# *Adapting Crops to Climate Change*

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Why do we expend a lot of effort in improving plant responses to sub-optimal environmental conditions? I don't have to go into detail on the growing world population, the need for alternative energy from biofeedstocks, or climate change. At Bayer BioScience, I am responsible for linking our efforts with academic research worldwide. We at Bayer recognize that we have to cooperate with academia to be successful in business because public-sector scientists often cover the early stages in the pipeline of crop development by breeding, including discovery and technology testing of various traits of interest.

Only about 3% of the Earth's surface area is arable land. The breeder's task is to increase productivity of this small area. The other limiting factor is water. Some 70% of global freshwater is used by agriculture. If we can use less water for irrigation and maintain crop yields, we would save resources and enormous amounts of money worldwide.

Our major objective is to close the gap between theoretically attainable crop yields and their actual yields. Abiotic stresses, which reduce attainable yields, can be grouped in terms of genes that are expressed under various stressed conditions, for example heat and drought form a group as do cold, drought and salinity. Drought, which occurs in both groups, has a major effect on plant growth, therefore we need to either adapt or acclimate our crops to resist these stresses.

## Energy a Key Factor

When we first considered how to breed plants with tolerance of various stresses, we thought that, regardless of the stress acting on the plant, energy is needed. This is true not only for plants, but also for humans: if we are stressed, we are likely to run out of energy. Would a plant tolerate stresses in general by maintaining energy homeostasis?

Cotton, for example, may have to face cold early in the growing season, and later drought, heat and then again cold. Genes may be introduced and expressed for each of these stresses individually or, thinking more generically, we were led to consider energy. When we started thinking about stress tolerance at the end of the 1990s, the question was, "Are pathways in the plant switched on in stress conditions that use high amounts of energy, which, in turn, is not available for normal physiological processes like photosynthesis and growth?"

Our research strategy was to maintain energy homeostasis in our crops (cotton, canola and rice). We quickly came to consider the poly(ADP-ribose) polymerase (PARP) pathway, which had been studied in animals and humans, but not in plants. It was not even known whether plants contained PARP-pathway enzymes. PARP is an enzyme that, under stress conditions, modifies nuclear proteins like histones and protects DNA. Studies on animals showed that, regardless of the stress an animal cell has to face, PARP is induced strongly, using a lot of NAD which is a major energy-source cofactor in many biochemical pathways.

In collaboration with scientists at the University in Ghent, PARP genes were discovered in plants and the work was published in the *Proceedings of the National Academy of Sciences* (PNAS) (Babiychuk *et al.*, 1998). We wanted to test the possibility of reducing the expression of PARP under stress, thereby saving energy. We know of other enzymes that do the same, for example glycohydrolases and other nuclear proteins that are linked to one pathway that is well known. In yeast, the NAD-salvage pathway is responsible for rechanneling products made during PARP activity, nicotinamide for example, into the $NAD^+$ pool.

We cloned and identified the plant genes for the NAD-salvage pathway, and over-expressed and down-regulated them, which resulted in a complex picture that we published 2 years ago in PNAS (Vanderauwera *et al.*, 2007). We know now that PARP plays an important role in controlling gene-expression patterns. This is a good example of starting from one idea—down-regulation of PARP to help the plant—and ending up with a network of genes, all of which somehow play a role in the response of plants to stress conditions.

Thus, we had a pool of genes, each linked to PARP, which could be over-expressed or down-regulated to test whether the change in gene expression results in stress tolerance. In microchip arrays, we found that a number of well characterized genes responded to abiotic stress with up-regulation, those regulated by abscisic acid (ABA), for example, which play a role in stomatal control. Although these results were not surprising, this was the first proof that down-regulation of PARP in plants affects gene-expression which, in turn, results in stress tolerance.

## RNA Interference

Down-regulation of genes may be achieved by various technologies. RNA interference (RNAi)—now widely used to control the expression of genes—was employed to modify the activity of PARP-pathway genes. An important advantage of this approach is that it can be highly specific. We can target the region of a gene of interest that shares homologies

with other genes to down-regulate that group of genes, or the regions specific for a single gene may be targeted. Thus, RNAi contrasts with the knockout-mutant approach with which all of the gene product is lost. Often the latter method doesn't work well; some *Arabidopsis* mutants are partially lethal and the plant hardly grows, whereas, with RNAi, gene expression can be dimmed rather than eradicated. We can reduce gene expression to a certain level and even come down from 80% to 60% to 40%.

This works even better with microRNAs. All organisms have genes for microRNAs that control other genes. We isolated natural microRNA genes, replaced the region of homology for controlling the gene and put in our PARP sequence. This is even more specific and works highly efficiently. Alternatively, we used transdominant-negative mutants to change the binding sites for proteins; using a protein that competes with the natural protein, also results in a down-regulation of a given gene product, but not total knock out.

We screen the plants we produce—either by genetic engineering or by mutation—by treating with high light densities, up to 300 µE, which stress plants. We chose high light because it is easy to apply and modify. It's more difficult to apply drought stress, for example. Analysis of our PARP plants confirmed that they saved energy; they had higher levels of ADP and showed less respiration, and so were less stressed.

We are doing field trials with canola, some of which are in Saskatoon. Under drought conditions, the RNAi-PARP plants visibly grow better. They can continue to develop under moisture-deficiency conditions that curtail the growth of wild-type plants.

