ASPB Pioneer Member

L. Curtis (Curt) Hannah

I was born and raised on a farm in central Indiana. From a young age, my father told me I was going to go to Purdue University and study agriculture- and I complied. It was a different time. I did have the choice though of a major, and because I was interested in chemistry and biochemistry, I enrolled in the School of Agriculture (satisfying Dad's criteria) majoring in biochemistry. This major has served me well. I took a course in genetics taught by Dr. Wayne Keim, and it changed my life. I knew then that graduate studies in biochemical genetics would be in my future.

Ongoing work at Purdue by Oliver Nelson in the Botany and Plant Pathology Department and Ed Mertz in Biochemistry caught my eye. They showed each of two single gene mutations in maize doubled the lysine content of the seed. Because lysine is the first most limiting amino acid when maize is consumed by monogastric animals, these mutations had, potentially at least, remarkable implications. I wanted to do research on this topic, and so I applied to graduate school in Botany and Plant Pathology to work with Oliver Nelson. Another Purdue student also applied for this position, and he had worked in Oliver's lab as an undergraduate. Consequently, Oliver chose him rather than me. Instead, I did a Masters' degree with Dr. Mark

Tomes in that department, studying carotenoids and chlorophylls in tomato mutants. Mark knew how to design projects for MS students. My question was whether mutations that alter tomato fruit pigments also alter other pigments in the plant. I assayed leaf chlorophylls and carotenoids in segregating F_2 populations that had been classified for fruit flesh mutants. The beauty of a project like this is that there is a publishable answer in the end. Yes, the flesh mutants alter leaf pigments, or no, they don't. Either answer is important to know, and I carried this lesson with me throughout my career.

I thought I should switch universities for a PhD, and as luck would have it Oliver Nelson was leaving Purdue for the University of Wisconsin, and he invited me join him. Because I moved a week before Oliver, my story is that I brought Oliver with me! Ironically, I never worked on the opaque mutants of corn or the zein proteins affected by them. My project was to determine how two maize genes, *shrunken-2 (sh2)* and *brittle-2 (bt2)*, control a starch biosynthetic enzyme, ADP-glucose pyrophosphorylase (AGPase), in the seed. This project started in 1969, when there was not a DNA cloning and sequencing option. Rather, there was just enzymology. Studies of the mutant enzymes revealed differences in their heat stability and kinetic parameters, as expected if the mutations were structural rather than regulatory or had indirect effects. The icing on the cake was a mutant allele of *sh2* caused by insertion of the transposable element, *Dissociation (Ds).* The mutant enzyme that resulted had unique structural properties, suggesting the *Ds* element inserted into the gene altered the protein's amino acid sequence. This idea went against Barbara McClintock's notion that transposable elements controlled expression of the gene in which they resided by regulating its expression. Not surprisingly, my hypothesis was not well received by followers of McClintock, although she seemed okay with it. Oliver believed the result, and we published it!

After finishing my PhD at Wisconsin, I fully planned on doing post-doctoral research, but my old MS professor, Mark Tomes, sent me the advertisement for an assistant professorship in biochemical genetics in the Vegetable Crops Department at the University

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of Florida. I applied, and to my surprise I was called for an interview. Again, as luck would have it, the sweet corn breeder in that department had just released one of the first sweet corn varieties based on the *sh2* mutation, rather than the then standard, *sugary-1.* My seminar of course was about how *Sh2* and *Bt2* controlled sugar levels in the developing kernel, and the timing was perfect. I was offered (and accepted) the job during the interview. It was a different time.

I have spent my career at the University of Florida, and my research has not wandered too far from *Sh2* and *Bt2*. I guess my career could be coined "non-genomic"! Not only are loss-of-function and near loss-of-function mutants of these genes important in the sweet corn industry, but the allosteric enzyme they encode, AGPase, is critically important in starch synthesis. Consequently, this research has had relevance for both sweet corn and field corn.

The major obstacle for commercialization of *sh2*-based sweet corns is poor seed and seedling vigor. No doubt this is due, directly or indirectly, to the reduced amount of starch in the mature seed. Before leaving Wisconsin, Oliver Nelson told me he thought leaky alleles of *Sh2* and *Bt2* might be important for sweet corn. Following his advice, I looked for a leaky allele of *Sh2*, and an allele termed *sh2-I* (I or intermediate) reported by Gerry Neuffer caught my attention. This allele now appears in several commercial sweet corns, and more are coming. While I get credit for the leaky allele approach, the idea originally came from Oliver.

