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Paclobutrazol leads to enhanced antioxidative protection of sweetpotato under flooding stress

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ABSTRACT. The aim of this research was to study the effect of paclobutrazol pretreatment on the changes of antioxidative enzymes and antioxidants in the flooding-stressed sweetpotato *Ipomoea batatas* (L.) Lam. 'Tainung 57' was grown in plastic boxes in a screenhouse and maintained in optimal water conditions for 45 days followed by PBZ treatments (0 and 0.5 mg/plant) for 1 day. Then flooding was induced by raising the water level to 5 cm above the soil medium surface for a 5-day period followed by drainage for 2 days. A factorial experiment in randomized complete blocks with three replications was conducted. Young fully expanded leaves from each plant were clipped to measure enzyme activities and antioxidant contents. Increased ascorbate peroxidase activity, total glutathione, oxidized ascorbic acid, and total ascorbic acid amounts on different days of flooding provided the sweetpotato with increased flooding tolerance. The levels of glutathione reductase, ascorbate peroxidase, total glutathione, oxidized ascorbic acid, and malondialdehyde were regulated and elevated by paclobutrazol pretreatment under non-flooded conditions. Paclobutrazol pretreatment increased the levels of all antioxidative enzymes and antioxidants following different flooding durations and drainage, and boosted the flooding tolerance of the sweetpotato.

Keywords: Antioxidant; Antioxidative enzyme; Flooding; *Ipomoea batatas* (L.) Lam.; Paclobutrazol.

INTRODUCTION

Environmental stress severely affects plants because it can throw the production and scavenging of reactive oxygen species (ROS) in plants out of balance (Gratão et al., 2005). One of the major biological consequences of soil flooding is oxygen deficiency. Roots suffer from periodic or prolonged deprivation of oxygen, which interferes with respiration at the level of electron transport. The lack of a suitable electron acceptor leads to saturated redox chains, accumulation of the reduced form of nicotine adenine dinucleotide phosphate (NADPH), and a decline in the generation of adenosine triphosphate (ATP). In plant cells, oxidative stress reactions are associated with toxic free radicals from the reduction of molecular oxygen to superoxide radicals (O_2^-), singlet oxygen ($\cdot O_2$), hydroxyl radicals ($\cdot OH$) and hydrogen peroxide (H_2O_2). These free radicals can inactivate various Calvin-Benson cycle enzymes and are involved in oxidative systems, marking proteins for degradation (Kennedy et al., 1992; Chaudiere and Ilious, 1999). The toxic radicals can be removed both enzymatically and chemically to protect plant cells against oxygen toxicity and counter the hazardous effects of ROS under stress (Gratão et al., 2005). The complex antioxidative defense system that has evolved in plants is composed of antioxidative enzymes (i.e., ascorbate peroxidase (APX), catalase (CAT), superoxide dismutase (SOD), glutathione reductase (GR)) and metabolites (i.e., ascorbic acid (ASA), as well as reduced glutathione (GSH), oxidized glutathione (GSSG), and vitamin E) (Gratão et al., 2005). High levels of some antioxidative enzymes and antioxidants are found to be important in tobacco (Hurng and Kao, 1994a; Hurng and Kao, 1994b), corn (Yan and Dai, 1996), wheat (Biemelt et al., 1998), soybean (VanToai and Bolles, 1991), rice (Ushimaro et al., 1992), tomato, eggplant (Lin et al., 2004), and sweetpotato (Lin et al., 2006) survival of oxidative stress after being subjected to different levels of flooding. Some oxidative enzymes or oxidants have been useful in screening for flooding-tolerant plants (Lin et al., 2004).

PBZ (paclobutrazol; (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 protect plants against various stresses, including drought,

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low and high temperatures, UV-B radiation, air pollutants, fungal pathogens, and flooding. Therefore, the triazoles have been characterized as plant multi-protectants (Kraus and Fletcher, 1994; Voesenek et al., 2003). In plants, chloroplasts are a major site of free radical production, and PBZ protects plants by increasing antioxidant defense systems. PBZ-treated plants have a more-efficient free radical-scavenging system to detoxify active oxygen (Kopyra and Gwozdz, 2003). Even though PBZ-induced metabolic stress tolerance or protection is reportedly due to some increased antioxidant enzymes (Pinhero et al., 1997), less is known about the extent to which the antioxidative response of PBZ application differs in the flooding tolerance of sweetpotato.

The sweetpotato is the world's fifth most important crop and is a major source of food and nutrition in developing countries (Food and Agriculture Organization, 2002). Heavy rain storms and floods can leave the soil saturated for days before drainage, making flooding a problem in many parts of the world. Attempts have been made to breed for increased flooding tolerance and modify crop cultivation or management practices and avoid flooding injury. PBZ has been reported to confer protection on plants experiencing stress by reducing oxidative damage via elevation of antioxidants or reducing the activity of oxidative enzymes. We hypothesized that pretreatment with PBZ would increase the activities of antioxidative enzymes or levels of antioxidants under flooding stress, leading to higher flood tolerance in the sweetpotato. The antioxidative system of the leaves of sweetpotato exposed to waterlogged conditions was studied. The results provide information that PBZ pretreatment increases sweetpotato tolerance to waterlogging stress.

