April 2014

iCommunity
NEWSLETTER

Breeding Climate-Resilient Cowpea for Improved Food Security in West Africa

Illumina high-density genotyping expected to accelerate marker-assisted breeding.

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.

UC RIVERSIDE UNIVERSITY OF CALIFORNIA

2014 Illumina Greater Good Initiative Award Winner

Introduction

The cowpea {*Vigna unguiculata* (L.) Walp} is an important source of protein in Sub-Saharan Africa, where it is well adapted to the hot, drought-prone climate of the region. Recognized as the black-eyed pea in the United States, this ancient legume was domesticated by African gatherers, cultivators, and farmers from its wild forms dating back to Neolithic times. The modern-day cowpea is grown mainly by smallholder farmers in semi-arid Sub-Saharan Africa where rainfall is unpredictable. The threat of climate change and the expansion of desert areas is fueling efforts by the USAID* Feed the Future program to tap into the cowpea's genetic diversity and maximize the environmental tolerance of this important food source.

In West Africa, the cowpea growing season begins with the first seasonal rains around April and ends with the harvest in October. The plant is an essential part of the nutritional profile for inhabitants of the region. As with other legumes, cowpea seeds are rich in the essential amino acids lysine and tryptophan. They support a balanced diet when combined with cereals such as sorghum and millet, which are rich in methionine. Cowpea is also rich in folic acid, which is important for fetal development. All parts of the cowpea plant are eaten. The leaves can be cooked like spinach and the immature pods are eaten as fresh vegetables. The mature dry seeds are processed into a nutritive flour, or cooked and eaten whole. The residual dry matter after pod harvesting can be stored and used as animal feed[1].

As one of the most drought- and heat-tolerant crops in the region, cowpea plays an important role in breaking the "hungry time," which can occur toward the end of each off-season when food reserves may become depleted. Seeds produced during the annual growing season must last throughout the year until the next cowpea crop. If the previous harvest was poor or the rains arrive late, the hungry time can become more severe, causing malnutrition and starvation that increases fatalities from malaria and other diseases [2].

Timothy J. Close, Ph.D., professor of genetics in the Department of Botany and Plant Sciences at University of California, Riverside (UCR), is interested in the adaptation of crop plants to environmental stresses. Dr. Close, a plant geneticist with extensive experience in genome resource development, is now engaged in the breeding activities of cowpea, with a specific interest in adaptation to drought-prone environments.

The cowpea plant and seed diversity make it an essential part of the nutritional profile for inhabitants of West Africa.

Timothy J. Close, Ph.D., is professor of genetics in the Department of Botany and Plant Sciences at University of California, Riverside.

---

*U.S. Agency for International Development that works to end extreme global poverty.

"The central objective is to identify more climate-resilient cowpea varieties for each production environment," said Dr. Close. "We make crosses between parents carrying favorable traits and look for progeny with a more optimal combination than either parent. It's all about using the normal reproductive system of the cowpea plant and the genetic diversity of cowpea that nature has provided. Our work is not about the creation of transgenic plants, also known as GMOs. The use of genotyping during breeding helps to keep pre-determined crossing and progeny selection plans on track. It gives us the ability to recognize and remove off-track individuals arising from unintended outcrosses, while minimizing the size of breeding populations and the number of generations required to achieve the intended outcomes. The overall goal is to improve the nutrition and livelihood of the people in West Africa by developing hardier cowpea varieties."

Drawing upon Nearly 40 Years of Cowpea Genetic Research

"The cowpea breeding program at UCR was started nearly 40 years ago by Professor Emeritus Anthony E. Hall," Dr. Close said. "He established strong, long-lived connections with West African cowpea breeders and initiated training programs and partnerships, several of which are still in place today. Those of us who comprise the current cowpea team are proud to continue the cowpea legacy."

The core UCR cowpea team has expertise in agronomy, agricultural biology, seed systems, general genetics, molecular genetics, and computer sciences. Professor Philip A. Roberts, Ph.D., in the Department of Nematology, has been a leader in the UCR cowpea team for nearly 30 years. He currently directs field-level decisions and brings practical agronomy, plant pathology, nematology knowledge, and decades of experience in the African context. Bao-Lam Huynh, Ph.D., handles a range of day-to-day operations spanning genotype data analysis, genotype-informed advice on crossing and selection for their African partners, and management of their local field and greenhouse assistants. The USAID Innovation Lab for Climate Resilient Cowpea project under the USAID Feed the Future program also benefits from the expertise of Professor Stefano Lonardi, Ph.D., in the Department of Computer Sciences.

