Botanical Studies (2006) 47: 417-426.

The effects of flooding and drought stresses on the antioxidant constituents in sweet potato leaves

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(Received January 12, 2006; Accepted April 14, 2006)

ABSTRACT. Environmental stress results in generation of reactive oxygen species in plants and causes oxidative stress. The aim of this work was to study the changes to the antioxidative system in the leaves of three sweet potato varieties, Taoyuan 2, Simon 1, and Sushu 18 as affected by flooding and drought stresses. The experimental design was completely randomized with a split plot arrangement of treatments. Young, fully expended leaves from each plant were clipped for antioxidant activity measurement. We concluded that genotypes exhibited their abilities and specificities on porphyrins, polyphenol, flavonoids, reduction power and scavenging DPPH radical and superoxide anion. The polyphenol content and scavenging superoxide anion percentage of the three sweet potato varieties under the stresses declined significantly. However, the conjugated dienes inhibition percentage increased markedly under the stresses. Inhibiting the conjugated dienes could mitigate flooding and drought stress effects and be useful as a flooding and drought-tolerant index.

Keywords: Antioxidative activity; Drought stresses; Flooding stress; Sweet potato.

INTRODUCTION

Drought and flooding are considered to be predominant factors determining the global geographic distribution of vegetation and restriction of crop yields in agriculture. Environmental stress severely affects plants because the production and scavenging of the reactive oxygen species (ROS) in plants loses its equilibrium (Crawford and Brandle, 1996). Symptoms of flooding or drought injury include chlorophyll breakdown, protein degradation, membrane permeability decrease, peroxidation, slower leaf expansion, petiole epinasty, and stomatal closure (Moran et al., 1994; Gogorcena et al., 1995). Stomatal closure causes a decrease in internal CO_2 concentration. Subsequently, a concomitant decline in photosynthesis resulted from the diminished availability of CO_2 for carbon fixation. Reduction of CO_2 concentration increases the amount of harmful ROS within the leaf due to ongoing light reaction, which leads to senescence and even death of the plant (Schwanz et al., 1996; Carvalho and Amancio, 2002; Keles and Dunl, 2002; Sairam et al., 1997). Roots suffer from periodic or prolonged deprivation of water or 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 NAD(P)H, and a decline in the generation of ATP (Asada, 1992; Kennedy et al., 1992). In plant cells, the oxidative stress reactions are associated with toxic free radicals from the reduction of molecular oxygen to superoxide radicals ($O_2^{\cdot -}$), singlet oxygen ($^1O_2$), hydroxyl radicals ($\cdot OH$), hydrogen peroxide (H_2O_2), and peroxyl radicals ($ROO\cdot$). These radicals can inactivate various Calvin-cycle enzymes and are involved in oxidative systems (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 (Perata and Alpi, 1993).

In recent years, increasing attention has been paid

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by consumers to the health and nutritional benefits of sweet potato leaves. A diet rich in leafy vegetables, including sweet potato leaves, offers production against some common diseases such as cardiovascular and cerebrovascular events, cancer, and other age-related degenerative diseases (Hayase and Kato, 1984; Huang et al., 2004; Scalzo et al., 2005). These protective effects are considered, in large part, to be related to the various antioxidants contained in them. Evidence that free radicals cause oxidative damage to lipids, proteins and nucleic acids is overwhelming. Antioxidants, which can inhibit or delay the oxidation of an oxidizer in a chain reaction, would therefore seem to be very important in the prevention of these diseases (Yen and Chung, 2000; Leong and Shui, 2002). These benefits have stimulated research to investigate the content, ability, capacity, and function of antioxidative systems in leafy sweet potato. Leafy vegetables contain antioxidant nutrients, in addition to vitamin C or E and carotenoids, which significantly contribute to their antioxidant capacity. Phenolic substances such as flavnoids are the most common compounds in leafy vegetables and have strong antioxidant activity (Prior et al., 1998; Chu et al., 2000; Hollman and Arts, 2000; Proteggente et al., 2002).