One unexpected pleasure in my career has been the characterization of mutants. I am convinced that every mutant is interesting and informative. It is extremely difficult to get funding for mutant characterization, but you don't know if a mutant is interesting until you have the data. And once you get the data, you don't need funding! So, we did lots of work with "rainy day" money, and a number of interesting things came from this. One discovery of significance was a transposable element termed *Helitron*. At the time, these elements had been hypothesized to be mobile, based on repetitive sequences found in a number of organisms. In our case however, the border sequences, a palindrome at one of the ends of the element and the site of insertion in a newly created *sh2* allele, were predicted from what was known at the time about *Helitrons*. There were many other "first" findings from our analysis of plant mutants, including that the transposable element *Ds* could function as a perfect intron, a change of the intron acceptor site from AG to AA could sometimes allow recognition as a splice site, and the first isolation in a plant of the branched lariat structure produced by RNA transcript splicing.

The last 20 years of my career were spent engineering AGPase to increase starch synthesis and improve yield in agriculturally important crops. To do this we used an *E. coli* gene expression system, because the end product -- glycogen – can be easily stained with iodine and we could apply classic and quick bacterial mutant screens for effects on the enzyme. By engineering the genes encoding AGPase, we and others were able to increase starch synthesis and enhance the yield of a number of agriculturally-important plants.

Surprisingly, in corn the engineered genes improved yield by increasing seed number, rather than by increasing individual seed weight. We don't understand how this happens, but I have some ideas. Hopefully, the research funding agencies will continue to support this work. Another unexpected outcome of these experiments was the observation that the yield increased only if it was hot during early seed set. And finally, while the engineered transgenes were expressed in the endosperm, they functioned in a tissue of maternal origin to increase seed number.

Effectively, these transgenes provide as an insurance policy. If it is hot during early seed set, they create yield increases usually in the 15 to 30% range. We actually saw a 68% increase in one trial. And while we could identify consistent relationships with high daily temperatures during early seed set, later it became apparent that the high night temperatures we experience

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in Florida likely play a major role in the transgene yield enhancement effect.

Were these engineered genes "natural", I have no doubt they would already be in farmer's cornfields. The major reason they aren't is due to the cost of genetically modified organism (GMO) regulation. Even though the products of these genes catalyze a reaction already occurring in plants, the transgenes must go through the same expensive deregulation process as genes that create unique products. Hopefully, these rules will change.

Having been in this profession for a while, I am sometimes asked for advice from people starting their career. First, I must say the pace at which plant genomes and RNA transcripts are being deciphered is just amazing. We still fall far short, though, in knowing what all the genes *really* do. Clearly, there is need for lots more research in this area. Unfortunately, this type of research is not "high-output", and consequently it does not get the level of funding and attention it deserves.

The application of molecular and genomic knowledge to real life problems is becoming far more important. A major employment opportunity is plant breeding programs that utilize molecular techniques to track important genes, some of which can be improved by engineering. This might seem ironic, since plant breeders were once dead set against plant biotechnology, as they viewed this approach as competition to their funding and employment.

Advocates for plant genetic enhancement technologies are sorely needed. As a group, plant scientists have not been effective battling with anti-GMO groups. Perhaps this is because we did not appreciate there was money to be made by opposing GMO crops. For example, market share can be increased if you sell non-GMO crops (organic growers) and convince consumers that GMO's are dangerous. Being an effective advocate for genetically engineered plants and foods is challenging. Understanding the science is simply not enough. Effective advocates must be trusted by their audiences and the public. They must be good listeners. And really effective advocates become targets for the anti-GMO groups. For the most part, plant scientists prefer to avoid confrontation and are busy doing their day job; plus they don't want to be targeted by hate groups. Nonetheless, it is important that we do it. In my view, this activity is more challenging than doing the science. Hopefully, the plant community (and university administers) will recognize the importance of effective science communicators and reward those who do it well. It is time.

Looking back, my career has been a fun ride. If I did anything well, it was picking good people with whom to work. The vast majority of our significant findings came from bright, hard-working and clever people who worked in the lab and the field. But by far, the best thing about my career was all the great friends I made along the way.