CASE STUDY

Understanding the genetics of important traits in hemp to help breed improved cultivars

Dr Jacob Toth is a Plant Breeding and Genetics Post-Doc at the School of Integrated Plant Science at Cornell University, working in the research group of Professor Larry Smart. We recently spoke with Jacob to find out more about the group's research and how they are using **PACE**® in their workflow.

What is your current research focus?

"Our lab investigates the genetics of important traits and uses these insights to breed new cultivars. Until 2018 the primary focus of the research group was investigating the genetic basis of traits relevant to willow breeding for bioenergy, but the focus of the lab has since switched to mainly studying hemp genetics. In hemp, important traits include cannabinoid type and content, plant sex, flowering time, seed size, plant colour and architecture, and resistance to pests and pathogens such as powdery mildew. To this end, we evaluate commercial cultivars, develop genotyping assays and platforms, develop segregating crosses that we genotype and phenotype, and are developing high-throughput methods for phenotyping"

How does SNP genotyping fit into your research/workflow?

"The main use of single SNP genotyping in our plant breeding workflow is for early selection or determination of essentially qualitative traits that are usually only expressed at maturity or under certain conditions. Beyond that, we also use single SNP genotyping for determination of allelic status for recessive loci, for fine mapping genes, and for stacking genes difficult to distinguish phenotypically."

How do you use PACE?

"We develop **PACE** assays based on interesting or informative SNPs or indels and use them for breeding and research purposes. We generally use **PACE** assays in 96-well PCR plates with an optically clear cover in 10 µL reactions. We generally run the samples on a Biorad C1000 Touch Thermocyler and read them in on a Biorad CFX96 real-time system. We then usually do analysis of the reads using CFX Maestro".

Dr Jacob Toth, School of Integrated Plant Science, Cornell University

How does PACE benefit your work?

"There are several qualitative traits in hemp that have a simple genetic basis but are difficult to assay. For instance, cannabinoid type is a major issue for hemp breeding, as if high-THC plants are grown, the crop is considered marijuana and not permitted to enter commerce. The standard way to assess chemotype is through HPLC analysis, which is slow and expensive. The cannabinoid type is largely governed by a single locus, and with PACE assays, we can screen thousands of plants for chemotype in a timeline of days rather than months using HPLC, and at a fraction of the cost.

There are also several major flowering time genes in hemp, but some are recessive. With **PACE** screening, we can identify homozygotes and heterozygotes resulting in a more uniform cultivar not segregating for these major genes. Knowing allelic states can also inform us about flowering time when the plant is young, rather than waiting months to see flowering under field conditions. We have also used PACE assays to show that there are at least two distinct loci conferring photoperiod insensitivity in hemp by crossing representative individuals and tracking the loci with PACE assays.

We also use **PACE** assays to determine the sex of young plants. This trait (male, XY, vs female, XX) is usually not expressed until the plant is mature, but with PACE assays we can separate out males from females at a young age, allowing us to manage crossing blocks in a more effective manner. We have also used **PACE** assays to determine if "supermale" (YY) plants are viable. We designed PACE assays that look at the ratio of X chromosomes to Y chromosomes and were able to collect seed off of XY plants that are normally male. Using PACE assays, we saw that there were only XX and XY plants in the XY-derived plants, with no YY group as determined by the marker".

What are your research aims in the future?

"From a genetics standpoint, we are investigating seed size (remarkably, we have seen thousand seed weights vary from 8-60 grams!), plant color, minor cannabinoid production, late flowering genes, and plant sex determination. We hope to release grain and fiber cultivars with improved cannabinoid profiles better adapted to more southern latitudes in the near future, as well as develop equatorially adapted high-cannabinoid cultivars".

What are the benefits and challenges of working with hemp?

"Hemp is a wonderfully diverse crop. We have plants that grow 16 feet tall in a season, plants that flower when they are 2 weeks old, and plants whose aroma ranges from gasoline to cherries. With the only very recent legalization of hemp, there is much to learn to bring it into 21st century cropping systems and being able to quickly and inexpensively determine the allelic state of major genes (which might determine market class) is a huge boon. The newness of the crop does bring its own challenges however, as markets are tumultuous, and legislation is inconsistent in the legality of the crop and its resultant products. On more practical notes, hemp is somewhat challenging to work with given its dioecious nature making desired crosses more difficult and inbreds less viable. The passion of the people working with hemp or Cannabis more broadly is a bit of a double-edged sword in that people get excited about the science and the plant in a way that you don't see for other crops, but there are a lot of strong personalities and misinformation available online leading to obfuscation and questionable practices".