Sweet potato is the world's fifth most important crop and is a major source of food and nutrition in developing countries (The International Potato Center, Lima, Peru, 1998). It is a plant species resistant to drought stress because its deep-root system accesses moisture. The increasing demand for food with growth of the world population has resulted in an increasing use of flooding-sensitive or drought-sensitive crops in wetland, lowlands, or drylands. In Taiwan, waterlogging is one of the primary physiological constraints to sweet potato production in the summer season every year due to short, intensive rainfall. Moreover, the wet-dry tropical environment of Taiwan is characterized by prolonged seasonal drought. During the dry season (October to January), little or no rain falls, and temperatures, solar radiation, and vapor pressure deficits are high. Plants possess different antioxidant properties, depending on their content of antioxidant molecules, which is, in turn, strongly affected by the specific plant genotype and environmental conditions of the plant. The interaction of these different factors in determining the antioxidant capacity and ability of a plant should be established to better characterize agronomic production. Little has been done to study antioxidative activity in response to flooding or water-depletion stresses in sweet potato. The long-term goal of our work is to help breed flooding-tolerant and drought-tolerant sweet potatoes to be grown in the summer season and winter season, respectively. The present research project studied the antioxidative systems of the leaves of sweet potato exposed to flooding and drought conditions. The system may be useful in screening for flooding-tolerant and drought-tolerant plants. The results provide information on the effects of various amounts of antioxidative capacity and ability on the flooding and drought tolerances in the sweet potato.

MATERIALS AND METHODS

Plant materials, cultural practice, experimental design and treatments

Three sweet potato (*Ipomoea batatas* [L.] Lam) cultivars, Taoyuan 2, Simon 1, and Sushu 18 were used. 'Taoyuan 2' is a variety grown in Taiwan that is popular for the consumption of its leaves. Sushu 18 is a drought-tolerant variety from China (JAAS and SAAS, 1984). The storage roots of Simon 1 contain high amounts of vitamin K. The planting was conducted in the screen house of Taoyuan District Agricultural Research and Extension Station in Taiwan. Terminals about 30 cm in length were taken from sturdy vines and cultivated with 25×25 cm density in June, 2001. The plants were watered three times per week to the field capacity by drip irrigation system and allowed to grow for 45 days before water stress and flooding imposition. The soil was sandy loam with pH 6.8. Average day/night temperature was 34/26°C and the average day length was 14 h during the study period. The size of the experimental field was 450 m^2. Water treatments were carried out in a split-plot design with four replications of completely randomized design. The three varieties were arranged as the sub-plot with five plants per replication. The main-plots included a 3-day flooding (watering to the soil level), no water irrigation to the field for a two-week period (drought treatment), and non-flooding treatments (control). After each treatment, the youngest fully expanded leaves of each plant were washed and clipped for antioxidant activity measurement.

Sample preparation and extraction

The cut leaves of each treatment were lyophilized and ground to powder. Five milligrams of powder were extracted with a 5-fold volume of methanol at room temperature, and then filtered through Whatman #1 filter paper. The remaining residue was re-extracted thrice until the residue was colorless. The three extracts were combined, concentrated to a powder by freeze dryer (LabConco, Japan), and then stored in a -20°C freezer for later analysis.

Determination of polyphenol content

Polyphenol content was measured by the Folin-Ciocalteu method (Taga et al., 1984; Singleton et al., 1999). One hundred microliters of the methanolic extract was added to 1 mL distilled water and 2 mL of Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 min, and 2 mL of 2% sodium carbonate was added to the mixture followed by gentle mixing. After standing at room temperature for 30 min, the absorbance was read at 750 nm. Deionized water was used as a blank. The standard calibration curve was plotted using gallic acid. The content of polyphenol was expressed as mg gallic acid equivalent / g extract.

