

# World of Knowledge

## Striving for a Blue Revolution in Plant Science

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### Summary

The combination of plant breeding and increased use of irrigation and fertilizer that dramatically boosted agricultural production over the last half century is often referred to as the “Green Revolution”. However, limitations on water and land availability demand that we find ways to use these resources more efficiently to ensure that the gains of the Green Revolution can be sustainable in the long term. There is great interest in using genomics and biotechnology to produce drought-resistant plants that can be productive using less water. Many hope that this will bring forth a “Blue revolution” in agriculture to build on the “Green Revolution”. However, we still lack the comprehensive understanding needed to rationally design stress-tolerant plants. Here I briefly discuss plant responses to water limitation and our work on proline metabolism in the model plant *Arabidopsis thaliana* to illustrate both the challenges and the research tools we have in working toward the Blue Revolution in plant science.

Many of us have heard of the “Green Revolution”, the convergence of improved varieties of rice, wheat and maize along with increased use of fertilizer, irrigation, and agricultural chemicals that dramatically increased crop yields beginning in the 1960s. The main innovation that launched the Green Revolution was the use of plant breeding to produce varieties of rice and wheat containing dwarfing genes. The shorter, sturdier plants that resulted were able to produce higher grain yields when supplied with inputs of water and fertilizer. The Green Revolution was, and is, one the great technological achievements of the 20<sup>th</sup> century and many believe that it staved off what would otherwise have been an era of widespread famine. At the same time, we now know that the increased use agricultural inputs, particularly irrigation water and chemical fertilizers, that made the Green Revolution possible are not sustainable in the long term (see for example commentary in Mann, 1999). We are also realizing that the future may bring further challenges: water supplies can be threatened by the effects of climate change and the amount of arable land available for crop production is likely to decrease. These factors have led to calls for a “Blue Revolution” to develop plants that can be productive on drier lands or with reduced amounts of irrigation (Pennisi, 2008). If the Blue Revolution is to come, it will be based not only on work with the staple crops of rice, wheat and maize but also on work with a small plant that has become hugely important for plant science.

### Water for carbon: the fundamental compromise of plant life

Managing the exchange of water and CO<sub>2</sub> is the defining compromise in the life of land plants and the availability of water is one of the major factors limiting crop productivity. Plant leaves must take up CO<sub>2</sub> for carbon fixation; however, while CO<sub>2</sub> is entering the leaf, water vapor is being lost from the leaf. Because of the diffusion coefficients of water vapor and CO<sub>2</sub> and their relative concentrations in air, the plant can lose as many as 500 molecules of water for each molecule of CO<sub>2</sub> that is successfully assimilated into reduced carbon. Thus, most of the water taken up by the plant is lost through transpiration while only a very small portion is retained in the plant tissue.

The exchange of CO<sub>2</sub> and water occurs through stomatal pores on the leaf surface, the opening and closing of which is intricately controlled by the plant. When the plant senses a lack of water (drying of the soil), the stomatal pores are closed to conserve water. While this restricts water use, it causes several problems for the plant. The main problem is that CO<sub>2</sub>

in the leaf soon is depleted and carbon assimilation stops. Not only does this restrict the supply of reduced carbon needed for synthesis of other molecules, causing changes in metabolism, it also means that there must be another way, instead of the reduction of CO<sub>2</sub> to carbohydrates, to dissipate the light energy being absorbed by the leaf.

Even when the stomates are closed to conserve water, the plant faces the further possibility that if the soil becomes too dry, water will be drawn back out of the plant tissue into the soil. This possibility can be counteracted by osmoregulation: accumulating extra solutes inside the cells to maintain osmotic balance with the environment. Osmoregulation in plants is very similar to that done by bacteria and yeast when they encounter hyperosmotic growth conditions or by marine organisms that must cope with high levels of salt and high external pressures. In all of these organisms, it is not a general increase of all metabolites that increases cellular solute content. Instead, certain specific compounds are synthesized and accumulated to high levels. These compounds share certain characteristics that are thought to have protective effects such as stabilizing protein structure or detoxification of reactive oxygen and are often referred to as “compatible solutes” (Yancey, 2005). Osmoregulation and synthesis of compatible solutes is another way in which plants must adjust their metabolism to cope with restricted water supply. Both compatible solutes and stomatal regulation have been key targets in attempts to produce drought resistant plants by genetic engineering.

#### **Applying genetics and genomics in the model plant *Arabidopsis thaliana* to understand plant drought resistance: proline metabolism as an example**

In terms of the research challenges involved, it has been said that drought stress is to plant scientists what cancer is to mammalian biologist (see commentary in Pennisi, 2008). Once we get past the surface, what seemed like one big problem is actually a widely varied range of more specific problems involving many genes and molecular pathways. Just as different cancers require different treatments, the factors needed to enhance plant drought tolerance are likely to vary depending on the plant species, the specific environment we want the plant to grow in and what part of the plant is of economic importance (for example seed yield or biomass production). In my laboratory we study stress-responsive changes in metabolism and function of the stress-responsive plant hormone abscisic acid (ABA). We are also part of the global community of plant scientists using the model plant *Arabidopsis thaliana* in our studies. We have a particular interest in the metabolism of the compatible solute proline and this as serves as an apt example of the experimental advantages of *Arabidopsis*.