Dr. Close interacts regularly with each of these team members, along with numerous students and other staff, to coordinate the use of informatics tools for genetic marker development and the deployment of markers to guide the breeding activities. "We have several excellent students at UCR whose contributions should not be overlooked. Mitchell Lucas is a graduate student in the Genetics, Genomics and Bioinformatics program whose Ph.D. thesis research includes recent publications on the genetics of heat tolerance and thrips resistance in cowpea. Seyed Mirebrahim is a Ph.D. student in Computer Sciences who has been pivotal in the development of the new Illumina iSelect® custom genotyping assay, working closely with Professor Lonardi and me, along with Programmer Steve Wanamaker. There are quite a few more people at UCR who are part of the cowpea team, but there is not sufficient space in my brief comments to do justice to everyone."

The UCR team also works with partners in Africa who are instrumental in delivering the improved varieties to small-holder farmers. "Dr. Ndiaga Cisse in Senegal, Dr. Issa Drabo in Burkina Faso, Dr. Ibrahim Atokple in Ghana, and Drs. Ousmane Boukar and Christian Fatokun in Nigeria are the leading cowpea breeders in these four West African nations. We have additional partners in Mozambique, China, and other nations, and I wish I had the time to acknowledge them all."

Leveraging Cowpea Natural Genetic Variation and Market-Assisted Breeding

The breeding team leverages natural genetic variation within cowpea. "Cowpea offers a multitude of wonderful targets with extensive genetic variability," said Dr. Close. For example, the team recently identified single nucleotide polymorphisms (SNPs) that mark five quantitative trait loci (QTL) for heat tolerance during reproductive development. According to Dr. Close, cowpea pollen won't develop properly when the night temperature is 34°C (93°F) or higher[3]. "At sustained elevated nighttime temperatures, cowpea pollen is not shed from the anthers, impeding pollination and thus preventing pod development and seed production," explained Dr. Close. This was shown in cowpea more than 30 years ago by now-retired Professors Hall and Dareleen DeMason and their students at UCR, and is a common phenomenon in plants. The opportunity for cowpea breeding lies in the fact that significant genetic variation exists for tolerance or susceptibility at an intermediate night temperature of 28°C (82°F), which is relevant in production environments. "We're starting with a species that is already well adapted to hot, drought-prone areas relative to most other crop plants," Dr. Close said. "But, there is still a range of tolerance within cowpea. We're trying to combine the best heat-tolerant and drought-tolerant alleles within individuals that also have favorable seed characteristics, along with insect and pathogen resistance."

The team applies marker-assisted breeding, which involves matching a trait of interest to a corresponding set of tightly linked DNA markers. The genetic markers, or SNPs, are used to determine the position of major loci in the genome that have the strongest influence on a trait. "Having a state-of-the-art genetic marker system is essential to tracking and combining (pyramiding) several traits simultaneously in our breeding activities," Dr. Close said. "This may seem like it needs a miracle to succeed, but the reality of marker-assisted breeding is that it actually does work for multiple traits. Cowpea has a simple diploid genetic system that can inbreed or outcross, and there is ample genetic variation. With an improved genotyping capability it becomes a matter of using good math to extract relationships between excellent phenotype data that African breeders can provide and volumes of genetic data generated using SNP genotyping. The fact that nature has provided us with such a simple genetic system in cowpea is a bonus."

For example, Dr. Close's team compared progeny derived from a cross of two parents that differed in their heat tolerance. They located five QTL regions of the genome that contribute significantly to heat resistance. "We have also identified one major and one minor QTL responsible for resistance to a California aphid biotype. We can mark these positions and recognize the resistant and sensitive allelic variants by virtue of a set of tightly linked markers."

Greater Good Award Enables Increased Genotyping Density and a Simple Workflow

The cowpea team uses high-density genotyping to map traits and mark favorable and unfavorable haplotypes**, leading the way toward new varieties. They previously developed an Illumina GoldenGate® SNP assay for cowpea and used it to mark traits such as bacterial blight resistance, aphid resistance, Macrophomina resistance, drought-induced senescence, heat tolerance during reproductive development, thrips resistance, and leaf shape. "The GoldenGate assay gave our genetic mapping a tremendous boost, enabling us

---

** A group of markers inherited together.

to map more traits and at a much finer level of resolution than was possible before," said Dr. Close. "However, there have been several instances when our nearest marker was a few centimorgan† away from a trait determinant. It was clear that we simply didn't have enough markers packed together to define a clear haplotype. We don't need to know exactly which gene controls a trait. However, for breeding we need enough markers packed together around favorable and unfavorable alleles so that we can reliably track haplotypes."

