

## Brief Report

# Efficient Rebalancing of ROS Levels in Plants Links to Temporarily Enhanced Aerobic Fermentation, Distinct Cell Restructuration, and Resilience in Field

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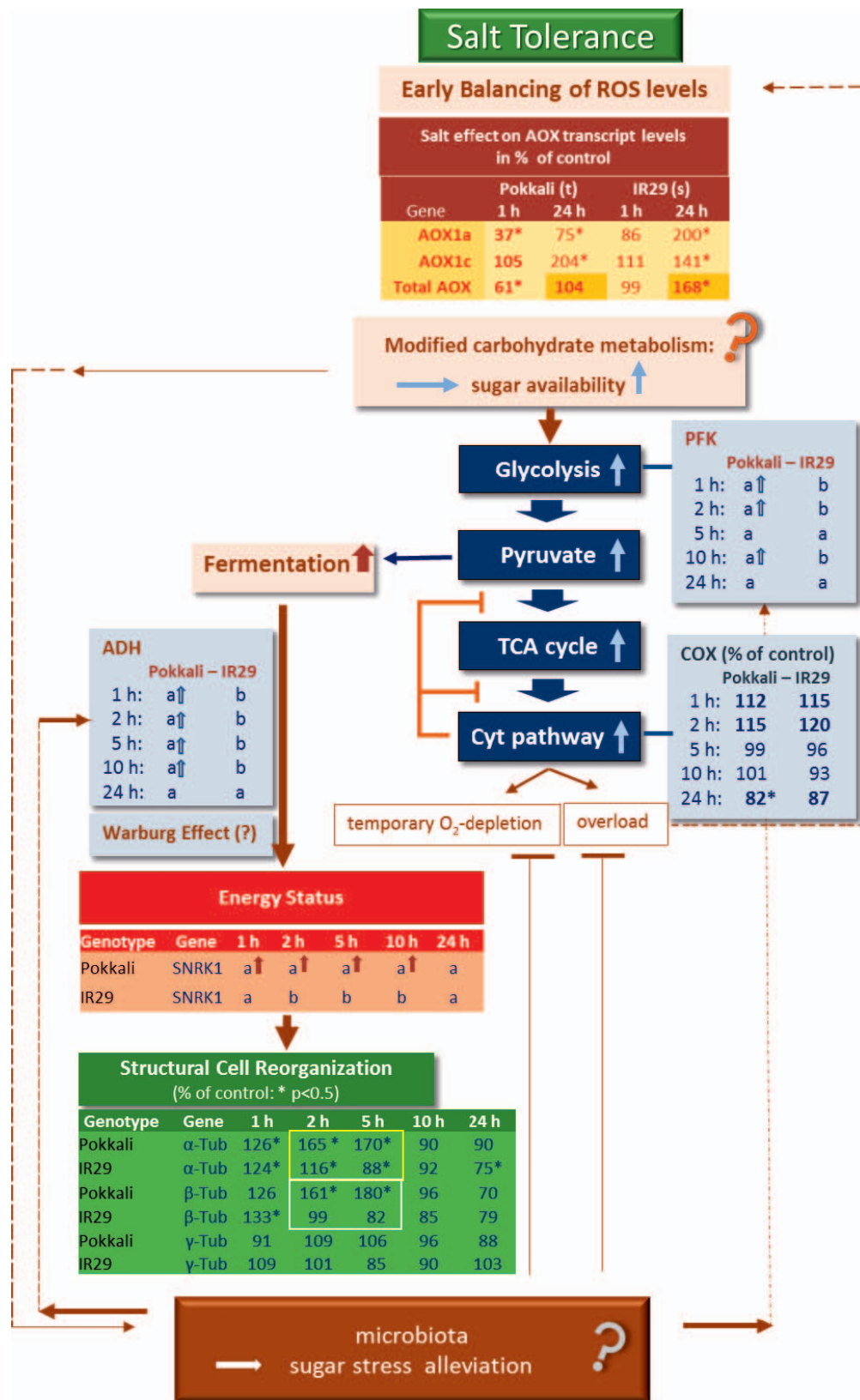
Recently we identified a major complex trait for early de novo programming (CoV-MAC-TED) in virus-infected human cells by using an interdisciplinary approach that integrates research in plant and human cell systems.<sup>[1,2]</sup> CoV-MAC-TED included unbalanced reactive oxygen species (ROS)/reactive nitrogen species (RNS) levels connected to increased aerobic fermentation, which was linked to alpha-tubulin-based cell restructuring and control of cell cycle progression. We suggested that adaptive ROS equilibration during the first hours of stress perception might be critical for disease performance; thus, genes involved in ROS equilibration might help target therapeutic tools.<sup>[3,4]</sup> It is easier to validate traits for resilience in plants than in humans. For validation, we considered well-established unambiguous measures, such as the differential performance of plant varieties in terms of yield height and stability, based on a priori experimentation under multiple field conditions as part of the breeding and variety registration procedures. The genetic capacity for efficient cell reprogramming on isolated and combined stresses can then be studied separately in simple laboratory experiments to explore associations and a posteriori predictability. In this way, plant research can increase the efficiency of knowledge gain for the principal determinants for resilience of holobiont organisms that live under similar environmental conditions, such as water, oxygen, nutrient availability, and temperature.

