



# Paclobutrazol pre-treatment enhanced flooding tolerance of sweet potato

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## Summary

The objective of this experiment was to study changes of antioxidants and antioxidative enzymes in the flooding-stressed sweet potato leaf, as affected by paclobutrazol (PBZ) treatment at 24 h prior to flooding. Sweet potato 'Taoyuan 2' were treated with 0 and 0.5 mg/plant of PBZ, afterwards subjected to non-flooding and flooding-stress conditions for 0, 1, 3, and 5 d, followed by a 2 d drainage period. The study was conducted as a factorial experiment in completely randomized blocks with three replications maintained within a screen house. Plants with various antioxidative systems responded differently to flooding stress according to the duration of the flooding period and subsequent drainage period. The increased levels of antioxidants and antioxidative enzymes observed on different days of flooding afforded the sweet potato leaf with improved flooding tolerance. Glutathione reductase activity in the leaf was significantly enhanced over 5 d continuous flooding followed by a drainage period, in comparison with non-flooding conditions. Under non-flooding conditions, antioxidative system of leaf was regulated and elevated by PBZ pre-treatment. PBZ treatment may enable sweet potato 'Taoyuan 2' to maintain the balance between the formation and the detoxification of activated oxygen species. Our results also show that under flooding-stress conditions, the level of 'Taoyuan 2' antioxidative system is linked to PBZ treatment. Pre-treating with PBZ may increase levels of various components of antioxidative systems after exposure to different durations of flooding and drainage, thus inducing flooding tolerance. PBZ exhibited

*Abbreviations:* APX, ascorbate peroxidase; ASA, ascorbic acid or ascorbate; CAT, catalase; GR, glutathione reductase; GSH, reduced glutathione; GSSG, oxidized glutathione; MDA, malondialdehyde; PBZ, paclobutrazol; SOD, superoxide dismutase

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the important function of enhancing the restoration of leaf oxidative damage under flooding stress after the pre-application of 0.5 mg/plant. These findings may have greater significance for farming in frequently flooded areas.

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## Introduction

Plants are severely affected by flooding, because under such stress the production and the quenching of the reactive oxygen species (ROS) in plants will fail to be kept in a balanced state (Crawford and Brandle, 1996). One of the major consequences of soil flooding in plants is oxygen deficiency; consequently, the roots suffer from periodic or prolonged deprivation of oxygen and this interferes with respiration. The lack of suitable electron acceptors leads to the saturation of redox chains, the accumulation of NAD(P)H, and a decline in ATP generation (Kennedy et al., 1992; Perata and Alpi, 1993). In plant cells, oxidative stress reactions are associated with toxic free radicals yielded by the reduction of molecular oxygen leading to the production of superoxide radicals ( $O_2^-$ ), singlet oxygen ( $^1O_2$ ), hydroxyl radicals ( $\cdot OH$ ) and hydrogen peroxide ( $H_2O_2$ ). These radicals can deactivate various Calvin-cycle enzymes and are involved in oxidative systems, in which they mark proteins for degradation (Chaudiere and Ferrari-Illous, 1999). The toxic radicals can be removed through the mobilization of antioxidant reserves, which react both enzymatically and chemically with the toxic molecular species and their products. Chemical constituents have been identified to scavenge free radicals and thus protect active plant cells against oxygen toxicity (Sairam et al., 1998; Ahmed et al., 2002). In order to counter the hazardous effects of ROS under stress, plants have evolved a complex antioxidative defense system composed of both antioxidant enzymes and metabolites, such as ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR), ascorbic acid or ascorbate (ASA), reduced glutathione (GSH), oxidized glutathione (GSSG), malondialdehyde (MDA) and vitamin E (Sairam et al., 2002). The ASA–glutathione cycle has been shown to be of great importance in multiple stress reactions (Drazkiewicz et al., 2003).

The interaction between the production and the scavenging of activated oxygen species maintains the plants in a relatively stable state. Plants are adapted to minimize radical damage using their natural defense mechanisms. Thus, maintaining the balance between the formation and detoxification of activated oxygen species is critical to cell

survival during flooding stress. When roots are submerged, the anoxic conditions inhibit aerobic respiration and less energy is yielded. The roots transport less water to the leaves. The antioxidative enzymes, hormones, and other solutes entering the leaves via the transpiration stream may also undergo changes (Grichko and Glick, 2001; Voese-nek et al., 2004). These changes may constitute physiologically active messages that modify leaf physiology and development; such modifications may include chlorophyll breakdown, protein degradation, decreased stem elongation coupled with increased stem thickness, a decrease in membrane permeability, peroxidation, slower leaf expansion, petiole epinasty and stomatal closure. Stomatal closure causes a decrease in internal  $CO_2$  concentration. Subsequently, a concomitant decline in photosynthesis results from diminished availability of  $CO_2$  for carbon fixation (Carvalho and Amancio, 2002). Reduction of  $CO_2$  concentration increases the amount of harmful ROS within the leaf due to ongoing light reactions that can lead to senescence and even the death of the plant (Else et al., 1995; Kato et al., 2001). Flooding tolerance can be achieved using several adaptive mechanisms, and the modulation of antioxidative system levels may be part of the whole mechanism. High levels of some antioxidant enzymes and metabolites, such as SOD, CAT, APX, GR, GSH, ASA, MDA, AOX or GPX, are important in tobacco (Hurng and Kao, 1994a, b), corn (Yan and Dai, 1996), wheat (Biemelt et al., 1998), soybean (VanToai and Bolles, 1991), rice (Ushimaro et al., 1992), tomato and eggplant (Lin et al., 2004) and sweet potato (Hwang et al., 1999) survival under oxidative stress, after being subjected to different degrees of waterlogging. Some oxidative enzymes or oxidants have been useful in screening for flooding-tolerant plants (Lin et al., 2004).

Paclobutrazol (PBZ) ((2RS, 3RS)-1-(4-(chlorophenyl)-4, 4-dimethyl-2-1, 2, 4-triazol-1-yl)-penten-3-ol) is a member of the triazole family. Triazoles have both fungitoxic and plant-growth regulatory effects. In addition, they can also protect plants against various stresses, including drought, low and high temperatures, UV-B radiation, air pollutants, and fungal pathogens. Therefore, the triazoles have been characterized as plant multi-protectants (Kraus and Fletcher, 1994; Pinhero and Fletcher,

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