There's an interesting link between the NAD-salvage pathway and a well known chemical marketed by Bayer, the insecticide imidacloprid. For many years farmers observed that plants treated with imidacloprid are more vigorous and produce more leaf material. So, together with our colleagues in crop protection, we looked for links between the insecticide and our well studied NAD-salvage pathway. We found that the intermediate metabolite, fluoro-nicotinic acid, occurs in the NAD-salvage pathway and in the metabolism of imidacloprid. When we treat RNAi-PARP plants with imidacloprid, there is a combinatorial effect: they are more protected against stresses than are the transgenic plants alone or wild-type plants treated with the insecticide.

These findings have produced a new line of research. Since imidacloprid is a toxic insecticide, farmers should not use it in large quantities as a growth enhancer. However, our chemists in Germany are now looking for compounds of similar structure that are less toxic but retain the growth-enhancing effect.

## Decreasing Photorespiration

Another relevant field is photosynthesis, which is less than optimally efficient because of photorespiration, whereby a third of the fixed carbon is lost. In collaboration with scientists at Aachen University, Germany, we addressed the possibility of decreasing photorespiration, thereby saving energy and improving the plant's resistance to stresses.

We cloned bacterial glycolate dehydrogenase—which converts glycolate to glycerate which then goes into the Calvin cycle—into *Arabidopsis* as a model plant, which grew more vigorously. They had larger leaves, more leaves and, even more importantly, longer roots. We are repeating this with some of our crops.

## Systems Biology

Over the past decade we have dissected plants into small units, the single genes. We now have to put these together again—returning to plant physiology and plant biochemistry—to understand the interaction of all these pieces of the puzzle within the functioning plant. Changing the expression of one gene can result in changes in hundreds or ever thousands of other genes. This is systems biology, which, in my teaching, I like to compare to Sudoku. Changing one number in a stable system forces many other changes in order to achieve stability again. Similarly with genes: a change in the expression of one gene can lead to changes in expression of many others.

Thus plant-systems biology enables mathematic modeling of dynamic networks that underpin crop productivity and sustainability, and mathematics is becoming an important part of biology.

With the increasing speed of development of new technologies, DNA sequencing is getting faster and faster. In a few years, it will be easier to sequence a plant genome directly than than to draw up genetic maps, thus facilitating the systems-biology approach.

## Epigenetics

As every breeder knows, regardless of whether classical or transgenic methods are used, good germplasm is a fundamental requirement, *i.e.* with broad variation in traits. Also, every breeder understands the potential role of genetic engineering, which will continue to be used in selected cases. Increasingly important is expression engineering, which includes manipulating a gene to be expressed constitutively in all tissue all of the time, using appropriate promoters. One of the most useful, in this regard, has been the 35-S promoter from cauliflower mosaic virus; however, it is now known that this approach doesn't work well for some new traits. Instead, we need to express genes in specific tissues at specific time points, and not all the time (which worked well for achieving insect tolerance from *Bt* genes). For traits like stress tolerance and improved yield, we need to be more cautious and more precisely modulate gene expression.

This is where epigenetics comes in—everything above the DNA level influencing gene expression, including well studied biochemical pathways such as histone acetylation and de-acetylation, and methylation and de-methylation of DNA, which result in changes in expression patterns.

Epigenetics may be illustrated in terms of two symphony orchestras with the same instruments playing the same piece. However, they may sound different because, for example, a trumpet player had a bad night and is playing too loudly. In other words, the music sounds different due to the change in the expression of one of the components in one of the orchestras. We can go further and say that the trumpet player's colleagues may try to compensate and play louder so that his bad playing is concealed. Although a simplification, this, indeed, happens in plants and other organisms. If the expression of one gene is changed then other genes may "compensate," to help stabilize the system.

My colleague, Mark De Block, took canola plants of a particular variety and grew them under stress and non-stress conditions and separated good performers (low respiration rate) from bad performers (high respiration rate) over several generations, producing a

population with higher energy-use efficiency under stress conditions. When he had a closer look at what had happened, he found that he had selected epigenetic variants, not mutants, with DNA methylation changes that correlate with good and bad performance. These changes occurred in coding regions of genes involved in stress response. This is opening a new approach to breeding stress-tolerant plants. De Block went a step farther and combined epigenetic variants with hybrid lines. Heterosis resulted in more leaf material, and better growth under a range of stress conditions.

## Second Green Revolution

We need a second Green Revolution. We now have tools in hand for a deeper holistic approach to plant breeding, looking at plants as a whole again rather than dissecting them into small pieces.

## References

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**FROM 1975** to 1980, Michael Metzlaff studied biology at the Martin-Luther University, Halle-Wittenberg, Germany, specializing in plant genetics. In 1983, he graduated with a PhD from the Institute of Genetics at the same university, researching chloroplast DNA modification.

Throughout the 1980s and the early 1990s, Dr. Metzlaff taught plant molecular genetics and genetic engineering. From 1993 to 1999 he was a senior scientist at the John Innes Centre in Norwich, UK, where his team elucidated gene-silencing mechanisms in plants. In 1999, he moved to Belgium to take up a senior scientist position at the biotech company Plant Genetic Systems in Ghent, which, in 2002, became the Innovation Centre of Bayer CropScience-BioScience. As crop-productivity group leader, with a steadily growing team of researchers and scientists, he resumed his research on gene silencing/RNAi/epigenetics and initiated studies on abiotic-stress-response mechanisms with the objective of improving stress tolerance in major crops. In 2008 he became the research liaison manager at Bayer BioScience, coordinating global research including joint efforts with leading academic universities and research institutions.

He has authored over fifty scientific publications and holds a number of patents.