MATERIALS AND METHODS

Plant materials, cultural practices, experimental design, and treatments

The sweetpotato (*Ipomoea batatas* (L.) Lam.) cultivar, Tainung 57, was used as the experimental material in this study. Tainung 57 is a popular variety grown in Taiwan for its storage roots. Cuttings about 30 cm in length were planted in plastic boxes 60 cm long, 22 cm wide, and 15 cm deep, containing medium consisting of sand, vermiculite, and loamy soil in a volume ratio of 2:1:1. Plants were planted in September 2001 in a greenhouse of Chinese Culture University, Taipei. Plants were evenly spaced every 50 cm to encourage similar growth rates and sizes. Plants were watered with a half-strength Hoagland solution (Lin et al., 2006) every other day to maintain optimal irrigation and growth for 45 days before imposition of flooding stress. The average day/night temperatures were 33/23°C, and the average day length was 13 h during the period of study.

A factorial experiment of three factors with different levels was used in this investigation. Two concentrations of PBZ (trade name, Bonsi; Zeneca Agrochemicals, Fermhursk Haslemere Survey, UK), aqueous solutions at 0 and 0.5 mg/plant level, were sprayed to study the plant responses to flooding stress based upon our previous experiments (Lin et al., 2006). Twenty-four hours after PBZ treatment, plants were subjected to two water conditions (non-flooded and flooded by tap water) for 0, 1, 3, and 5 days followed by drainage for 2 days. Three plants from each flooding period were harvested at the same time of the day and used for the enzyme measurements. All boxes of the same replication in each flooding time treatment were placed in a 140 × 50 × 35-cm plastic bucket containing a water level 5 cm above the soil medium surface. Plants without PBZ treatment in a non-flooded condition were considered the control to provide a basis to compare the effects of PBZ under flooded and non-flooded conditions. The experiment was performed twice independently for a randomized design of growth environment, sampling day, and biochemical analysis. Young, fully expanded leaves from each plant were clipped for measurement of enzyme activities and antioxidant contents.

Enzyme extraction and activity determination

The cut leaves of each treatment were carried in an icebox to the laboratory less than 5 min away and immediately frozen in liquid nitrogen. They were then stored in a -70°C freezer for later analysis. Samples were prepared for SOD, CAT, APX, and GR activity analyses by homogenizing 0.2 g of frozen leaf in 990 μL of an ice-cold 100 mM HEPES buffer (pH 7.0) containing 1 mM PMSF (phenylmethysulfonyl fluoride) and 0.03 g PVP (polyvinylpyrrolidone). The extracts were centrifuged at 13,000 *g* and 4°C for 15 min. The supernatants were then collected in a fresh tube for the enzyme assays. Enzyme activities were determined using a spectrophotometer.

CAT activity was assayed by measuring the initial rate of the disappearance of H_2O_2 according to the method of Hwang and VanToai (1991). GR activity was performed by oxidized GSH-dependent oxidation of NADPH using the protocols described by Foyer et al. (1997). The assay for APX activity was carried out as described by Nakano and Asada (1981). SOD activity was determined using a SOD Assay Kit-WST (Dojindo Molecular Technology, Gaithersburg, MD). The specific activity of SOD was calculated using the equation described in the protocol of the kit.

Antioxidant extraction and content measurement

The contents of total ASA and total glutathione were determined by dissolving 0.2 g of homogenates in 1 mL of a 5% m-phosphoric acid solution. The extract was then centrifuged at 13,000 *g* for 10 min and 4°C. The supernatant was used for the total ASA and total glutathione assays. Both total ASA and reduced ASA contents were determined according to Cakmak and Marschner (1992). The content of oxidized ASA (DHA,

dehydroascorbate or vitamin C) was calculated by subtracting the reduced ASA content from the total ASA content. Total glutathione content (GSH + GSSG) was quantified as described by Anderson (1985). MDA is a final decomposition product of lipid peroxidation and has been used as an index for the status of lipid peroxidation. MDA concentration was determined by the methods of Kosugi and Kikugawa (1985).

The general chemicals used in the study were purchased from Sigma (St. Louis, MO, USA). All spectrophotometric analyses were conducted on a 530 UV/VIS spectrophotometer (Pharmacia Biotech, Uppsala, Sweden). One unit of enzyme was defined as the amount of enzyme required to decompose 1 μmole of substrate min^{-1} g^{-1} fresh weight (FW).

Determination of the leaf water potential (WP) and total chlorophyll content (TCH)

The WP was measured on the third leaf from the top of each plant using a pressure chamber (Plant Water System, Skyeskpm 1400, Tokyo, Japan) (Sairam et al., 1998). A leaf sample (0.2 g) was homogenized with 1.25 ml of 80% acetone and incubated for 10 min followed by centrifugation at 13,000 *g* for 15 min at 25°C. Absorbances of the supernatant were measured at 663.6 and 646.6 nm, the major absorption peaks of chlorophyll a and b, respectively. The TCH was calculated as 8.02 $A_{663.6}$ + 20.2 $A_{646.6}$ as described by Porra et al. (1989).

Statistical analysis

Measurements of enzymes were analyzed by a three-factor completely randomized ANOVA that compared the PBZ concentrations, flooding conditions, and duration of treatment. For significant values, means were separated by the least significant difference (LSD) test at $p \leq 0.05$, 0.01 or 0.001, using PC SAS 8.2 (SAS Institute, Cary, NC).

