

# Effects of photosynthetic variations on the grain content of iron (Fe) and zinc (Zn)

## Background

Dietary deficiencies in Fe and Zn affect rural and urban communities with limited access to a diverse diet. Biofortification of rice comprises a potentially sustainable approach for alleviating these deficiencies as part of existing interventions (diverse diet, supplementation, and food fortification). Recently, we reported on a biofortified rice line grown under field conditions that satisfies the prerequisites to meet approximately 30% of the estimated average requirements.<sup>1</sup>

To assess the effect of a possible reduction in photosynthesis due to climate change on grain micronutrient content, agronomic characteristics, and gene expression profiling of selected candidate gene events, our Seed Grant proposed to study three high-Fe/-Zn lines grown under different irradiances in a biosafety screenhouse setting. Overall, the study tested the effect of climate on the nutrient content of grain, specifically the resilience of new transgenic lines that have high Fe and Zn content in the polished grain.



## What was done

Previously published work<sup>2</sup> and preliminary results from our studies in high-CO<sub>2</sub> chambers of non-GM lines indicated a reduction in Fe and Zn content in polished grains.

To test the effect of climate on grain nutrient content, we grew selected high-Fe/-Zn lines along with their azygous and wild-type controls under different irradiances (0%, 50%, and 75% shading) in screenhouse conditions during the 2017 wet season in the Philippines. For each of the respective irradiances, 20 homozygous T3 plants per event were included (see images for the experimental setup). Measurements, including determination of biomass and leaf or canopy nitrogen distribution, leaf gas exchange measurements, instantaneous measurements of leaf photosynthesis, determination of chlorophyll content, and determination of leaf chlorophyll and Rubisco content, etc., were conducted. Leaves at the vegetative stage from selected events were collected for subsequent gene expression (RNA seq) analysis. The agronomic performance (including plant height, number of panicles to be harvested, number/weight of field seeds per plant, etc.) of the transgenic events grown under the different irradiance conditions was assessed. Last but not least, the Fe and Zn content of harvested polished grains was determined through inductively coupled plasma



## Partners

The study was primarily performed in collaboration with Dr. Hsiang-Chun Lin, postdoctoral fellow in the C<sub>4</sub> Rice Center, who designed and oversaw the photosynthesis-related experiments. Dr. William Paul Quick, head of the C<sub>4</sub> Rice Center, and Dr. Inez Slamet-Loedin, head of the Genetic Transformation Laboratory, participated in the planning of the study and supervised its overall progress. In addition, several individuals from the two laboratories contributed at various stages of this study and assisted with the performance and data collection as in-kind contributions of the respective heads of the laboratories.



## Results and Lessons Learned

Preliminary analysis of the results from the study indicates differences in the agronomic characteristics as well as the Fe and Zn content of the harvested polished grains between the different biofortified high-Fe/-Zn lines grown under different irradiances. However, a more thorough investigation of the results is underway to determine the extent of these differences and to suggest potential reasons why this is occurring. Moreover, an overlay of these data with the photosynthesis- and gene expression-related results will enable a more holistic and detailed overview of the reasons underpinning these changes.

## Next Steps

It has been shown that the Fe, Zn, and protein contents of polished rice grains are negatively affected under elevated CO<sub>2</sub> conditions.<sup>2</sup> By modulating the irradiance levels under which the biofortified high-Fe/-Zn lines were exposed, the team aims to investigate how climatic factors affect the nutrient content of grain, especially in the new biofortified varieties being developed at IRRI with high Fe/Zn content. Such information will have a major impact in deciding the geographic locations for maximum impact and mitigating the effects of future climate change. In this respect, the study enabled the collection of novel data on the effect of climate on grain nutrient content and related data on the gene networks affected that underpins these changes. Hence, this study has provided and will continue to give novel data on a fundamental question that addresses the link between grain quality and environment that is poorly understood. In combination with earlier preliminary data, it will also provide data for a significant publication.

<sup>1</sup>Trijatmiko, K. R., Dueñas, C., Tsakirpaloglou, N., Torrizo, L., Arines, F. M., Adeva, C., et al. (2016). Biofortified indica rice attains iron and zinc nutrition dietary targets in the field. *Sci. Rep.* 6, 19792. doi:10.1038/srep19792.

<sup>2</sup>Myers, S. S., Zanobetti, A., Kloog, I., Huybers, P., Leakey, A. D. B., Bloom, A. J., et al. (2014). Increasing CO<sub>2</sub> threatens human nutrition. *Nature*. doi:10.1038/nature13179.