Carotenoid and porphyrin content measurement

Carotenoid and porphyrin concentrations were determined as decribed Lichenthaler (1987) and Porra et al. (1989) and modified by Yang et al. (1998). Five milligrams of samples were homogenized with 5 mL of 80% acetone in a cooled mortar. Extract was centrifuged for 5 min at 1,500 *g*, and the supernatant was stored. The pellet was re-extracted with acetone and centrifuged again. This process was continued until the supernatant was colorless, and then the supernatant was pooled.

1. Absorbance was measured at 663.6, 646.6 and 440.5 nm, the major absorption peaks of chlorophyll a and b and carotenoids, respectively. Carotenoids were calculated using the following equation: $(4.69 \times A_{440.5} - 1.96 \times A_{663.6} - 4.74 \times A_{646.6}) \times$ volume of supernatant (mL) × dilution factor / sample weight (g).

2. Absorbance was measured at 663.6, 646.6, 440.5, 575, 590 and 628 nm, the absorption peaks of chlorophyll a, chlorophyll b, carotenoids, protoporphyrin, magnesium-protoporphyrin and protochlorophyllide, respectively. Porphyrin contents were summed (A+B+C) by the following three equations:

$A = [(12.25 \times A_{663.6} - 2.55 \times A_{646.6}) \times$ volume of supernatant (mL) × diluted factor / sample weight (g)] / 892 × 1000

$B = [(20.31 \times A_{646.6} - 4.91 \times A_{663.6}) \times$ volume of supernatant (mL) × diluted factor / sample weight (g)] / 906 × 1000

$C = [(196.25 \times A_{575} - 46.6 \times A_{590} - 58.68 \times A_{628}) + (61.81 \times A_{590} - 23.77 \times A_{575} - 3.55 \times A_{628}) + (42.59 \times A_{628} - 34.32 \times A_{575} - 7.25 \times A_{590})] \times$ volume of supernatant (mL) × dilution factor / sample weight (g).

Determination of flavonoid content

The flavonoids were determined according to the method of Geissman (1995). 80% ethanol containing 1% HCl of solvent was used to extract 0.05 g of the powder samples. The mixture was vigorously shaken for 30 min, followed by centrifuging at 4°C, 1,500 *g* for 15 min. Sampling of the supernatants was taken to measure the absorbance at 540 nm. Flavonoid contents were calculated as $A_{540} \times$ volume of supernatant (mL)/sample weight (g).

DPPH free radical-scavenging assay

The free radical scavenging ability of the sweet potato was measured using the protocols described by Shimada et al. (1992) and Yoshiki et al. (2001). Briefly, an aliquot of 4 mL of the methanolic extract (4, 6 and 8 mg/mL) was added to 1 mL of 10 mM DPPH (2, 2-diphenyl-1-picrylhydrazyl) solution freshly prepared in methanol. The mixture was left in the dark for 30 min, and decolorization of DPPH donated H^+ was followed by measuring the absorbance at 517 nm. DPPH radical-scavenging activity was calculated from the absorption according to the following equation: DPPH radical-scavenging activity % = $[(A_{control} - A_{sample}) / A_{control})] \times 100$.

Measurement of reducing power

The reducing property of the crude extract was determined according to the method of Pulido et al. (2000). Briefly, an aliquot of 0.5 mL of the methanolic extract (2, 4 and 8 mg/mL) was mixed with an equal volume of 0.2 M sodium phosphate buffer (pH 6.6) and 1% potassium ferrocyanide. The mixture was incubated at 50°C for 20 min. Then an equal volume of 10% trichloroacetic acid was added to the mixture, which was then centrifuged at 1,500 *g* for 10 min. One milliliter of the supernatant was mixed with equal volume of distilled water and 0.2 mL of 0.1% $FeCl_3 \cdot 4H_2O$. After 10 min, the absorbance at 700 nm was measured. Increased absorbance of the reaction mixture indicated elevated reducing power. Reduction capacity = sample of A_{700} - control of A_{700}.