RESULTS

The effects of PBZ pretreatment (P) on sweetpotato subjected to flooding conditions (F) were monitored by measuring changes in CAT, GR, APX, SOD, total ASA, oxidized ASA, total glutathione, MDA, TCH, and WP under various durations (D) of treatment. SOD, CAT, APX, GR, MDA, TCH, and WP levels displayed significant differences ($p \leq 0.001$ and 0.05) by the main effect of PBZ (Table 1). For the main effects of flooding, there were significant differences in the activities of SOD and APX, and in the contents of total ASA, oxidized ASA, total glutathione, TCH, and WP. Moreover, all the enzymes and antioxidants, as well as TCH and WP, were significantly affected by the duration of treatment. Table 1 also illustrates that the SOD activity appeared to significantly differ in terms of both the main effects (P, F, and D) and the interaction effects (P×D, P×F, F×D, and P×F×D).

Figure 1a shows the SOD activities of plants with and without PBZ pretreatment after 0, 1, 3, and 5 days of flooding and the subsequent 2 days of drainage. SOD activity with non-PBZ treatment under a non-flooded condition (-P/-F) increased, reached its maximum value (1.33 unit/g FW) after 3 days of treatment, and dropped thereafter. In the case of PBZ-treated and non-flooded conditions (+P/-F), the activity of SOD increased up to day 5 of treatment followed by a decrease. The trend of change in the SOD activity of untreated plants under flooding conditions (-P/+F) was that it remained low before drainage, then increased to its maximum (1.09 unit/g FW) after drainage. In addition, pretreatment with PBZ followed by flooding stress (+P/+F) resulted in a significant increase in SOD activity on days 3 and 5 of flooding and drainage, compared to the -P/+F condition.

Table 1. ANOVA of paclobutrazol concentration (P), flooding condition (F), duration of treatment (D), and their interactions (P × D, P × F, F × D, P × F × D) for superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), glutathione reductase (GR), total ascorbic acid (ASA), oxidized ASA, total glutathione, malondialdehyde (MDA), total chlorophyll (TCH), and water potential (WP) contents in sweetpotato leaves.

Source of variance	Degrees of freedom	Significance									
		SOD	CAT	APX	GR	Total ASA	Oxidized ASA	Total glutathione	MDA	TCH	WP
PBZ (P)	1	***	*	*	***	NS	NS	NS	***	***	***
Flooding (F)	1	***	NS	*	NS	***	***	*	NS	**	***
Days (D)	4	***	***	*	**	***	***	***	*	***	**
P × D	4	***	NS	**	**	***	*	**	***	***	**
P × F	1	***	*	*	*	***	***	NS	***	NS	NS
P × F	4	***	NS	NS	NS	***	***	***	***	***	**
P × F × D	4	***	NS	**	***	***	***	***	***	***	NS

***: $p \leq 0.001$; **: $p \leq 0.01$; *: $p \leq 0.05$; NS: non-significant difference.

Therefore, adding PBZ before flooding stress may promote SOD activity in the sweetpotato.

As shown in Figure 1b, the -P/-F condition produced significantly higher CAT activity than the -P/+F condition after 3 and 5 days of flooding. This observation suggests that flooding stress can result in reduced CAT levels and the occurrence of oxidative damage. On the other hand, CAT activity in the -P/-F condition was significantly higher than that in the +P/-F condition after 1 day of flooding and drainage. This implies that pretreatment with PBZ may decrease CAT activity under non-stressful conditions.

Figure 1c presents the effects of PBZ pretreatment on leaf APX activity during flooding and drainage. The pre-addition of PBZ to plants in a non-flooded condition on day 0 of flooding caused a significantly higher level of APX. One day after the plants received PBZ in a non-flooded condition, PBZ immediately induced an increase in APX activity. Plants are highly regulated by PBZ, which can drastically elevate APX activity. Compared to plants without PBZ treatment and a non-flooded condition (-P/-F), PBZ-treated plants under a non-flooded condition (+P/-F) had higher APX activities on all days of treatment except for day 1 of flooding. This observation reveals that pretreatment with 0.5 mg PBZ per plant in a non-flooded condition could lead to an increase of APX activity.

When across-day comparisons were made, the +P/+F condition consistently displayed significantly higher GR activity than the -P/+F condition after flooding was imposed (Figure 1d). Higher GR activity was shown in the +P/+F condition on days 1, 3, and 5 of flooding than for the -P/+F condition. Pretreatment with 0.5 mg PBZ per plant enhanced higher GR activity in the sweetpotato during flooding stress. GR stimulated by pretreatment with PBZ may play an important role in overcoming flooding stress.

The total glutathione content in the -P/+F condition was significantly higher than that in the -P/-F condition on days 1 and 5 of flooding (Figure 2a). It is noteworthy that the -P/+F condition (230.1 nmol/g FW) displayed an increase threefold greater than did the -P/-F condition (65.0 nmol/g FW) on day 5 of flooding. In the absence of PBZ pretreatment, the total glutathione content in the sweetpotato was induced by flooding stress, and glutathione could be considered a flooding-tolerant enzyme against flooding stress.

Figure 2b demonstrates comparisons of oxidized ascorbate (or DHA) contents under PBZ/flooding conditions throughout the period of treatment. The oxidized ASA content in the +P/-F condition accumulated at different rates from day 0 (0.2 μmol/g FW) to day 7 (4.0 μmol/g FW). Therefore, pretreatment with PBZ followed by non-stressful conditions may increase the level of oxidized ASA in a 7-day period.

