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Different forms of osmotic stress evoke qualitatively different responses in rice

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ABSTRACT

Drought, salinity and alkalinity are distinct forms of osmotic stress with serious impacts on rice productivity. We investigated, for a salt-sensitive rice cultivar, the response to osmotically equivalent doses of these stresses. Drought, experimentally mimicked by mannitol (single factor: osmotic stress), salinity (two factors: osmotic stress and ion toxicity), and alkalinity (three factors: osmotic stress, ion toxicity, and depletion of nutrients and protons) produced different profiles of adaptive and damage responses, both locally (in the root) as well as systemically (in the shoot). The combination of several stress factors was not necessarily additive, and we even observed cases of mitigation, when two (salinity), or three stressors (alkalinity) were compared to the single stressor (drought). The response to combinations of individual stress factors is therefore not a mere addition of the partial stress responses, but rather represents a new quality of response. We interpret this finding in a model, where the output to signaling molecules is not determined by their abundance *per se*, but qualitatively depends on their adequate integration into an adaptive signaling network. This output generates a systemic signal that will determine the quality of the shoot response to local concentrations of ions.

1. Introduction

In the agricultural reality, drought and salinity stress are often accompanied by alkalinity stress as third stressor. Saline soils frequently not only contain high amounts of Na^+, but also HCO_3^-. The impact of hydrogen carbonate accentuates the damage imposed by sodium ions, and has therefore attracted a lot of attention. The economic consequences of alkalinity are substantial because relatively large areas of agricultural land suffer from this problem as a consequence of progressive desertification (Liu and Guo, 2011). Globally, about 434 million ha of arable soil are estimated to be impaired by this combination of salinity and alkalinity, which exceeds the 397 million ha of land affected by salinity alone (Wang et al., 2011).

Due to the fact that soil salinization and soil alkalinization frequently co-occur in nature (Patil et al., 2012), plants under alkalinity stress actually face a combination of three stress factors: physiological drought, ion toxicity, and a depletion of protons in the rhizosphere (Ibraheem et al., 2011). Although present in saline soils, water is not physiologically available to plants because the sodium ions in the soil decrease the water potential ψ, such that water is withdrawn from the roots. The resulting dehydration will damage membranes, impair enzyme activities, and disrupt cellular metabolism (Horie et al., 2012). Dehydration stress is accentuated by the toxicity of sodium ions that enter the cell through non-selective cations channels (reviewed in Tester and Davenport, 2003). Perturbation of K^+ homeostasis represents a crucial target of sodium toxicity (Szczerba et al., 2008), but also many enzymes are negatively affected because the electrostatic interactions necessary to maintain a functional protein structure are affected leading to metabolic imbalance (Hasegawa et al., 2000). For instance, strong downregulation of the tricarboxylic acid cycle had been found in maize leaves under salt stress (Richter et al., 2015). Alkalinity will result in the precipitation of metal ions (especially calcium and magnesium), and the depletion of phosphate (Lajtha and Schlesinger, 1988). Furthermore, direct impact on root cell structure and physiology has been reported (Yang et al., 2008a).

Adaptation to stress requires specificity in the cellular and physiological responses depending on the *context* of the stress factor. For instance, in order to reinstall turgidity in the context of

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water scarcity, production of compatible solutes is a good strategy, whereas in the context of salinity, translocation of sodium into the vacuole is more efficient, since it removes ionic stress and at the same time maintains turgor as driving force for growth (reviewed in Martinoia et al., 2012). The specificity of responses at first sight would imply a specificity of signaling transduced by specific molecular components. However, the number of molecular players that convey stress signals in plants is rather limited, and many of these molecular players are shared between different stresses (reviewed for salinity stress in Ismail et al., 2014). Alternatively, specificity might be generated by particular spatiotemporal patterns (so called signatures) of these overlapping signals and their signaling pathways. For instance, the role of calcium influx in cold, drought and salinity stress differs due to different temporal signatures for this signal resulting in the activation of different downstream events (reviewed in Xiong et al., 2002). Also shifts in the relative timing of primary signals will result in qualitatively different responses of cortical microtubules (reviewed in Nick, 2013). The three aspects of alkalinity stress (water scarcity, ionic stress, proton depletion) must differ in their primary inputs, in order to be discriminated. Water scarcity will affect membrane tension, which can be perceived by mechanosensitive ion channels (reviewed in Kung, 2005). In plant cells, unknown mechanosensitive channels drive an influx of calcium. The recently discovered calcium channel OSCA1 (Reduced Hyperosmolality-induced [Ca^{2+}] Increase 1) which is gated by hyperosmotic stress (Yuan et al., 2014) might be a molecular candidate for such channels. Calcium-dependent kinases can translate this calcium influx into the activation of membrane-located NADPH oxidases that produce superoxide in the apoplast such that the primary calcium influx is followed (with some delay) by a transient oxidative burst (Dubiella et al., 2013). In case of salinity stress, the osmotically induced Ca^{2+} influx is accompanied by influx of sodium ions via nonselective cation channels, which activates the adaptive SOS (for salt-overly sensitive) module that not only will extrude sodium from the cytoplasm, but also links cytosolic sodium with the activity of calcium binding signaling proteins (reviewed in Ismail et al., 2014). Alkalinity will affect the apoplastic pH, which is normally kept slightly acidic (at ~5.5) by proton ATPases localised in the plasma membrane to sustain cell expansion growth (Haruta et al., 2010). Also, alkaline pH will impair the activity of osmotically induced calcium influx, because calcium enters the cell by co-transport with protons. In addition, the superoxide anions generated by the NADPH oxidases will accumulate to higher levels, because they are not dissipated due to the absence of protons as electron acceptors. Thus, the oxidative burst induced by ionic stress is expected to be more persistent under alkalinity.