Determination of scavenging activity of superoxide radical

The superoxide anion scavenging activity of the methanolic extracts was determined according to the method of Robak and Gryglewski (1988) with modifications. An aliquot of 1.0 mL of methanolic extract (0.5 and 1 mg/mL) was added to an equal volume of 120 μM phenazine methosulphate (PMS), 936 μM dihydronicotinamide dinucleotide (NADH) and 300 μM nitro-blue tetrazolium (NBT) in 0.1 M phosphate buffer (pH 7.4). The mixture was left at room temperature for 5 min, and the absorbance at 560 nm was measured. The lower absorbance indicated higher scavenging activity. Superoxide radical-scavenging activity % = $[(A_{control} - A_{sample}) / A_{control})] \times 100$.

The inhibition of conjugated dienes formation in linoleic acid emulsion

The inhibition of conjugated dienes formation was determined according to the method of Mitsuda et al. (1966). An aliquot of 0.05 mL of each methanolic extract (0.3125 mg/mL) was added to 1 mL of 10 mM linoleic acid emulsion (pH 6.6). The mixture was shaken and incubated at 37°C for 15 h. One hundred microliters of the solution from a 0- and 15-h incubation period was separately added to 3.5 mL of 80% methanol. The absorbance at 234 nm was then measured. The percent inhibition of linoleic acid peroxidation was calculated as: Inhibition % = $[(A_{control} - A_{sample}) / A_{control})] \times 100$

The general chemicals used in this study were purchased from Sigma Co. (MO, USA) unless otherwise noted. All spectrophotometer analyses were conducted on a Hitachi U-2000 type spectrophotometer (Japan).

Statistical analysis

The measurements of antioxidants were analyzed by a two-factor completely randomized ANOVA that compared the water conditions and the varieties. For the significant

values, means were separated by the least significant difference (LSD) test at P≦0.05, 0.01 or 0.001, using PC SAS 8.2 (SAS Institute, Cary, NC). Among three varieties, means with the same small letters were not significantly different and presented in Tables 2 to 8. Among three water treatments, means with the same capital letters were not significantly different and shown in Tables 2 to 8. Each value was the mean of four replicate analyses.

RESULTS

The effects of water treatments on the three sweet potato varieties were monitored by measuring changes of antioxidant contents and capacity. Except for the contents of carotenoids and conjugated dienes inhibition, main effects of variety (V) on the measured components of the antioxidative system displayed significant differences across varieties (P≦0.01 and 0.05) (Table 1). Across different water treatment (W), there were significant differences in the polyphenol, reduction power, scavenging of superoxide anions, and conjugated dienes inhibition. Additionally, the amounts in each component of the antioxidative system exhibited a non-significant difference in the interaction effect (V × W), except for those of polyphenol and scavenging superoxide anion.

Table 2 presents the effect of water conditions on the antioxidant content in the leaves of three sweet potatoes. Porphyrin content did not show any significant difference among water treatments. However, Sushu 18 showed a significantly higher porphyrin content than Taoyuan 2 or Simon 1. Thus, different genotypes displayed variations in their porphyrins. When polyphenol content under three water treatments with different varieties was compared, normal water treatment had significantly higher polyphenol content than either flooding or drought stresses (Table 2). Furthermore, in Sushu 18, polyphenol content

Table 1. ANOVA of main effects of variety (V), water treatment (W) and their interaction (V × W) for porphyrin, carotenoid, polyphenol and flavonoid contents, and scavenging DPPH radical, reduction power, scavenging superoxide anion, and conjugated dienes inhibition capacities.

Source of variance	Degree of freedom	Significance							
		Porphyrins (μg/g)	Carotenoids (μg/g)	Polyphenol (μg/g)	Flavonoids (A540/g)	Scavenging DPPH radical (%)	Reduction power	Scavenging superoxide anion (%)	Conjugated dienes inhibition (%)
Variety (V)	2	*	NS	**	**	*	*	*	NS
Water (W)	2	NS	NS	**	NS	NS	*	**	**
V × W	4	NS	NS	**	NS	NS	NS	NS	NS

**: P≦0.01; *: P≦0.05; NS: non-significant difference.

Table 2. The effect of drought and flooding treatment on the porphyrin, carotenoid, polyphenol and flavonoid contents of sweet potato leaves.

