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The Role of an Absisic Acid Activated Protein Kinase in Drought Tolerance

Drought is the major environmental factor limiting crop productivity. Therefore, there is a great need for crop improvements that will increase drought tolerance and stabilize crop yield under drought conditions. When it comes to mediating the adaptation of the plant to drought stress, Abscisic acid is an important hormone. The phytohormone abscisic acid mediates drought and other stress responses. When plants are subjected to drought, the concentration of absisic acid in the leaves begin to increase. Increased abscisic acid then causes the stomata (minute aperture structures in the surface of the leaf to allow water vapor to move out of the leaf) to close, which enables plants to reduce water loss under drought conditions. Protein phosphorylation catalyzed by protein kinases is a major mechanism of signal transduction in animal and plant cells. Plants have more than twice as many protein kinase genes than mammals, which may allow plants to more efficiently respond and adapt to their constantly changing environment. Previously we have identified an abscisic acid activated protein kinase (AAPK) in fava beans and have shown that AAPK is a positive regulator for abscisic acid-mediated stomatal closure. The aim of this project was to determine the role of a rice AAPK- like protein kinase in plant responses to drought stress.

Along with AAPK being a positive regulator in stomatal closure, we have also previously found that it is a positive regulator that can enhance abscisic acid signaling. To this end, we have generated overexpression line of SAPK10 (an AAPK homolog gene in rice). Analysis of SAPK10 overexpression lines shows that these transgenic plants exhibit enhanced drought tolerance, suggesting that the rice SAPK10 is a positive regulator of drought tolerance.

All of the rice plants used in this project was grown in a greenhouse under natural light conditions. The control and SAPK10-overexpressing plants were grown in pots that contained the same weight of soil. The same amount of water was applied to the two pots. Once the plants were each fully

grown, I took measurements of the total height of the plant, the flag, panicle, and 1st, 2nd, and 3rd nodes. I measured them in centimeters as accurate as I could possibly get it. The original measurements of these plants were so that we could tell a growth difference in the plants that had plenty of water and of plants that we were testing the drought tolerance of. I had a total of seventeen wild type plants and a total of thirteen genetically produced plants, SAPK10. Once all the measurements were taken, I entered them in to Microsoft Excel so that I could figure out what the standard deviation was of the wild type plant (WT) and the SAPK10 plant. In more simple terms, the standard deviation is the average of the measurements of all the plants. When I measured the plants, there was room for error because I couldn't be 100% accurate, which is why figuring out the standard deviation helped me to get a more overall accurate average. In the rice plants, total RNA was isolated from different tissues of rice plants using TRIzol Reagent (Invitogen). Complementary DNA (cDNA) was synthesized from the DNase-treated RNA using SuperScript II reverse transcriptase (Invitogen). Polymerase chain reaction (PCR) amplification of the cDNA was performed using gene specific primers. The constitutively expressed rice actin-1 was used as a control for RT (reverse transcriptase)-PCR. Real-time quantitative PCR was performed using the fluorescent dye SYBR Green 1 on a LightCycler 2.0 System (Roche) according to the manufacturer's instructions. All real-time PCR reactions were performed in triplicate. The complete coding sequence of the SAPK10 gene was amplified from rice leaf cDNA by PCR and cloned into the overexpression vector pU1301. Transgenic rice plants carrying the construct for overexpression of SAPK10 were generated by *Agrobacterium*-mediated transformation of rice calli. Once all of this was finished, the next step was to get measurements of the stomata of the leaf. This turned out to be a very difficult task because we didn't realize until we started just how small rice stomata are. Even under a max zoom microscope, the rice stomata are almost too small to get an accurate reading of. However, before we could examine the stomata of the leaf, we had to first remove the leaf peel (green layer on the outside of leaf) so that we could get a better visual of the leaf. This also turned out to be very tricky

because there is really no good way to do it. At first I tried cutting the leaf in to small sections and putting it in a grinder to remove the leaf peel. This only worked so well and by doing this, the stomata of the rice plants were near impossible to see and could not get an accurate reading off of them. We did some research and found a better method of looking at the stomata by using quick dry glue. We took a drop of clear quick dry glue, and applied in directly to the plant while the leaf was still attached. Once the glue was spread out evenly on the plant, I took a glass cover we use with a microscope and pressed it against the glue and rice plant. I waited roughly 45 seconds to a minute for the glue to dry, and then slowly peeled the glass cover off with the glue stuck to the glass cover. What this did was it basically took a microscopic picture of the rice stomata using the glue. When put under the microscope, it was much easier to get a clearer picture and a more accurate measurement. This then allowed us to be able to measure the maximum opening of the rice stomata. So far throughout the entire project, that has seemed to be the hardest part. Like I said before, rice stomata are much smaller than that of other plants, so it was difficult for us to be able to see whether or not they were open or not when we looked at them through a microscope. Thus the "picture" taken with the glue, was a much better approach to being able to see and record the data that we needed. This seemingly simple step had to be done so that when we were testing the drought tolerance of the plants, we would be able to see the difference in measurements between the separate stomata's.

To further explore the function of abscisic acid-activated protein kinases (AAPK) in rice, we searched the rice genome for proteins with sequence similarities with the fava bean AAPK. We identified an AAPK homolog gene named SAPK10 in rice that encodes a serine/threonine protein kinase phylogenetically related to the fava bean AAPK. SAPK10 was found to be expressed in various rice tissues. The housekeeping gene actin acted as internal positive control for all PCRs from three separate plants. SAPK10's transcript levels were also higher in leaves and seeds than in the stem, panicle and roots, suggesting that SAPK10 may play an important role in leaf and seed biology. To uncover the

function of SAPK10, we generated overexpression lines of SAPK10 in rice. We obtained six independent transgenic lines that showed much higher expression of SAPK10 than control plants. All the SAPK10-overexpressing plants displayed reduced stature. Compared to the control plants containing the empty vector, the SAPK10- overexpressing plants have shorter flag leaf and internodes. The SAPK10-overexpressing plants displayed enhanced drought tolerance, suggesting that the rice SAPK10 is a positive regulator of drought tolerance. Further studies are warranted on the molecular mechanisms of drought tolerance in plants.

In conclusion, the abscisic acid-activated protein kinase SAPK10 is a positive regulator of drought tolerance in rice. After eight days without watering the plants, the control plant exhibited leaf rolling and wilting, a characteristic response of rice plants to drought. Therefore, SAPK10 is a positive regulator. However, we still have further testing to do with new rice plants that we have grown in the green house. Same conditions, but measurements and testing must be done on them as well to confirm our findings. In these further studies, the target proteins of the SAPK10 kinase are going to be identified within the rice plants.