Antioxidant Activities and Cold Stress Phenotypes of 370 *Oryza sativa* Cultivars

Huy Phan, Michael Schläppi
Department of Biological Sciences, Marquette University

Introduction

Low temperature stress in crops endangers the global crop production every year. Low temperature induces the accumulation of H_2O_2, which is detrimental and lethal to plant cells. Together with other undesired effects on plant physiology, this leads to a significant yield loss due to chilling stress. As global population grows and climate fluctuates, it is crucial improve the ability to cold temperatures. To do so, it is important to understand pathways and mechanisms the plant uses for cold stress responses.

Oryza sativa, or Asian rice, is a great model to study cold stress tolerance in crops. Rice is largely cultivated as a staple grain for half the world population. Yet, growing rice in high altitude regions faces a critical challenge: chilling condition. Depending on genetic backgrounds, different rice cultivars can have different tolerance against low temperature. For example, cultivars from *Oryza sativa INDICA* sub-species, containing three subgroups: *aus*, *indica*, and *INDICA-mixture*, is annotated as cold sensitive. Meanwhile, *temperate japonica, tropical japonica, and JAPONICA-mixture* cultivars, members of *Oryza sativa JAPONICA* sub-species are annotated as cold tolerant. Similarly, *aromatic japonica* and *admixture* cultivars between two sub-species are thought to be intermediately cold tolerant. Taken together, this diversity in cold tolerance in Asian rice makes it a promising crop system to learn about cold stress responses in order to aid in the development of crops for better tolerance against unstable climate.

Objectives

The objectives of this study include determining the antioxidant activities of 370 cultivars under cold treatment, as well as membrane damage and low temperature seedling survivability. Additionally, correlations between antioxidant activities and membrane damage and survivability are also determined to further understand whether the ability to turnover H_2O_2 contributes to the overall survivability of the plant under low temperature.

Materials and Methods

USDA Rice Diversity Panel 1

A smaller subset of the Rice Diversity Panel 1 (RDP1), containing 370 cultivars, was utilized in this study to investigate the catalase and anthocyanin activities, membrane damage, and low temperature seedling survivability of 2-week-old seedlings after a 7-day-10°C treatment. The germplasm has representatives from all eight sub-groups of Asian rice.

Experimental Design

Seeds were germinated in 37°C. Germinated seedlings were grown hydroponically in triplicate in a randomized block design at 28°C/25°C with a 12-hour photoperiod. Assasys for catalase activity, anthocyanin activity, and membrane damage were done at the end of the stress, while thelow temperature seedling survivability was assayed after a 7-day recovery period.

Catalase and Anthocyanin Activities

Tissues for catalase and anthocyanin activities were ground with liquid nitrogen. Assay for catalase activity was done accordingly to Shi et. al. 2020 with phosphate buffer and 1% H_2O_2 solution. Assay for anthocyanin activity was done accordingly to Shi et. al. 2020 with methanol:water:trifluoroacetic acid extraction buffer and DPPH solution.

Electrolyte Leakage

Leaf samples were cut from live plants after cold treatment and shaken for 60 minutes. Conductivity was measured after shaking and boiling for initial and total conductivity measurement, similar to Shimoyama et.

Low Temperature Seedling Survivability

The percentage of living seedlings after cold treatment were determined by observation after a 7-day recovery period.

