Case Study

Investigating the Role of Alternative Splicing for Rice with the M4 TORNADO Micro-XRF Spectrometer

Determing ways to breed crops with a higher nutrient-use efficiency

Available mineral nutrients are essential for plant growth and development. However, many agricultural sites do not have adequate amounts of required minerals for optimal growth which adversely effects crop productivity. Consequently, the use of fertilizers to enhance growth has been common for over one hundred and fifty years. In addition to an increase in production costs, the widespread use of chemical fertilizers has resulted in environmental and ecological problems worldwide.

Regenerative agriculture practices are used to increase the crop productivity without sacrificing the environment or ecological systems. One area of research for this is determining ways to breed crops with a higher nutrient-use efficiency, either by conventional approaches or through genetic engineering. Since plants have evolved flexible and complex regulatory systems which allow them to adapt to changes in soil nutrient availability, researchers first wanted to understand the adaptive strategies used by plants to withstand a deficiency or excess of a specific nutrient.

Alternative splicing (AS) is a mechanism that plays important roles in a wide range of biological processes in plants. AS mechanisms are finely regulated by a group of splicing factors called serine/arginine-rich (SR) proteins which appear to function in plant responses to environmental stress.

How did the M4 TORNADO help?

Collaborating research scientists at Nanjing Agricultural University, Nanjing, China and La Trobe University, Melbourne, Australia chose rice shoots to analyze the role of SR proteins in the alternative splicing method. They generated mutants of SR genes of the rice family to determine if they had a regulatory role in mineral uptake and mobilization in rice shoots.

The research scientists performed whole transcriptome RNA sequencing of rice (Oryza sativa) roots that were grown in either the presence or the absence of various mineral nutrients including iron (Fe), zinc (Zn), copper (Cu), manganese (Mn), and phosphorus (P). The M4 TORNADO micro-XRF spectrometer is capable of simultaneous, multi-element analysis from carbon to americium.

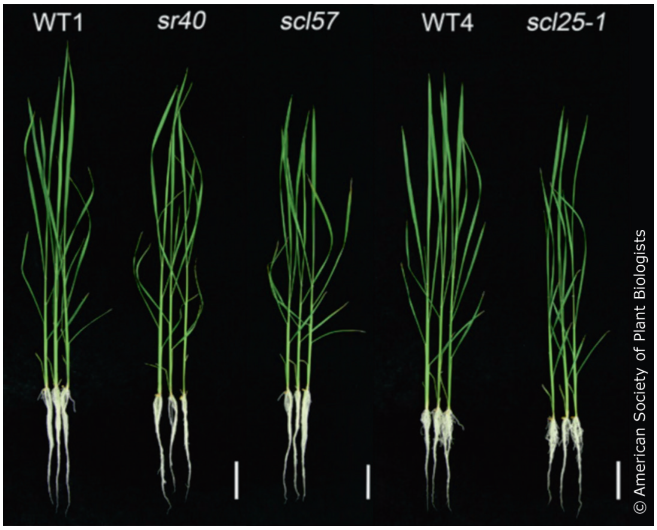

Fig. 1 Photographs of 24-day-old mutant phenotypes sr40, scl57, and scl25-1 rice seedlings and their corresponding wild type WT1 and WT4 plants grown in solution with excess phosphorus.[1]

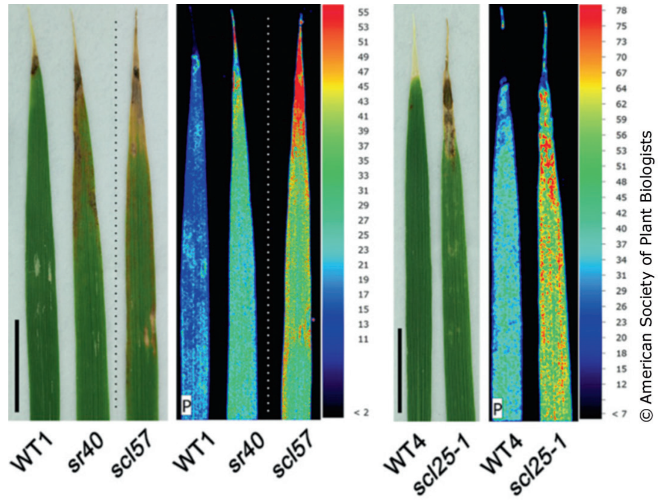

Fig. 2 Photographs and phosphorus distribution maps of the mutant and wild type plant leaves with relative abundance of concentration according to the color scale at the right.[1]

[1] Alternative Splicing Plays a Critical Role in Maintaining Mineral Nutrient Homeostasis in Rice (*Oryza sativa*), C. Dong, F. He, O. Berkowitz, J. Liu, P. Cao, M. Tang, H. Shi, W. Wang, Q. Li, Z. Shen, J. Whelan and L. Zheng, *Plant Cell* 2018, 30, 2267 - 2285, DOI 10.1105/tpc.18.00051.

Samples can be measured in high resolution with a spot size < 20 µm. The M4 TORNADO can be used to create quantifiable elemental distribution maps for a variety of sample types with little to no preparation; hence, it was ideal for this study.

Identifying proteins as mineral nutrient regulators in rice

When the seedlings were grown in control conditions, all three mutants sr40, scl57, and scl25-1 showed a significantly lower shoot height and biomass as well as leaf tip necrosis when compared with the wild type WT1 and WT4 (see Fig. 1).

To confirm that necrotic spots in the leaves only arise under excessive accumulation of phosphorus, the phosphorus distribution in selected leaves was analyzed with micro-XRF scanning at high resolution using the M4 TORNADO. It was found that the three mutants sr40, scl57, and scl25-1 accumulated more phosphorus, especially in the region of the necrotic spots than did the wild type (see Fig. 2).

It was proven that the targets of alternative splicing (AS) are highly nutrient-specific. The research scientists verified the role of AS in mineral nutrition by characterizing mutants in genes encoding SR proteins that function in AS. They showed that three SR protein-encoding genes regulate the phosphorus uptake and remobilization between leaves and shoots of rice, demonstrating that AS has a key role in regulating the mineral nutrient homeostasis in rice.

"The mineral nutrient distribution maps of phosphorus in the rice plant leaves confirm that the necrotic spots in leaves of the mutants were caused by the accumulation of excess phosphorus. This information provides a piece of the puzzle concerning the role of AS in maintaining mineral nutrient homeostasis in plants."
stated Professor Luqing Zheng at the College of Life Sciences, Nanjing Agricultural University, Nanjing, China

Bruker Nano GmbH

Berlin · Germany
Phone +49 (30) 670990-0
Fax +49 (30) 670990-30
info.bna@bruker.com

www.bruker.com/m4tornado