"The Illumina Agriculture Greater Good Award enables us to reach higher heights than would have been possible otherwise," Dr. Close added. "The Infinium custom iSelect 60K genotyping BeadChip should provide about 40 times as many markers and 40 times the resolving power as the 1536-plex GoldenGate assay that we developed previously. We are excited about progressing to 60,000 bead types with the iSelect assay. We anticipate about 40,000 technically successful SNP assays using the same workflow for DNA preparation and data analysis that we used with the GoldenGate assay."

"The goal in designing the iSelect 60k BeadChip assay has been to annotate every known gene in the cowpea genome with two markers so we can detect up to four haplotypes," Dr Close said. "We'll also scatter markers at various positions throughout the genome in non-genic regions, ensuring that we have positional coverage. Not every attempted bead type will succeed, but overall the numbers are very much in favor of achieving a level of resolution sufficient to mark every major allele with a reliable haplotype. The iSelect 60k assay design will also support genome-wide association mapping and map-based cloning of trait determinants. We are confident that the higher resolution will provide the needed increase in accuracy of marker-trait associations to meet our USAID Feed the Future project objectives."

Data Analysis Training with GenomeStudio®

According to Dr. Close, the Agricultural Greater Good Award has stimulated consortium interest around a higher density, off-the-shelf genotyping assay, expanding the consortium group and building momentum for future studies. "The iSelect assay offers a simple workflow, including easy data analysis and export tools in the GenomeStudio software," Dr. Close said. "The portability of relatively small, digested datasets and software enables us to put more raw forms of data into the hands our partners, just in case the less processed data may be useful."

Through UCR's partnership under the USAID Innovation Lab for Climate Resilient Cowpea[4], Dr. Close's team extracts DNA from leaves sent by breeder partners in Burkina Faso, Ghana, Senegal, and Nigeria. After genotyping with the iSelect assay, the data are packaged in the GenomeStudio software, and exported to spreadsheets that are formatted for other analytical tools. The Agricultural Greater Good Initiative is providing a license for the GenomeStudio software to each African partner site. "The GenomeStudio interface is informatics-light and thus easy for all of us to use," said Dr. Close. "It doesn't require an informatics specialist, and this simplicity is valuable at each breeder location. Knowing how, and when, to use GenomeStudio will help our partners understand what happens to their samples, and what we routinely do with the data. This familiarity will build confidence in the whole genotyping workflow. We anticipate that our West African host country partners will become local sources of expertise regarding genotyping and cowpea genetics, which will further secure cowpea's future."

Sowing New Seeds

Climate-resilient cowpea varieties that will eventually result from intensified breeding activities will be distributed through existing networks in the partner countries. Favorable progeny from breeding programs typically progress through standard stages of seed multiplication and distribution. This begins with breeders seed, then proceeds through carefully controlled increases to produce foundation seed, then further increased for larger-scale production as certified seed.

"Our breeder partners are engaged in their local seed systems, and well linked to local outreach groups and networks," said Dr. Close. "They have experience with adoption of new varieties. Cowpea is an inbreeding crop, so the mature seeds taken from a plant will be genetically identical to the plant from which they were collected, as long as there has been no cross-pollination in the field. This means that farmers don't need to buy certified seed every year. However, the accumulation of outcrossed progeny will eventually cause collected seeds to diverge genetically from the original variety."

Understandably, there is resistance to buying a new seed variety and the farmers need data that convinces them of its higher value. "By using the iSelect assay, we can provide detailed visual displays of the genotypes of known varieties. This type of knowledge can help assure that seeds which progress through the seed system will be what they are supposed to be."

References

1. https://www.hort.purdue.edu/newcrop/afcm/cowpea.html
2. http://www.fao.org/ag/agp/agpc/doc/publicat/cowpea_cisse/cowpea_cisse_e.htm
3. http://feedthefuture.gov/article/feed-future-innovation-labs
4. Lucas MR, Huynh B, Diop N, Roberts PA, Close TJ (2013) Markers for breeding heat tolerant cowpea. Mol Breeding 31: 529-536.

---

†A unit for measuring genetic linkage.

Pub. No. 1370-2014-002 Current as of 08 April 2014