

Abiotic Stress in Rice. An "Omic" Approach

Abiotic stress can impose limitations on crop productivity and also limit land available for farming, often in regions that can ill afford such constraints, thus highlighting a greater need for understanding how plants respond to adverse conditions with the hope of improving tolerance of plants to environmental stress. More is becoming known about the physiological and molecular effects of environmental stress, and many groups are turning to "omic" studies with the goal of identifying the genes involved and their expression patterns during the course of stress perception and response. If analyzed properly, the information gleaned from these studies will help identify the interactions between signaling pathways, along with novel cis-acting elements, in the promoters of coregulated genes (Provart and McCourt, 2004). With the goal of initiating such a study, the laboratory of Kazuko Yamaguchi-Shinozaki analyzed the transcriptome of whole rice (*Oryza sativa*) seedlings exposed to cold, drought, high salinity, or application of abscisic acid (ABA) in their work titled "Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and ABA application using cDNA microarray and RNA get-blot analyses." This article appeared in our December 2003 issue and as of March had 44 citations.

BACKGROUND

As sessile organisms, plants cannot physically move away from environmental stresses that can negatively affect growth. Therefore, plants have had to evolve strategies to cope with abiotic stress. Indeed, the ability to respond and ultimately adapt to abiotic stress may be a driving force in speciation (for review, see Lexer and Fay, 2005).

Plants must be able to "sense" the environmental cues before being able to respond appropriately to the abiotic stress. Due to the complex nature of stress, multiple sensors, rather than a single sensor, are more likely to be responsible for perception of the stress. After the initial recognition of the stress, a signal transduction cascade is invoked. Secondary messengers relay the signal, ultimately activating stress-responsive genes generating the initial stress response. Stress-induced gene products can be divided into two major groups: those involved in stress tolerance and those involved in signal transduction. Stress-tolerance genes enable plants to cope with the stress situation, in terms of both short- and long-term responses. These can include the synthesis of chaperones and enzymes for osmolyte biosynthesis and detoxification, to a

change in the composition of membrane lipids as is found with cold stress. Gene products can also act as transcription regulators controlling sets of stress-specific genes or be involved in the production of regulatory molecules, such as the plant hormone ABA.

It has been demonstrated that multiple signaling pathways can be activated during exposure to stress, leading to similar responses to different triggers. For example, drought, low temperature, and high salinity, three common abiotic stresses, all cause an accumulation of compatible solutes and antioxidants (Hasegawa et al., 2000). Studies such as this one by Rabbani et al. (2003) have also demonstrated the overlap in gene expression between environmental stresses.

WHAT WAS SHOWN

In their study, Rabbani et al. (2003) used microarray analysis to determine rice genes induced by the stresses of cold, drought, high salinity, and by the hormone ABA, a regulatory molecule generated by stress. The accuracy of the transcriptome data was confirmed by RNA gel-blot analysis of candidate genes, narrowing it to 73 genes up-regulated by exposure to the abiotic stress. Of these, 20% are already known to be responders to abiotic stress in rice, confirming their up-regulation during stress exposure. Among these were those involved in stress tolerance, such as compatible solutes and proteins involved in carbohydrate metabolism and detoxification, and those involved in regulation, such as transcription factors. Additionally, a gene involved in the biosynthesis of ABA was also identified. This is interesting, as ABA is known to be a regulatory molecule in stress response and functions to regulate plant water balance through guard cells as well as regulate osmotic stress tolerance via cellular dehydration tolerance genes. The remaining 80% have not previously been reported as stress responders in rice.

One objective of this experiment was to identify cis-acting elements involved in stress-inducible gene expression. These regulatory elements interact with transcription factors and act as molecular "switches" ensuring an appropriate response (in this instance, to environmental stress). In *Arabidopsis* (*Arabidopsis thaliana*), for example, the dehydration-responsive element has been identified as a cis-acting promoter element and has been implicated in regulating gene expression under drought, high-salinity, and cold stress. To determine how many of the candidate rice genes contained a dehydration-responsive element or an ABA-responsive element, the 5'-end sequences were compared to rice genomic sequences in both the GenBank and Rice Genome Project databases in order to obtain the complete coding sequence. The complete genomic sequences of 95% of the identified stress-responsive genes were

present in the databases, allowing analysis of the promoter regions. Of these, almost 40% contained a dehydration-responsive element or ABA-responsive elements in their promoter region. The authors interpreted the lack of either element in the remaining 60% to suggest the presence of novel cis-acting elements.