In plants, the small enzyme family of mitochondrial alternative oxidase (AOX) is involved in regulating ROS/

RNS homeostasis and equilibration (for example, see Scheibe<sup>[5]</sup>). Its relevance for adaptive metabolism and stress behavior was widely explored across diverse plant species and environmental threats (see detailed recent reviews in Arnholdt-Schmitt et al,<sup>[1]</sup> Costa et al,<sup>[2]</sup> Scheibe,<sup>[5]</sup> Mohanapriya et al,<sup>[6]</sup> and Bharadwaj et al<sup>[7]</sup>). Its beneficial effect in respiration-related deficiencies was also studied in transgene mammals missing this enzyme.<sup>[1,2]</sup> AOX acts at the level of early cell reprogramming and maintenance. It senses stress levels and coordinates rapid metabolic reorganization for plant plasticity and adaptive plant robustness by permanently optimizing respiration.<sup>[1,2,6–9]</sup> We hypothesize that plant genotypes, which acclimate more efficiently to changing environmental conditions, have a better chance of maintaining a healthy state that translates into the desired stable higher yield and better food-quality characteristics.<sup>[7]</sup>

Here, we disclose essentials of the novel insights we obtained using two rice plant genotypes, which are known through field experience to differ in multistress (such as that caused by dehydration, cold, abscisic acid application) performance and, especially, in salt tolerance.<sup>[10,11]</sup> Our results confirm the growing understanding that rapid transcript level changes during early cell reprogramming provide appropriate markers for predicting stress-tolerant phenotypes.<sup>[1]</sup>

Figure 1 presents a simplified scheme that highlights our complex concept (developed and discussed in detail by Bharadwaj et al<sup>[7]</sup>) by integrating the summarized



**Figure 1.** AOX transcript levels at salt-induced early cell reprogramming and their link to aerobic fermentation and tubulin-marked cell restructuring in two rice varieties with differential stress tolerance. Detailed materials and methods are available online in Supplemental File 1. t: tolerant; s: susceptible; a and b: indicate significant differences between genotypes at  $p < 0.5$ ; \*: significantly different from the water control at  $p < 0.5$ ; ↑: points to higher transcript accumulation; TCA, tricarboxylic acid; Cyt, cytochrome.

results for rice achieved during the first 24 h after seedlings were stressed by high-salt (300 mM) treatment compared with a watered control. The watered control demonstrated high transcript levels of both expressed AOX genes, *AOX1a* and *AOX1c*, from the first hour of watering in the stress-tolerant rice variety Pokkali. These levels always remained higher than that in the salt-susceptible genotype IR29 at all observed time points (1, 2, 5, 10, and 24 h; not shown, 2–10 h). Under salt treatment, we observed the following.