Results

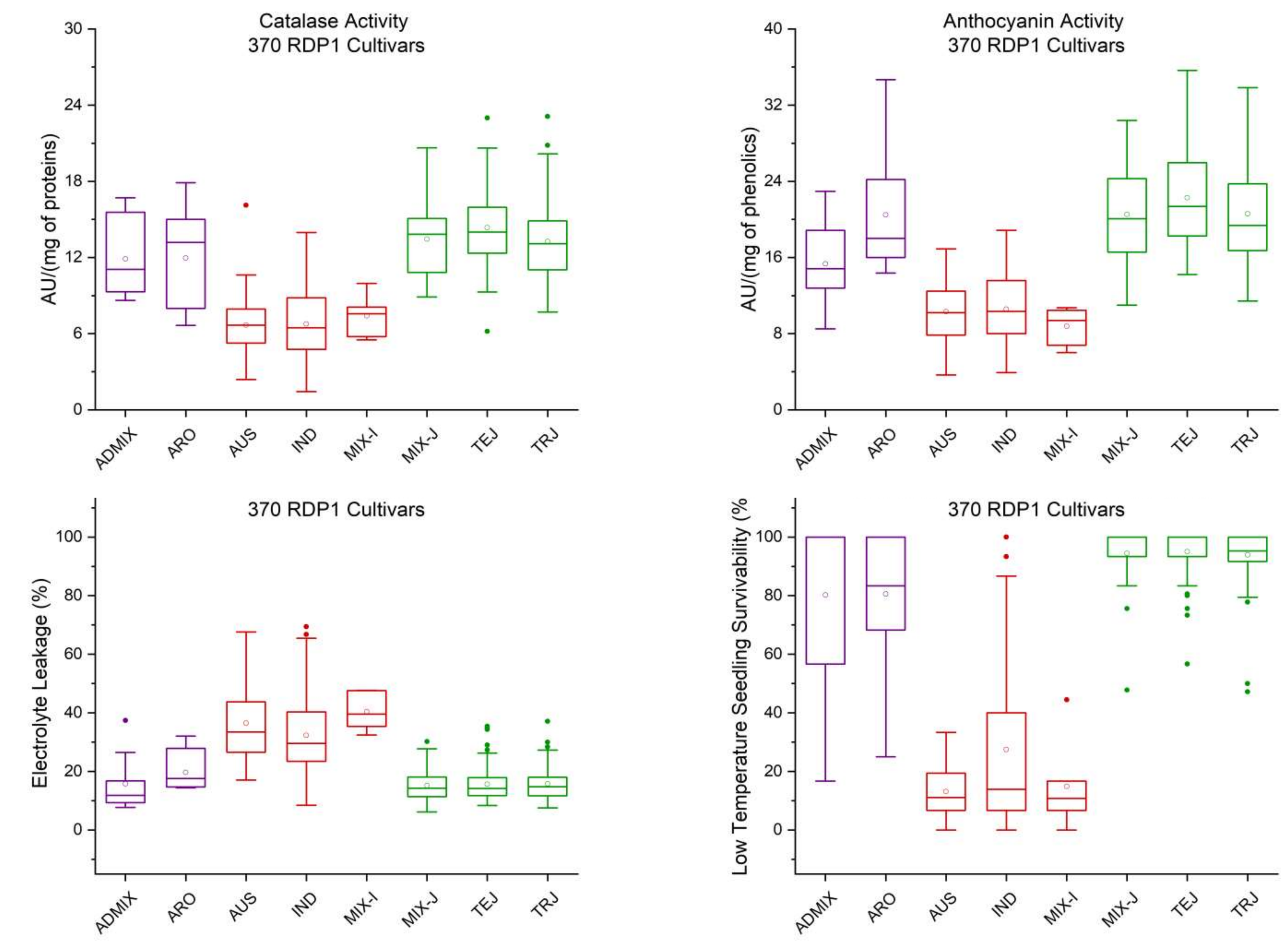

Figure 1. Phenotypes of 370 RDP1 cultivars after a 7-day-10°C treatment. (Top left) catalase activity (top left), anthocyanin activity (top right), and electrolyte leakage (bottom left) were done at the end of the treatment; low temperature seedling survivability (bottom right) was done after 7 days recovery. (Violet) intermediately cold tolerant; (Red) cold sensitive; (Green) cold tolerant.