All four PBZ/flooding treatments had the highest level of total ASA on day 0 of treatment with no significant difference (Figure 2c). The -P/+F condition (3.1 μmol/g

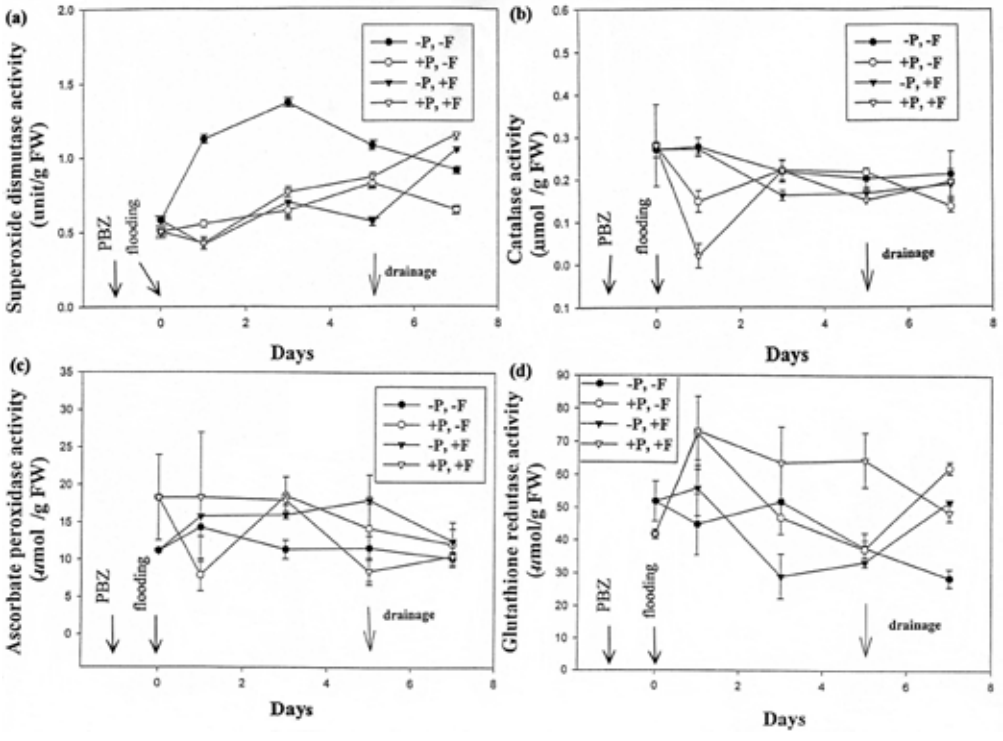

Figure 1. Effects of pretreatment with paclobutrazol on the (a) superoxide dismutase, (b) catalase, (c) ascorbate peroxidase, and (d) glutathione reductase activity in leaves of sweetpotato subjected to flooding stress for different durations of treatment. Vertical bars represent the mean ± standard error. +P, paclobutrazol pretreatment; -P, no paclobutrazol treatment; +F, flooding stress; -F, non-flooded condition.

FW) had a significantly higher total ASA level than the +P/+F condition (1.5 μmol/g FW) on day 5 of flooding. This implies that PBZ addition may reduce the total ASA content under long-term flooding stress.

A significantly lower MDA content under the +P/-F and +P/+F conditions compared to the -P/-F control condition with 3-day flooding duration is shown in Figure 3a. Therefore, PBZ pre-addition increased lipid peroxidation under flooding stress, and might afford protection to the plants by decreasing flood-induced oxidative stress.

As shown in Figure 3b, the level of the leaf WP in both -P/+F and +P/+F plants decreased at different rates as the flooding duration was extended followed by drainage. The water status of PBZ-treated plants was better than that of untreated plants under flooding stress. For example, the WP of -P/+F plants was -6.7 bar, and it was -9.2 bar for +P/+F plants on day 5 of flooding. Data for the WP in both +P/+F and +P/-F plants showed significantly higher negative values on days 3 and 5 of flooding compared to the control -P/-F plants. Therefore, PBZ pretreatment under flooding may induce an increase in the plant tissue water level, subsequently affecting the leaf WP.

