break. It's an excellent protein source, and is often referred to as the "poor man's meat." India produces 90% of the pigeonpea grown in the world and is also its largest importer. While pigeonpea is important for food security in India, it's an income-generating crop in Africa, particularly Kenya, Tanzania, and Rwanda. In these countries, farming pigeonpea enables families to send their children to school, something that would be otherwise impossible.

"When I joined ICRISAT, we had just 10 molecular markers in pigeonpea," Dr. Varshney said. "These were simple sequence repeats or SSRs. We had no genomic resources such as advanced molecular markers or single nucleotide polymorphisms (SNPs), and no mapping populations. However, when you have a crop like pigeonpea, which has narrow genetic diversity in the germplasm, even if you have 10,000 SNPs, there are only about 200 to 300 that will be polymorphic in germplasm. For the last several years, we have been collaborating with different partners to develop genomic resources in pigeonpea using next-generation sequencing (NGS)."

Rajeev Varshney, Ph.D., is Research Program Director-Grain Legumes and Director, Center of Excellence in Genomics at ICRISAT.

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In 2011, the ICRISAT team, Beijing Genomics Institute (BGI), and other partners used Illumina sequencers to generate a draft genome of pigeonpea[2]. The Greater Good initiative award ICRISAT received in 2013 enabled it to accelerate projects to enhance pigeonpea genetic diversity and support development of superior varieties.

Upon receiving the Greater Good Initiative award, the ICRISAT team had several rounds of discussions with its stakeholders about the range of problems afflicting pigeonpea crops. "We realized we couldn't tackle all of them in a single go," Dr. Varshney said. "We decided to use the Greater Good award to support development of pigeonpea varieties resistant to sterility mosaic disease (SMD) and *Fusarium* wilt (FW). These diseases negatively impact pigeonpea yield and quality, and have spread to farms throughout India, Uganda, Kenya, and Tanzania."

"The award has also enabled us to focus our research funds on developing pigeonpea varieties with higher yield, protein content, and early maturity," said Dr. Varshney. "In general it takes about 140 to 180 days for a pigeonpea crop to be harvest-ready. In India, we would like to grow two crops in about 120 days. This would ensure greater food security and support income generation, enabling farmers to make the shift away from subsistence farming."

Pigeonpea is grown in marginal environments where water is scarce. There it is also exposed to several biotic and abiotic stresses, reducing crop productivity to >1 ton per hectare. "In the face of climate change, our goal is to establish climate-resistant pigeonpea varieties," said Dr. Varshney. "Molecular breeding enables us to develop plants that can withstand drought conditions and environmental stresses, and ultimately generate much higher yields[3]".

By leveraging NGS systems, such as Illumina MiSeq® and HiSeq® systems, ICRISAT intends to enhance the genetic base of pigeonpea. "The great thing about the HiSeq system is that we can generate a huge amount of sequence data in a very cost-effective manner. We use the MiSeq system for smaller projects, such as pathogen (bacteria, fungi) resequencing. We're in the process of optimizing genotyping-by-sequencing and hope to be employing that soon."

Molecular breeding will enable ICRISAT to increase the allelic diversity in the cultivated gene pool from landraces (traditional pigeonpea breeding populations), the gene bank, and existing wild varieties. It's developing introgression libraries in the genetic background of the cultivated varieties, performing extension phenotyping, and developing specialized genetic stock called MAGIC (Multi-parents Advanced Generation Inter-Cross). "We are making some complex two-, four-, and eight-way crosses, creating varieties that possess different haplotype blocks," Dr. Varshney said. "The set of 500 MAGIC lines we developed possess a huge amount of genetic diversity, because we are reshuffling the haplotype blocks."

"We're using three different approaches to identify genomic regions responsible for various economically important traits," Dr. Varshney added. "The first is through linkage mapping, second is genome-wide association studies (GWAS), and the third is nested association mapping (NAM). For GWAS analysis, we have already completed the resequencing of 300 plant lines at 5–10× coverage."

This work also supports capacity building. "The research is being conducted by eight Ph.D. students, two from Africa (Uganda and Tanzania) and six from India," Dr. Varshney said. "Through this work, we are developing the next generation of scientists."

By the end of this three- to five-year project, ICRISAT will have molecular markers associated with specific pigeonpea traits. "These markers will enable breeders to develop new varieties with improved traits in less time. In general, it takes 8 to 10 years to develop the varieties, but we believe that we can reduce this time to 4 to 6 years," Dr. Varshney added.

Reference

1. http://www.icrisat.org/index.htm#
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3. Varshney RK, Ribaut J-M, Buckler ES, Tuberosa R, Rafalski A and Langridge P (2012) Can genomics boost productivity of orphan crops? Nat Biotechnol 30: 1172–1176.

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