It has been observed since the 1950s that many plant species accumulate high levels of the compatible solute proline. Several types of environmental stress can cause increases in proline, but the highest levels are observed in drought stressed plants and high levels of proline have been associated with drought resistance (see for example Ben Hassine et al., 2008). It is also likely that adjusting proline metabolism has other, still unclear, benefits for the plant under stress in addition to its role in osmoregulation. Given our cancer analogy above, it is interesting to note that, indeed, proline metabolism in mammalian cells has also been implicated in cancer and proline dehydrogenase, an enzyme in the mitochondria, is regulated in part by the tumor suppressor p53 and linked to apoptosis (see for example Maxwell and Davis, 2000)

As befits sessile organisms that cannot flee from changes in their environment, plants are very facile in changing their growth and metabolism in response to changes in the environment. This can be illustrated in *Arabidopsis*: within 96 hours after transfer to stress conditions, proline content of *Arabidopsis* seedlings can increase by as much as 100-fold. If the seedlings are transferred back to the original, unstressed conditions, proline content decreases back to the original level over a similar time frame. While this basic observation is well known, the questions we are grappling with now are what molecular mechanisms are responsible for proline accumulation, how is it regulated and is altering proline metabolism a feasible strategy to increase drought resistance. The specific experimental advantages of *Arabidopsis* as a model plant can

help to address these questions. These new insights can then be used in experiments with other plants that are economically important but harder to manipulate experimentally.

One of the advantages of *Arabidopsis* is the availability of mutants and easy generation of new mutants. For example, the *Arabidopsis* mutant *aba2-1*, which is deficient in ABA, has only half as much stress-induced proline accumulation as the wild type (non-mutant) plant. ABA is known to accumulate in stressed plants and regulates many processes, such as the stomatal closing mentioned above, that plants use to resist drought. Interestingly, the fact that proline accumulation is not completely blocked in the ABA-deficient mutant means that it is also controlled by ABA-independent stress signaling which is less well understood. The advantage of *Arabidopsis* is that we can now generate more mutants, both by specifically targeting genes and doing screening of randomly mutagenized plants to find genes involved in proline metabolism and the stress perception and signaling mechanisms that control it.

A complementary strategy is to use the natural variation among different strains (ecotypes) of *Arabidopsis*. Traditionally, such an approach has been applied directly to crop plants to select useful variants for plant breeding programs. However, it has recently been found that *Arabidopsis* is an ideal organism this type of quantitative genetic study. *Arabidopsis* is distributed across the northern hemisphere and thousands of ecotypes have been collected from a wide range of natural environments. More than 1000 ecotypes readily available to researchers are currently being analyzed by 250,000 SNP Affymetrix genotyping chips (<http://walnut.usc.edu/2010/SNPs>) to detect polymorphisms throughout the genome. This explosion of genotype information is making new approaches possible (genome wide association mapping) and breathing new life into more traditional approaches (mapping of quantitative trait loci) that seek to link specific genome changes with changes in phenotype. The ecotypes of *Arabidopsis* vary in many traits including drought response (Bouchabke et al., 2008). In our laboratory, a screen of a small number of ecotypes revealed that one called Shahdara (originally collected in Tadjikistan) has a very low level of stress-induced proline. Here, the advantages of the large community working on *Arabidopsis* become apparent: there is already an existing, publically available population of recombinant inbred lines between Shahdara and *Landsberg erecta*, which accumulates five-fold more proline than Shahdara. The existence of an already genotyped set of recombinant inbred lines between parents which differ widely in our trait of interest means that we can directly work on finding quantitative trait loci explaining the variation in proline accumulation between *Landsberg* and Shahdara. The best outcome of this type of study is to find a single gene polymorphism which explains the variation in proline and tells us something about how natural selection has adjusted this trait to maximize fitness in a particular environment. In a crop plant, finding the one polymorphism underlying a QTL is a large undertaking. In *Arabidopsis*, it is a challenge, but the same characteristics of *Arabidopsis* that make it an ideal model for other types of genomic research also make it more feasible to find the gene underlying a QTL and easier to do subsequent experiments to understand the molecular mechanism by which the altered gene causes the change in phenotype.

### **How to build a better plant: many parts but no instructions**

The Green Revolution involved the use of plant breeding to manipulate a relatively small number of genes of unknown molecular function. Indeed, the main “Green Revolution genes” were not cloned until recently (Peng et al., 1999; Sasaki et al., 2002), more than 30 years after their use transformed agriculture. Increasing plant stress tolerance involves complex traits and more genes. A central tenant in most calls for the new “Blue Revolution” is that this time we will know the molecular function of stress resistance genes and use them in a rational way to design improved plants. But, we are not yet at this point and it is still the plant breeders who are making the most progress in the Blue Revolution (see for example commentary in Finkel, 2009). To get to the point where we can speed plant improvement by directly selecting the genes needed to make stress tolerant plants is going require a lot more knowledge about how plants work. Thus to ensure a more

stable future for agriculture, one of the things we need to do is to make use of a small plant which none of us will ever eat.

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