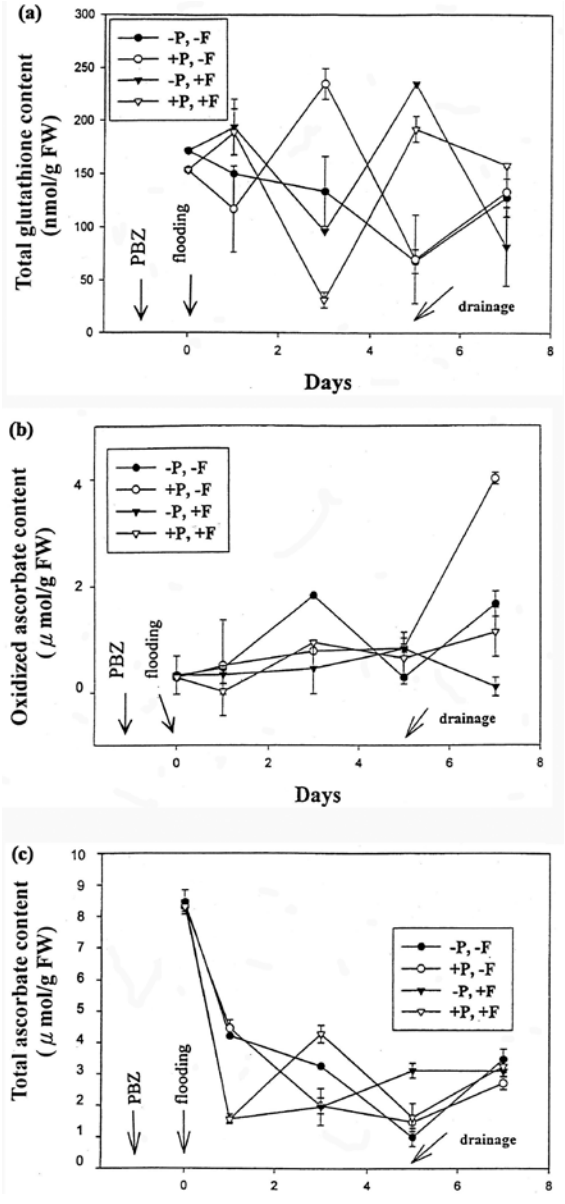

Figure 2. Effects of pretreatment with paclobutrazol on the (a) total glutathione, (b) oxidized ascorbate, and (c) total ascorbate content in leaves of sweetpotato subjected to flooding stress for different durations of treatment. Vertical bars represent the mean ± standard error. +P, paclobutrazol pretreatment; -P, no paclobutrazol treatment; +F, flooding stress; -F, non-flooded condition.

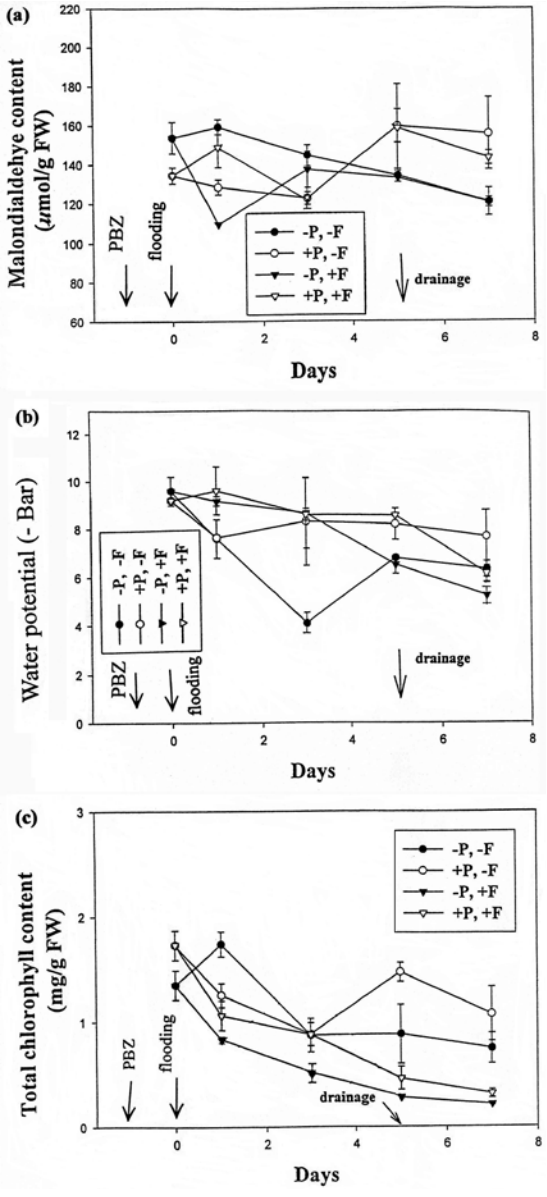

Figure 3. Effects of pretreatment with paclobutrazol on the (a) malondialdehyde content, (b) water potential, and (c) total chlorophyll content in leaves of sweetpotato subjected to flooding stress for different durations of treatment. Vertical bars represent the mean ± standard error. +P, paclobutrazol pretreatment; -P, no paclobutrazol treatment; +F, flooding stress; -F, non-flooded condition.

TCH was used as a physiological parameter to monitor the response of PBZ-pretreated sweetpotato under flooding stress (Figure 3c). The TCH contents in -P/-F, -P/+F, and +P/+F plants gradually decreased over the time course of the experiment, with the exception of elevated values from days 0 and 1 in -P/-F plants. During flooding stress, PBZ-treated plants (+P/+F) maintained significantly higher TCH amounts than did untreated plants (-P/+F). Plants under a non-flooded condition (both -P/-F and +P/-F) exhibited higher levels of TCH compared to those under flooded conditions (both -P/+F and +P/+F) on days 1, 5, and 7. Therefore, periods of flooding and drainage were accompanied by lower TCH values.

DISCUSSION

Comparisons between untreated plants under non-stressful conditions (-P/-F, as the control) and untreated plants subjected to a flooded condition (-P/+F)

Determination of the function of an observed response is one of the most complex issues in plant stress physiology. In this study, the involvement of the antioxidative system in the regulation of free-radical metabolism was followed by measuring changes in enzyme activities and antioxidant contents for comparisons between flooded and non-flooded conditions. On different days of treatment, plants responded differently to flooding stress according to the various components of their antioxidative system. Flooding conditions produced significantly higher APX, total glutathione, oxidized ASA, and total ASA contents than non-flooded conditions following no PBZ application on day 5 of flooding (Figures 1c, 2a-2c). In addition, after drainage, plants in the -P/+F condition exhibited significantly higher SOD and GR activities compared to those in the -P/-F condition (Figures 1a, 1d). In contrast, no signifcant differences for most of the treatment due to flooding stress appeared when CAT and MDA levels under -P/+F conditions were compared to those under -P/-F conditions (Figures 1b, 3a). Plants may prepare for oxidative damage by up-regulating APX, total glutathione, oxidized ASA, and total ASA under flooding conditions for 5 days. Therefore, increases in these enzymes and metabolites were observed before leaves became epinastic and senescent under flooding stress, and these identified systems could be used for rapid monitoring and early detection of flooding injury, i.e., in the seedling stage. This means that hundreds of individual plants might be screened per day, providing scope for the discovery of individuals that exhibit tolerance to flooding stress.