Cross talk between the abiotic-stress responses was also explored, revealing a high degree of similarity. Specifically, drought and salt stress were found to induce many of the same genes as did drought stress and ABA application. The similarity between drought and high-salinity response in rice also has been observed in *Arabidopsis*. Plant response to drought and salt stress is very similar, with both disrupting the ion and osmotic homeostasis of the plant, thus the signaling pathways would be expected to be similar. Both drought and salt stress result in increase in ABA expression, known to be involved in the tolerance of osmotic stress and regulation of plant water balance, where it is involved in guard cell regulation. This is illustrated in ABA-deficient mutants that grow relatively normally unless they are exposed to water stress. Under drought conditions, these plants rapidly wilt and will die if the stress remains. Contrary to what was seen with drought and high salinity, the overlap between the cold and high-salinity stress responses or cold and ABA application was not that high.

THE IMPACT

NAC domain-containing proteins are members of a large family of plant-specific transcription factors, of which only a small fraction has assigned function. These functions are diverse and are believed to be involved in defense, development, and abiotic-stress response (for review, see Olsen et al., 2005). Nogueira et al. (2005) identified 26 nonredundant NAC genes from sugarcane (*Saccharum officinarum*), including *SsNAC23*, which was previously shown to be up-regulated by cold (Nogueira et al., 2003). Molecular modeling of *SsNAC23* predicts it to contain a basic amino acid-rich protrusion, a conserved characteristic of NAC domain transcription factors, while transient β -glucuronidase fusion studies suggest targeting to the nucleus. Taken together, this supports the idea of *SsNAC23* being a transcription factor. *SsNAC23* is orthologous to rice *OsNAC6* (Kikuchi et al., 2000) and is strongly induced by extreme chilling stress and transiently by water stress and herbivory. In rice, *NAC6* expression was found to increase slowly after cold exposure (Rabbani et al., 2003). The induction of *SsNAC* by these stresses is in line with the known function of NAC domain-containing proteins. Osmocant-induced water stress also induced expression similar to what was observed with *OsNAC6* (Rabbani et al., 2003), but, unlike what was observed in rice, it did not respond to exogenous application of ABA.

Basic region/Leu zipper (bZIP) proteins are a family of transcription factors that, as their name implies, contain a region of basic amino acids followed by a

region containing at least three to four repeats of Leu or another hydrophobic amino acid. The hydrophobic region mediates homodimer formation, whereas the basic area is involved in DNA binding, usually to an ACGT sequence. A notable exception is the oncogene Fos, which does not bind to DNA but rather binds to another bZIP protein (Jun) to produce heterodimers. The cold-induced plant bZIP protein LIP19 (Aguan et al., 1993; Rabbani et al., 2003) was found to be similar to Fos in that it also does not form a homodimer and cannot bind to DNA. Shimizu et al. (2005) characterized rice LIP19 and identified its binding partner as *OsOBF1*, a new bZIP protein. Unlike LIP19, *OsOBF1* is abundant at normal growth temperatures, and has the ability to bind DNA and to form homodimers. This group proposed a model of how these two bZIP proteins, despite having opposite responses to cold, interact in low-temperature signaling. Under permissive temperatures, *OsOBF1* forms homodimers and binds to a specific DNA sequence. Upon exposure to low temperature, *OsOBF1* is down-regulated, while LIP19 is up-regulated and binds to the available *OsOBF1* protein. In the transcriptome profile by Rabbani et al. (2003), LIP19 expression, like that of *NAC6*, increased gradually after exposure to cold. This heterodimer then binds to yet unidentified promoter sequences different than what *OsOBF1* binds to, altering gene expression during the chilling episode. LIP19 is unstable at growth temperatures; thus, once returned to warmer temperatures, the genes activated by the heterodimer will no longer be activated. After *OsOBF1* is again expressed, those genes regulated by the *OsOBF1* homodimer will be activated. This is an interesting idea that is currently being explored by this group.

CONCLUSION

The transcriptome profiling of rice seedlings by Rabbani et al. (2003) has added to the foundation necessary for further understanding of how plants respond to abiotic stress. As brought up in this article, transcriptome profiling provides the ability to identify novel cis-acting elements and elements acting in tandem. As this field is further advanced, the accuracy of transcript profiling will become increasingly exact, allowing the pattern of the complex web of signaling to be uncovered.

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