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Article Addendum

Compatible solutes mitigate damaging effects of salt stress by reducing the impact of stress-induced reactive oxygen species

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Under abiotic stress conditions, rapid increases in reactive oxygen species (ROS) levels occurs within plant cells. Although their role as a major signalling agent in plants is now acknowledged, elevated ROS levels can result in an impairment of membrane integrity. Similar to our previous findings on imposition of salt stress, application of the hydroxyl radical (OH•) to Arabidopsis roots results in a massive efflux of K^+ from epidermal cells. This is likely to cause significant damage to cell metabolism. Since K^+ loss also occurs after salt application and salt stress leads to increased cellular ROS levels, we suggest that at least some of the detrimental effects of salinity is due to damage by its resulting ROS on K^+ homeostasis. We also observed a comparative reduction in K^+ efflux by compatible solutes after both oxidative and salt stress. Thus, we propose that under saline conditions, compatible solutes mitigate the oxidative stress damage to membrane transporters. Whether this amelioration is due to free-radical scavenging or by direct protection of transporter systems, warrants further investigation.

Reactive oxygen species (ROS) are continuously produced as by-products of various metabolic pathways.[1] Under unstressed steady-state conditions, cellular ROS levels are kept in check by the sophisticated antioxidant defence system.[2] However, under adverse environmental conditions, the balance between ROS production and its subsequent scavenging may be perturbed, leading to a rapid increase in ROS levels.[3] Although significant progress has been made in defining ROS as a major signalling agent in plants,[3] ROS can react with a large variety of biomolecules, causing lipid peroxidation and impairing membrane integrity.[4,5] One such abiotic stress is salt stress,[6] with ROS generation occurring within minutes of salt application.[7] Alleviation of oxidative damage may be, therefore, an important strategy of plant salt tolerance.[8]

One of the earliest measurable responses to salt stress is a massive K^+ efflux from plant roots.[9,10] Such K^+ efflux is initiated within seconds of acute salt stress and may last for several hours[11,12] reducing the intracellular K^+ pool[13,14] and significantly impairing cell metabolism. Consistent with the key role of K^+ homeostasis in salt tolerance mechanisms[15] a reduction of K^+ efflux correlates with increased salt tolerance.[11,12]

We have previously reported that hydroxyl radical ($OH^•$) application to *Arabidopsis* roots also results in a rapid efflux of K^+ from the epidermis.[16] In this report, we find a similar K^+ efflux response.[17] As is the case for salt stress,[9] we found that membrane depolarisation could be responsible for a substantial part of this efflux. However, an observed discrepancy between the membrane depolarisation and the pattern of K^+ efflux indicates that voltage-dependence is not the only factor influencing K^+ loss from the root cells after oxidative stress. Demidchik et al.[16] demonstrated that stress-induced K^+ efflux could be mediated by activation of K^+ outward rectifying channels directly by $OH^•$. This direct effect on K^+ transporters could also account for our observed delay before the peak efflux of K^+ is measured, indicating that a certain amount of time is required before maximal direct damage by $OH^•$ to transporters occurs. Because both K^+ channel blockers and non-selective cation channel blockers reduce this efflux, it indicates non-specificity in $OH^•$ attack. Furthermore, combinations of these channel blockers were effective in reducing K^+ efflux implying that, at least in the short term, the damaging effects of $OH^•$ is due to compromising the transporter systems as opposed to lipid peroxidation. Certainly, K^+ channels harbour reactive groups, thus are expected to be sensitive to ROS.[18]

We have previously shown that the exogenous application of low concentrations of a variety of compatible solutes reduces the salt-induced K^+ efflux.[19,20] Plants, when confronted with a saline environment, respond with a significant elevation in their compatible solute levels. This ameliorates the detrimental effects of salinity.[21] However, their original proposed role in cellular osmoregulation is under question: their concentration in transgenic plants overexpressing osmolyte biosynthetic genes is not significant for osmotic adjustment, despite showing improved salt tolerance.[8] Furthermore, one hallmark of the detoxification effect is its lack of specificity, that is, transgenic plants have increased tolerance not only to high salt, but also to drought, cold and heat shock,[22,23] stresses that also result in ROS production.[3] Certainly, ecotopic expression studies suggest that compatible solutes increase stress tolerance by protection of membranes and proteins against ROS.[6]

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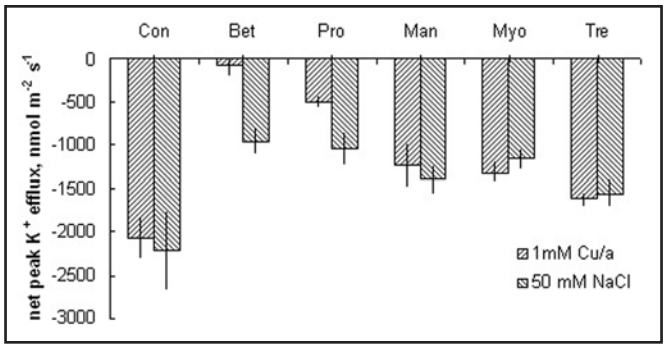

Figure 1. Effects of exogenous supply of compatible solutes on net peak K^+ efflux after application of either 1 mM Cu/a or 50 mM NaCl. Roots were pre-incubated for 1 h in 5 mM concentration of a number of compatible solutes prior to treatment. Mean ± SE (n = 6–8).

We show that in this work that exogenous application of low concentrations of a range of compatible solutes significantly reduces $OH^{\bullet}$-induced K^+ efflux,[17] a similar effect to that we reported after salt application to barley roots[19] and also observed in *Arabidopsis* (Fig. 1). Interestingly, we found that not only known free-radical scavenging osmolytes,[24] but also glycine betaine, previously found to be non-effective in ROS scavenging,[24] were effective in reducing $OH^{\bullet}$-induced K^+ efflux. Indeed, glycine betaine showed a greater mitigation of $OH^{\bullet}$-induced K^+ efflux compared to that induced by 50 mM NaCl (Fig. 1). However, it is open to speculation as to whether this mitigation is via direct channel blocking, a direct protection of ion channel proteins or by some other protective mechanism.

In our further investigations we have found that salt-tolerant barley show a reduced ROS-induced K^+ efflux compared to sensitive varieties.[25] This superior ability of salt-tolerant barley cultivars of preventing K^+ loss further indicates a possible causal link between salt and oxidative stress tolerance. We propose that upon the imposition of salt stress, the instantaneously resulting membrane depolarisation[9] results in activation of depolarisation activated K^+ outward-rectifying channels, leading to the initial massive K^+ efflux. Over the longer term, ROS levels within the plant cell increase,[7] resulting in direct damage to K^+ transporters and the longer-term sustained loss of K^+ from the cell. Due to mitigation of both NaCl- and $OH^{\bullet}$-induced K^+ efflux by compatible solutes, we propose that one of their primary amelioratory effects is through reducing the damaging effects of salt-produced ROS on K^+ transporter, and by this means, reducing the effects of stress damage. Whether this amelioration is achieved through free-radical scavenging or due to a direct protection of membrane transports warrants further investigation.

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