The effect of flooding on the plant's growth was also observed in this study. The lower leaves of the plants under flooding stress showed epinasty and senescence after 3 days of flooding; however, under a non-flooded condition, most leaves looked green and healthy (photos not shown). Flooding stress had a harmful effect on the sweetpotato, and changes in enzyme activities were related to the degree of chlorosis and reduced TCH content of the plant leaves during flooding. The leaves of flooded plants maintained a certain level of antioxidants in their systems, which scavenged at least part of the ROS. Antioxidative enzyme activities and antioxidant contents play major roles in maintaining the balance between free radical production and elimination. Enhancement of APX activity in a waterlogged environment may be an indicator of superoxide production (Figure 1c). High levels of APX should favor the scavenging of H_2O_2 produced by SOD and CAT. The APX found in organelles is believed to scavenge H_2O_2 produced from the organelles while the function of cytosolic APX is probably to eliminate the H_2O_2 that is produced in the cytosol or apoplasts and has diffused from organelles. In the study, we measured the variations of total enzyme activity. In most of the higher plants, algae and some bacteria, APX, SOD, POD, and CAT isozymes were distributed in four distinct cellular compartments: chloroplast (including stomata and thylakoid), microbody (including glyoxysome and peroxisome), mitochondria, and cytosome (Shigeoka et al., 2002; Jang et al., 2004; Kim et al., 2004). In the chloroplast of sweetpotato, H_2O_2 can be detoxified by the ASA-GSH-NADPH system catalyzed by swAPX1, and thus help to overcome the oxidative stress induced by abiotic and biotic stresses (Park et al., 2004). High levels of glutathione are the result of increased GSH biosynthesis. The level of MDA is one of the measures of whether plant cells are damaged by oxidative stress. Lower levels of MDA indicate better oxidative stress tolerance. In this study, MDA content of 1-day flooded plant (-P/+F) was lower (or non-significant different) than non-flooded (-P/-F) plant, indicating low cell damage in the flooded TN57 (Figure 3a). The increased level of total glutathione in 1-day flooded plant might help to reduce the MDA level while the increased APX activity might help 5-day flooded plant and drained plant show the same level of MDA content as the non-flooded plant, which would indicate that TN57 is tolerant to flooding stress. Plants are more flooding-tolerant if the increased ROS level under flooding can lead to an enhanced ROS-scavenging system. ROS scavenging is important in imparting tolerance against flooding stress (Noctor and Foyer, 1998; Blokhina et al., 2003).

Previously, we reported that the GR activity in Taoyuan 2 sweetpotato, the leaves of which are consumed as a popular vegetable, was significantly enhanced over 5 days of continuous flooding, in comparison with non-flooded conditions (Lin et al., 2006). The present study indicates that APX, total glutathione, oxidized ASA, and total ASA levels affected the defense mechanism of Tainung 57 plants under a flooded condition. Different varieties displayed variations in their antioxidative systems, and the differential expressions of each genotype were associated with the flooding stress response. Specific genotype responses to flooding stress were correlated with resistance to oxidative stress. Gratão et al. (2005) reported that responses to oxidative stress induced by biotic and

abiotic stress may vary depending on plant species, tissue, and length of stress, apart from other specific aspects. Almeselmani et al. (2006) studied the effect of high temperature stress on the antioxidant enzyme activity in five wheat genotypes. There was significant increase in the activity of SOD, APX, and CAT in the late and very late planting and at all stages of plant growth, i.e., vegetative, anthesis, and 15 days after anthesis. However, GR activity decreased under late and very late plantings compared to normal planting. Lee and Lee (2000) reported that chilling stress enhanced the activities of SOD, APX, and GR in the leaves of cucumber while inducing a decrease in CAT activity. Cho and Park (2000) demonstrated that substantial increases in H_2O_2 content and SOD and CAT activities occurred under mercury-induced oxidative stress in 30-day-old tomato plants in comparison with controls.

Enhancement of the aforementioned antioxidative systems favors flooding resistance. Antioxidative enzymes may augment antioxidants in the removal of ROS from plant cells. These findings are important for farming in frequently flooded areas, and also informative for further genetic and physiological studies on sweetpotato flooding tolerance.

Comparisons between untreated plants under a non-flooded condition (-P/-F, control) and pretreatment with PBZ followed by a non-stressful condition (+P/-F)

Figures 1a, 1b, and 2c showed reduced SOD, CAT, and total ASA contents and increased MDA content in the interval of days 0 to 7 under a non-flooded condition with 0.5 mg PBZ/plant pretreatment. However, Figures 1c, 1d, 2a, 2b, and 3a reveal that PBZ pretreatment significantly increased the levels of APX, GR, total glutathione, and oxidized ASA under 0 to 7 days of non-stressful conditions. PBZ pretreatment might lead to prodution of ROS. Although APX and GR activities were enhanced to detoxify ROS, the reduced SOD, CAT, and total ASA levels might be the reason behind the increased MDA content. In our observations, compared to control plants (-P/-F), PBZ-treated plants under a non-flooded condition (+P/-F) appeared healthy and had greener leaves throughout the duration of the experiment (photos not shown). More work needs to be conducted to confirm the effect of PBZ on the production of sweetpotato under non-flooded condition.