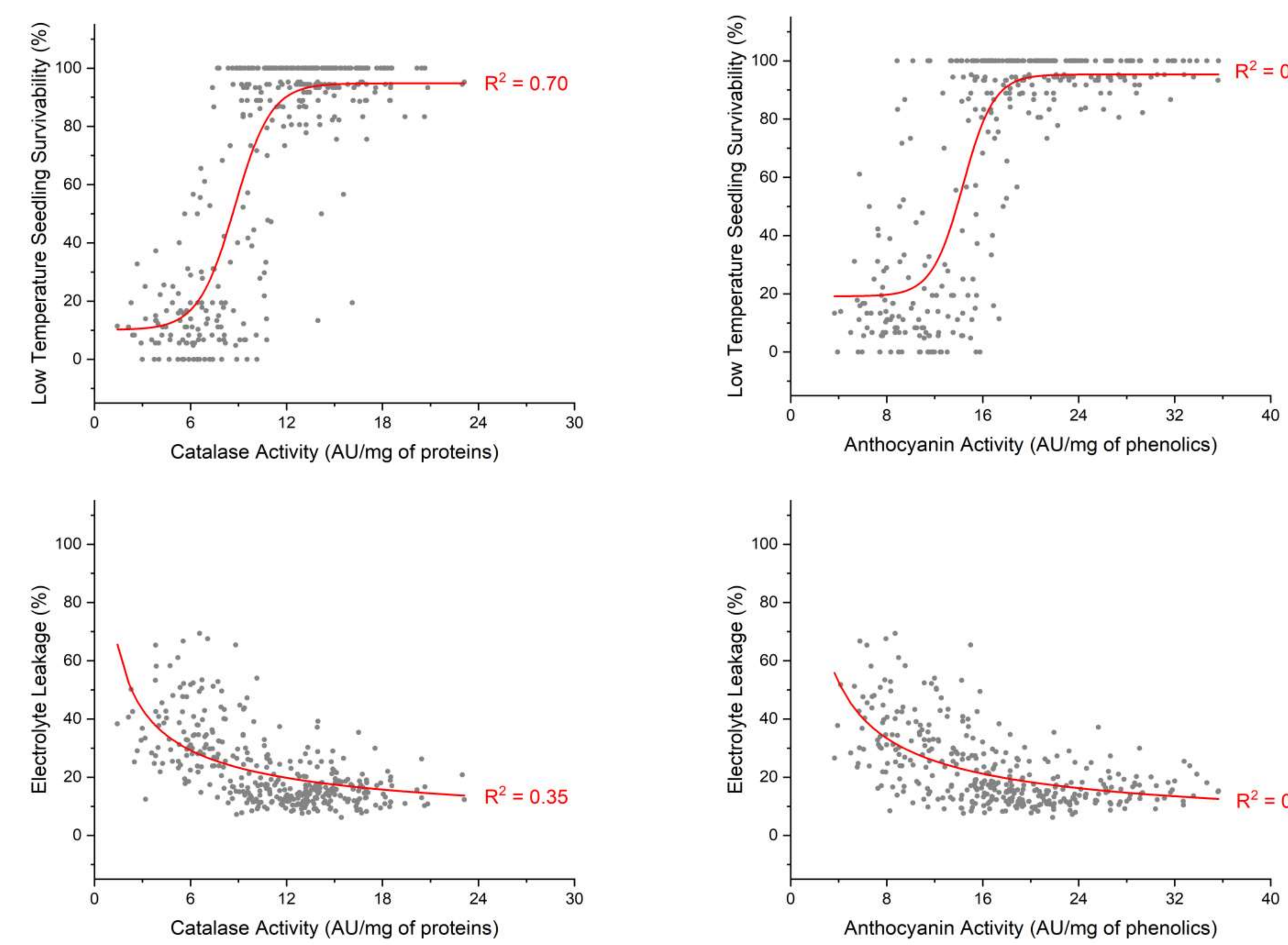

Figure 2. Correlations of catalase and anthocyanin activities against electrolyte leakage and low temperature seedling survivability. R2-value was calculated based on best fit models (Boltzmann for top panels; non-linear allometric for bottom panels).

Conclusions

- It seems to be certain plateau thresholds in correlations between catalase and anthocyanin activities with membrane damage and seedling survivability, around 10 (AU/mg of proteins) for catalase activity and 15 – 16 (AU/mg of phenolics) for anthocyanin activities
- Cultivars with high capability to turnover H_2O_2 (high catalase and anthocyanin activities) at the end of the cold treatment tend to have lower membrane damage (electrolyte leakage) and high survivability
- Cultivars possess catalase and anthocyanin activities less than these thresholds seem to have a more variable membrane damage and survivability
- Intermediately cold tolerant cultivars (*admixture* and *aromatic japonica*) have their phenotypes spread somewhat in between cold sensitive cultivars (*aus*, *indica*, and *INDICA-mixture*) and cold tolerant cultivars (*temperate japonica*, *tropical japonica*, and *JAPONICA-mixture*)
- Among the cold sensitive cultivars, *indica* seems to have a broad distribution of survivability, despite of not being much different compared to *aus* and *INDICA-mixture* cultivars

Future Directions

- Such diverse phenotypes based on different sub-groups are perfect for genome-wide association study mapping with all 370 cultivars
- The sub-group *indica* itself can be run via genome-wide association, given how broad its survivability distribution is after this cold treatment
- Similar approaches may be done under even more critical stress temperature, 8°C and 4°C to see if these sub-groups still cluster together based on their cold tolerance annotation
- Once genome-wide association study is done, further analysis will be done to find candidate genes for genetic modifications to boost cold tolerance in rice and other crops
- Genetic modifications, such as over-expressing and knocking-out candidate genes in rice will be done to assess the effects these genes have on cold stress tolerance

References

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Acknowledgements

- Funding was provided by USDA AFRI-NIFA grant no. 2016-67013-24587 and GAANN fellowship from US Department of Education
- Feedbacks and suggestions from Dr. Schläppi, M. R., Dr. Shi, Y., and Ph.D. candidate Shimoyama, N.
- Helps from these following undergraduates students: Johnson, M. and Beaumont, A.