Based on these differences in the primary inputs among drought, salinity, and alkalinity stress, a stress-signature mechanism should lead to outcomes that are not mere additions of the responses to the individual stress components. Instead, combinations of stressors should result in non-additive interactions and even qualitative differences of the stress response. To address this, we designed a comparative approach where the individual components of alkalinity stress were tested along with their combination. As an experimental system, we used the economically relevant Egyptian rice cultivar Sakha 102, and monitored cellular and organismic responses, while titrating the stress dosage such that each input was equivalent with respect to osmotic challenge.

2. Materials and methods

2.1. Plant materials, growth and stress conditions

In this study, *Oryza sativa* L. *ssp. japonica* cv. Sakha 102 was used, kindly provided by the Agricultural Research Center (ARC), Giza, Egypt. The caryopses were dehusked and surface sterilized according to Hazman et al. (2015). The seeds were sown on sterilized 0.5% phytoagar medium (Duchefa, Netherlands) containing 1/10X strength MS medium basal salt (Sigma Aldrich). After one week under continuous light of 120 μmol/m^2s at 25 C° the healthy well grown seedlings were transferred to custom made floating racks and moved to a glass container containing 1/20 X MS medium as nutrient solution for extra 5 days. Subsequently, the seedlings were transferred into a glass container containing 2 liters of 1/20X new fresh MS medium solution as control or the same solution containing mannitol (≈205 mM), NaCl (≈102 mM), or $NaHCO_3$ (≈102 mM), respectively. Thus, the stress treatment was administered in a step-up manner, and not by gradual increments of osmotic pressure. The treatments were chosen such that the osmotic challenge in all three conditions was −0.5 MPa. However, the treatments with NaCl and $NaHCO_3$ complemented this osmotic challenge by additional stressors (ionic stress in case of NaCl; ionic stress in combination with alkalinity stress in case of $NaHCO_3$). Osmotic potential (OP) level of mannitol, NaCl and $NaHCO_3$ were created based on the equation of Van't Hoff (Ben-Gal et al., 2009). The shoots of control and stressed plants were harvested after 24 and 72 h, frozen in liquid nitrogen, and then stored at −80 °C to be used for subsequent analysis.

2.2. Analysis of root elongation

Root elongation was evaluated as the mean of the seminal root length of seedlings raised in darkness (25 °C, 7 days). The seeds were surface sterilized as described above, and sown on 0.5% phytoagar medium with different osmotic pressure of, NaCl and $NaHCO_3$ (0, −0.2, −0.4, −0.6 and −0.8 MPa), for drought, salinity and alkalinity, respectively. The levels of osmotic pressure were calculated as described above. The seedlings were scanned and the root length was measured using Image J (http://imagej.nih.gov/ij/). The length of the seminal root was measured for n = 70 seedlings from at least 3 independent biological replications.

2.3. Carbon isotope discrimination

Plant materials (shoots) of five independent repeats were dried in an oven at 80 °C for 3 days. Subsequently they were ground to fine powder. For the analysis, 100 μg of the powder were weighed into a tin cartridge and compressed to free from air. Carbon isotope discrimination was calculated according to Cernusak et al. (2013).

2.4. Determination of lipid peroxidation

Lipid peroxidation of shoots (representing 3 independent biological replica) was estimated by the level of MDA (Malondialdehyde) using the thiobarbituric acid (TBA) method as described by Heath and Packer (1968). The value of the non-specific absorption at 600 nm was subtracted. The amount of MDA-TBA complex (red pigment) was calculated from the extinction coefficient 155 mM^{-1} cm^{-1}.

2.5. Measurement of ion content

Leaves and roots of control and treated plants were harvested, then washed gently several times with deionized water, and subsequently incubated at 80 °C for 3 days. The dry tissues were homogenized using a mortar and pestel and collected in digestion tubes (Gerhardt, UK), supplemented with 5 ml of concentrated nitric acid (HNO_3) and then incubated for at least 24 h at room temperature while vortexing at 6 and 24 h. The measurements were done at the Institute of Applied Geosciences, Karlsruhe Institute