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Hydrogen peroxide and nitric oxide reprograms signal transduction in leaves of citrus plants under natural and salt stress conditions

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Reactive oxygen- and nitrogen species (ROS/RNS) are involved in a wide variety of signalling cascades necessary for plant adaptation to abiotic stresses. Recent studies on salinity stress in citrus revealed regulation of adaptive responses by hydrogen peroxide (H_2O_2) and nitric oxide ($^{\bullet}NO$). From that observation, it was hypothesized that pre-exposure to H_2O_2 or $^{\bullet}NO$ altered endogenous plant ROS/RNS-related signal transduction mechanisms. Here, we tested this hypothesis by applying H_2O_2 or nitroprusside (SNP; nitric oxide donor) to citrus roots under natural and salt stress conditions and observing phenotypical, physiological, histochemical, biochemical and molecular changes in leaves. Data indicated that pre-treatment with H_2O_2 or SNP reduces the detrimental physiological effect of the direct NaCl stress. Root-applied H_2O_2, SNP or NaCl continuously triggered accumulation of both H_2O_2 and $^{\bullet}NO$ in leaves. Histochemical and confocal laser-scanning microscopy analysis revealed that the accumulation of ROS towards the leaf midrib was accompanied by generation of ROS and $^{\bullet}NO$ signals in vascular bundles. Time-course analysis showed that gene transcript and proteins levels of components of the antioxidant system were differentially regulated by pre-treatments. Pre-exposure to both molecules protected against salt-induced oxidative and nitrosative damage as analyzed by a hydroxyl radical or peroxynitrite-generating *in vivo* system. Expression patterns of carbonylated, tyrosine nitrated and S-nitrosylated proteins were altered by both pre-treatments under non-stress and salt stress conditions. This work provides a reference data set for the systemic role of oxidative and nitrosative signaling pathways that enables the adjustment of plants to stressful conditions.