

Tolerance to oxidative stress shields plants from other abiotic stresses

In nature, the environment of plants is highly variable. Plants have thus developed various strategies to respond to ever-changing environmental conditions, monitoring their surroundings and accordingly adjusting their metabolic systems to maintain homeostasis and survive stress. In addition to biotic stresses, abiotic stresses also have a great impact on plant growth and productivity. Plants that are tolerant to multiple stresses can perform successfully in stressful environments and maintain productivity, which is essential for food security. However, most of the crop varieties currently used in agriculture to enhance food security have been selected for their high productivity rather than for their tolerance to various abiotic stresses.

Plants can adapt to variable environments due to the plasticity of their cellular reduction/oxidation (redox) processes. To date, all kinds of biotic and abiotic stresses have been found to trigger a generalized stress response called oxidative stress that results from the accumulation of reactive oxygen species (ROS). Oxidative stress involves disruption of the redox state in cells due to the imbalance between ROS generation and detoxification. Plants that are tolerant to oxidative stress protect themselves from the damaging effects of ROS mainly through maintenance of redox balance triggered by increased detoxification. Hence, exploiting and enhancing these mechanisms in high-yielding or commercially important varieties could improve their performance under various types of stress. Such broad-spectrum stress tolerance resulting from oxidative stress tolerance is

referred to as cross-protection, a phenomenon in which plants that display tolerance to one kind of stress (here, oxidative stress) also display tolerance to one or more other stress factors (such as drought, salinity, heavy metals, and high temperatures).

In aerobic organisms, metabolic processes unavoidably lead to the production of ROS. Under optimal growth conditions, ROS are produced at low levels and are used as signaling molecules. However, under conditions of stress, ROS levels become elevated and are damaging to cells. ROS include active oxygen species and their intermediates, such as singlet oxygen ($^1\text{O}_2$), superoxide ($\text{O}_2^{\bullet-}$), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^{\bullet}). In addition to ROS, reactive nitrogen species also contribute to oxidative stress. In plants, cellular compartments such as chloroplasts, mitochondria, peroxisomes, and apoplasts and the plasma membrane are the major sites of ROS production. Changes in cellular redox status play pivotal roles in integrating external stimuli and stress signaling in plants, and excess ROS accumulation results in oxidation of major macromolecules, such as DNA, RNA, carbohydrates, proteins (causing denaturation) and lipids (lipid peroxidation).

Increased levels of ROS are regulated or controlled by ROS scavenging mechanisms. In plants, these are broadly classified as enzymatic and non-enzymatic mechanisms. The enzymatic scavenging system includes superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), monodehydroascorbate reductase (MDHAR), dehydroascorbate

reductase (DHAR), glutathione reductase (GR), glutathione peroxidase (GPX) and glutathione S-transferase (GST). The non-enzymatic system includes antioxidant molecules such as ascorbic acid/ascorbate (AsA), dehydroascorbate (DHA), glutathione (GSH), tocopherols, tocotrienols, proline, flavonoids, carotenoids, phenolics, quercetin, kaempferol glycosides, cytochromes, polyamines and proteins carrying redox-active S-groups (thioredoxins, peroxiredoxins and sulfiredoxins). The balance of these redox-active compounds contributes to general redox homeostasis in plant cells. Some plants have been genetically engineered for oxidative stress tolerance through targeting of genes coding for SOD, APX, CAT, MDHAR, DHAR, GR, GPX or GST. Most of these transgenic plants also display cross-protection against other abiotic stresses. Plants overexpressing multiple antioxidant genes (combinations of two or more genes) display higher levels of oxidative stress tolerance than single-gene transformants. Among the antioxidant systems, identification of the most important antioxidant system that contributes to maximum oxidative stress tolerance is important; for such identification, functional validation of each of the systems must be carried out in various crop species. Within the antioxidant system in plants, the ascorbate-GSH (AsA-GSH) pathway plays a central role in regulating ROS. This pathway combines both enzymatic (APX, MDHAR, DHAR, and GR) and non-enzymatic (AsA and GSH) antioxidant components. In the first step of the pathway, H_2O_2 is reduced to water by APX, with AsA as the electron donor. During this reduction, AsA is oxidized

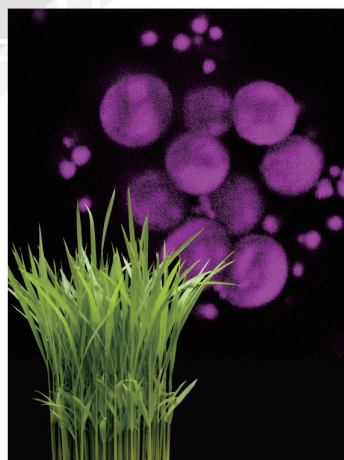


Figure 1. Salt-inducible rice GR 3 (OsGR3) is dual-targeted to chloroplasts and mitochondria in rice. Rice protoplasts were isolated from transgenic rice harboring a *ubiquitin promoter::OsGR3-GFP* construct. Confocal images of rice protoplasts showing the chloroplastic and mitochondrial localization of OsGR3-GFP are shown. (Photo credit: *Plant Molecular Biology*, 2015; 87:555-564.)



Figure 2. Complementation of *gr3* mutants with wild-type (WT) GR3 (C1) restores sensitivity to salt stress to the WT level. (Photo credit: *Plant Molecular Biology*, 2015; 87:555-564.)

to monodehydroascorbate, which is reduced back to AsA by the action of MDHAR or is spontaneously converted to DHA. DHA is reduced to AsA by the action of DHAR at the expense of GSH, yielding glutathione disulfide (GSSG). GR regenerates GSH from GSSG using NADPH as an electron donor. In the whole pathway, the net electron flow proceeds from NADPH to H_2O_2 . This pathway requires the maintenance of high levels of both ascorbate and GSH.

GR, also known as glutathione disulfide reductase (GSR), is a flavoprotein belonging to the family of NADPH-dependent oxidoreductases. GR catalyzes the reduction of GSSG to GSH and plays an essential central role in cell defense against ROS. GR, like other antioxidants, is differentially regulated under various environmental stress conditions. Studies on transgenic plants have shown that GR is important in providing resistance to oxidative stress caused by different environmental cues. In addition to conferring stress tolerance, increased GR activity can alter the redox states of electron transport chain components. GR plays a central role in maintaining the reduced GSH pool and in regulating cellular ROS scavenging pathways under conditions of stress. At the Department of Ag-

ricultural Chemistry, National Taiwan University (NTU), the focus of our laboratory has been on understanding the role of all 3 GR isoforms in rice, which include one cytosolic GR and two chloroplast/mitochondria dual-localized GRs. We study the effects of regulated GR expression on cross protection against salinity, heat stress and heavy metal stress. Our studies have provided clues suggesting that not all isoforms function equally in imparting stress tolerance. In addition, we are also trying to understand the molecular and physiological impacts of GSH metabolism under control and stress conditions. Elucidating the mechanisms of GSH-induced or GSH redox change-induced cross protection in plants will allow us to fine-tune ROS homeostasis in plants under abiotic stress, thereby helping to generate climate-resilient crops to improve global food security.

References

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