Comparisons between untreated plants subjected to flooding stress (-P/+F) and pretreatment with PBZ followed by a flooded condition (+P/+F)

Compared to the -P/+F condition, SOD (at 3 and 5 days), CAT, oxidized ASA, and total ASA (at 3 days), APX (at 1 and 3 days), and GR (at 1, 3, and 5 days) in PBZ-pretreated sweetpotato were significantly enhanced under flooding stress (+P/+F) (Figures 1a-1d, 2b, 2c). Furthermore, the +P/+F condition showed a significantly higher MDA content than the -P/+F condition on days 1 and 5 of flooding and drainage (Figure 3a). PBZ pretreatment caused changes in the levels of various components of the antioxidative system under flooding stress.

As waterlogging was prolonged, the leaf WP of plants decreased more rapidly in untreated plants (-P/+F) than in PBZ-treated plants (+P/+F) (Figure 3b), indicating that the development of flooding stress in leaves was more gradual or perhaps delayed by PBZ treatment. Chlorosis of most waterlogged and untreated plants was visually higher than in PBZ-treated plants subjected to flooding stress. These observations imply that PBZ application might reduce or delay flooding stress thereby allowing those plants to survive and function during flooding. This ability can perhaps be attributed to an avoidance of flooding stress, as indicated by the higher WP in +P/+F plants than in -P/+F plants during the flooding time course. It is not clear how PBZ pretreatment improves the water status of leaves, but it might cause more-efficient water uptake by plants, by retarding water loss from plants during flooding stress, or both.

Generally speaking, PBZ application before flooding protects plants from the adverse effects of flooding. PBZ application affected enzyme activities, which had an impact on the flood tolerance and health of the plant. An anoxic condition is one of the major problems for plants under flooding stress. As a plant encounters anoxic stress, a stronger antioxidative system illustrates a superior flood-tolerant mechanism in terms of the ability to scavenge H_2O_2, O_2^-, $\cdot O_2$, and $\cdot OH$. By removal of O_2^- and increases in SOD and GR, the redox potential and important components of the electron transport system may be altered, hence facilitating improved stress tolerance. Pinhero et al. (1997) showed that PBZ treatment of maize induced several changes in the antioxidative system profiles and especially enhanced the activities of SOD, GR, and APX, along with the induction of chilling tolerance. Kraus and Fletcher (1994) proposed that PBZ-induced protection of wheat from damage caused by heat stress was mediated by increased SOD, APX, and GR activities. In our study, pretreatment with PBZ could be an important strategy for altering the behavior and survival of the sweetpotato under flooding stress. PBZ apparently plays an important role in the antioxidative system under flooding stress. Flooding stress protection conferred by PBZ was mediated to some extent by an enhanced antioxidative system. Our results suggest that flooding stress effects on the sweetpotato can be mitigated by 0.5 mg PBZ/plant. Further characterization of the isoforms of each antioxidative enzyme will be helpful in elucidating whether or not there are distinct effects and responses among isozymes to the PBZ pretreatment under flooding stress. If the PBZ and flooding treatments have affected one particular antioxidative isozyme, this may indicate correlation to a cell physiological phenomenon due to the specific organelle localization of this isozyme. To know exactly which isoform is related to the stress response,

isoenzyme assays, i.e., activity staining is needed.

Comparisons between untreated plants under a non-flooded condition (-P/-F as the control) and pretreatment with PBZ followed by a flooded condition (+P/+F)

CAT and APX activities, and oxidized ASA and total ASA contents of the sweetpotato pretreated with PBZ, eventually showed the same levels after flooding stress and drainage as did untreated plants under a non-flooded condition (Figures 1b, 1c, 2b, and 2c). Moreover, pretreatment with PBZ eventually enhanced SOD and GR activities and total glutathione and MDA contents after flooding stress and drainage compared to the untreated and non-flooded condition (Figures 1a, 1d, 2a, and 3a). Generally speaking, pretreatment with PBZ improved the antioxidative system of the sweetpotato under flooding followed by drainage for 2 days to a level at least similar to that of the control. These results suggest that SOD, GR, total glutathione, and MDA values are flooding-specific and not expressed solely in response to PBZ pretreatment. Plants with various antioxidative systems respond differently to flooding stress according to the durations of the flooding period and subsequent drainage period. In ABA-treated turfgrass, SOD and CAT activities also markedly increased after ABA treatment and were maintained at higher levels during drought stress (Lu et al., 2003). Agarwal et al. (2005) reported that 0.05 mM of H_2O_2 increased the activities of SOD, APX, CAT and NADPH oxidase in wheat genotypes C306 and Hira. Liu et al. (2007) mentioned that hematin promoted both SOD and CAT activities of rice seeds under salt stress.