First, there was early downregulation of *AOX1a* transcript levels in the tolerant variety and recovery of total AOX to control level at 24 h. AOX downregulation indicates an important issue for AOX utility,<sup>[1,2]</sup> and the efficiency of downregulation from initially increased levels was shown to be important for the predictability of stress-tolerance performance.<sup>[6,7]</sup> To the contrary, in the stress-susceptible genotype, the same two AOX genes were transcribed, but no pronounced early response was observed at 1 h. Instead, total AOX transcript accumulation was significantly increased at 24 h after treatment initiation, and upregulated transcription levels were indicated for both transcribed AOX genes.

Second, transcript levels of phosphofructokinase (PFK), which represent glycolysis, were found at a higher level in the tolerant genotype during the first hours. This level was linked to higher transcript levels of alcohol dehydrogenase (*ADH1* and *ADH2*), which marks aerobic fermentation. In this context, respiration via the cyclooxygenase (COX) path remained more stable. Nevertheless, during the first 2 h, in both genotypes, a slight increase (nonsignificant) was indicated compared with the control. This seems to show that the cytochrome path began to be overloaded. However, this did not reach significance and was rapidly brought down to control levels, and then to slightly below control at 24 h, which became significant only for the tolerant variety Pokkali. In addition, we suspect that genotype-dependent microbiota interaction could influence the stress-inducible sugar availability for glycolysis or aerobic fermentation.

Third, during the first hours of salt treatment, the tolerant variety signaled higher energy depletion, marked by *SNRK1* transcript levels, than did the susceptible genotype. This indicates the more efficient energy-dependent cell reorganization in Pokkali.

Finally, in both genotypes, differential cell reorganization or restructuring was marked by distinct tubulin transcript level changes. Under salt stress, Pokkali demonstrated increased tubulin transcript accumulation against the water control between 2 and 5 h after salt treatment began, whereas IR29 tubulin transcription remained at the basal level observed at 1 h. Additional analyses indicated that cell proliferation was arrested during the first 24 h after treatment in both genotypes (not shown). This confirmed our recent conclusions from a different experimental system (induction of somatic embryogenesis by severe stress) in which we found that the cell cycle was suppressed during early cell

reprogramming.<sup>[1,2]</sup> Of note, our results show parallel transcript level changes for the two most frequently expressed tubulin genes, alpha- and beta-tubulin ( $\alpha$ -*Tub* and  $\beta$ -*Tub*) for both genotypes, which we also observed in the water control (not shown). Thus, these observations confirm the approach we applied in Costa et al,<sup>[2–4]</sup> in which we selected only alpha-tubulin transcript levels as a marker for differential cell reorganization during early cell reprogramming.

Overall, this report supports our hypothesis that adaptive ROS equilibration can provide a powerful means of predicting the capacity for resilient performance in plant and human virus cells when viewed during early cell reprogramming at the transcript level in crucial genes and in the context of functional marker-assisted description of energy-dependent cell reorganization and cell cycle regulation. Furthermore, our group studied exhaustively the diversity of polymorphisms in AOX genes related to diverse environments. We suggest that AOX gene diversity in target cells within an individual plant might be a trait that, by itself, allows rapid switching between diverse allelic sequences for acclimation. In parallel, we identified allelic transcript level profiles in the gene *ASMTL* (N-acetylserotonin methyltransferase-like) in human epithelial nasal cells, which link under viral stress to ROS/RNS balancing. Considering our overall results, we propose that these findings should be explored as promising sources of functional markers that could support prediction of differential disease development.<sup>[13,14]</sup>

## Supplemental Material

Supplemental data are available online with the article.

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