Flooding had effects on both the leaf WP and TCH. WP can be used as a parameter of flooding tolerance. WP decreased with progressive soil flooding, indicating that water relations of sweetpotato suffered from flooding injury. As PBZ application to flooding-stressed plants can improve the water status of plants (Figure 3b), it is reasonable to expect that this in turn may lead to a favorable effect on the TCH content (Figure 3c). Flooding may induce stomatal closure and consequently reduce WP and TCH levels. TCH has been widely examined in a number of plants to determine injury or tolerance to various environmental stresses including drought, chilling, heat, and radiation (Ahmed et al., 2002). Typically, the TCH amount is reduced by stressful conditions. This is consistent with our observations that flooding stress aggravated senescence in the leaves of -P/+F plants that was associated with a significant loss of TCH. The lower leaves of the plants under flooding stress showed senescence after 3 days of flooding while most leaves looked green and healthy under non-flooded conditions. Simultaneously, with the flooded plants (-P, +F), along with the characteristic visual symptoms, a significantly lower content of TCH was observed compared to controls (-P, -F) (Figure 3C). In addition, the light induced chlorophyll accumulation was gradually inhibited by the increasing flooding time. Stomatal closure causes a decrease in internal CO_2 concentrations. Subsequently, a concomitant decline in photosynthesis results from the diminished availability of CO_2 for carbon fixation (Carvalho and Anancio, 2002). Reduction of the CO_2 concentration increases the amount of harmful ROS within the leaf due to ongoing light reactions, which leads to senescence of the plant. Along with the visible symptoms, the reduced TCH could be used to monitor the flooding induced damage in both green or senescent leaves.

The present work studied changes in the antioxidative system involved in detoxification of ROS in sweetpotato responding to flooding stress. Compared to -P/-F plants, +P/+F plants had higher TCH contents (Figure 3c) in coordination with higher functions of APX (Figure 1c, on days 0, 1, and 3), GR (Figure 1d, on days 1, 3, 5, and 7), total glutathione (Figure 2a, on days 1, 5, and 7), and total ASA (Figure 2c, on days 3 and 7), and lower production of MDA (Figure 3a, on days 0, 1, and 3), implying that PBZ-treated plants had a better waterlogging tolerance capacity. Oxygen is required for flooding-induced inactivation of photosynthesis to take place, which suggests that the formation of ROS seemed to be the cause of the damage to chlorophyll during exposure to flooding. A decrease in the TCH use of radiation absorbed by pigments can lead of the production of potentially dangerous ROS (Ahmed et al., 2002). The ROS were then enhanced in the leaves of sweetpotato. High rates of electron transport together with a flooding-induced decrease in carbon metabolism increase the probability of electron transfer to oxygen in the Mehler reaction, thus forming superoxides and finally increasing oxidative stress. The ROS can potentially be detoxified by an efficient chloroplastic oxidative system. In order to keep the electron transport chain oxidized and prevent ROS formation, electrons must be efficiently consumed in the leaves of sweetpotato by the Calvin cycle or other electron sinks.

CONCLUSIONS

The APX activity and contents of total glutathione, oxidized ASA, and total ASA were characterized by an increasing trend for different durations of flooding. Leaves of the sweetpotato under flooding stress generate ROS that may then be removed by the aforementioned antioxidative enzymes and antioxidants. The presence of these antioxidants may be a useful criterion in the early screening of flooding-tolerant sweetpotato for the ability overcome flooding stress. Furthermore, the antioxidative system level under non-flooded conditions was highly regulated by PBZ pretreatment. APX, GR, total glutathione, oxidized ASA, and MDA levels in the sweetpotato were significantly induced by 0.5 mg PBZ/plant pretreatment for different durations of treatment. Under non-stressful condition, PBZ pre-addition increasedantioxidative enzyme and antioxidant levels against insults from ROS and rendered it better able to respond to flooding stress. Levels of all components of the

antioxidative system at different durations of treatment were enhanced by adding PBZ before flooding, and GR exhibited especially higher activity levels in plants subjected to flooding treatments and drainage. PBZ pretreatment showed significant effects on both leaf WP and TCH content under flooding stress. PBZ may protect the sweetpotato from flooding stress through its influence on the oxygen-detoxifying system. Therefore, applying 0.5 mg PBZ/plant 24 h before flooding may mitigate flooding stress.

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巴克素對甘藷在淹水逆境下抗氧化系統之效應

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本研究探討巴克素 (Paclobutrazol, PBZ) 前處理對淹水逆境甘藷之抗氧化酵素與抗氧化物的影響。甘藷品種臺農 57 號栽種於網室內之塑膠盆，維持適當水分 45 天後，以 PBZ (0 及 0.5 mg/plant) 處理 1 天，接著淹水至土表上 5 cm 至 5 天，而後排水 2 天，以多因子設計逢機完全區集 3 重複，每植株採完全展開之幼葉，測定抗氧化酵素活性與抗氧化物含量。結果顯示，經過不同天數淹水，增加的抗壞血酸過氧化酶活性、以及總穀胱甘肽、氧化態抗壞血酸與總抗壞血酸之含量提供甘藷較高之淹水耐受性。甘藷在無淹水狀態下，穀胱甘肽還原酶與抗壞血酸過氧化酶之活性以及總穀胱甘肽、氧化態抗壞血酸與丙二醛之含量受 PBZ 前處理之調節而增加，PBZ 可能促進甘藷維持活化氧族生成與解毒間平衡的能力。甘藷對淹水逆境具保護機制，PBZ 前處理使此潛力表現，PBZ 前處理增加甘藷不同淹水時間與排水後所有抗氧化酵素活性與抗氧化物含量，提高甘藷之淹水耐受性，尤其是丙二醛含量受高度調節。

關鍵詞：巴克素；抗氧化酵素；抗氧化物；淹水；甘藷。