Variety	Water treatment	Porphyrins (μg/g)	Carotenoids (μg/g)	Polyphenol (mg/g)	Flavonoids (A540/g)
Taoyuan 2	Control	31540 b	1553 NS	0.35 aA	132.8 b
	Drought	38570 b	1507 NS	0.20 aB	130.2 b
	Flooding	32104 b	1624 NS	0.21 aB	146.7 b
Sushu 18	Control	51980 a	1657 NS	0.11 bA	157.7 a
	Drought	43577 a	1714 NS	0.02 bB	176.0 a
	Flooding	43746 a	1538 NS	0.01 bB	163.7 a
Simon 1	Control	36051 b	1576 NS	0.39 aA	138.8 b
	Drought	28775 b	1480 NS	0.27 aB	132.7 b
	Flooding	32854 b	1315 NS	0.24 aB	144.2 b

Among three varieties, means with the same small letters were not significantly different by the least significant difference (LSD) at P≦0.05 with completely randomized design.

Among three water treatments, means with the same capital letters were not significantly different by the least significant difference (LSD) at P≦0.05 with completely randomized design.

NS: non-significant difference; Each value was the mean of four replicate analyses.

was significantly lower than Taoyuan 2 and Simon 1. The pattern and trend of flavonoid content appeared similar to those of porphyrin content in Table 2. A significantly higher level of flavonoid was observed in Sushu 18 than in Taoyuan 2 and Simon 1. Nevertheless, the flavonoid content in response to various water treatments displayed non-significant difference.

DPPH radical is scavenged by antioxidants through the donation of hydrogen, forming the reduced DPPH-H. The color changes from purple to yellow after reduction, which can be quantified by its decrease of absorbance at wavelength 517 nm. Table 3 indicates that Simon 1 exhibited a significantly higher scavenging DPPH radical percentage than Sushu 18 and Taoyuan 2 at 4 mg/mL and 8 mg/mL of the extract, respectively. For Sushu 18, the percentage of scavenging DPPH radicals was significantly lower than either Taoyuan 2 or Simon 1 at 6 mg/mL of the extract.

Table 4 illustrates that the reduction power percentage was different among the varieties under water treatments. Simon 1 demonstrated a significantly higher level of reduction ability compared to Taoyuan 2 at 2 and 4 mg/mL of the crude extract. Additionally, plants of 3 varities under drought stress induced significantly higher reduction ability than those under flooding treatment at 8 mg/mL of the extract. Flooding might result in a decreased reduction ability in 8 mg/mL of the extract following plant oxidative damage.

In Taoyuan 2, the scavenging superoxide anion percentage was significantly higher than Sushu 18 and

Table 3. The effect of drought and flooding treatment on scavenging DPPH radicals of sweet potato leaves.

Variety	Water treatment	Scavenging DPPH radicals %		
		4 mg/mL	6 mg/mL	8 mg/mL
Taoyuan 2	Control	31.2 ab	46.2 a	43.2 b
	Drought	27.8 ab	38.0 a	42.7 b
	Flooding	20.0 ab	36.3 a	41.0 b
Sushu 18	Control	11.2 b	29.1 b	48.7 ab
	Drought	19.3 b	25.9 b	51.9 ab
	Flooding	15.5 b	24.1 b	60.2 ab
Simon 1	Control	30.3 a	37.4 a	59.1 a
	Drought	41.1 a	40.3 a	65.0 a
	Flooding	25.9 a	42.2 a	67.5 a

Among three varieties, means with the same small letters were not significantly different by the least significant difference (LSD) at $P \leqq 0.05$ with completely randomized design.
NS: non-significant difference; Each value was the mean of four replicate analyses.

Table 4. The effect of drought and flooding treatment on the reduction power of sweet potato leaves.

Variety	Water treatment	Reduction power		
		2 mg/mL	4 mg/mL	8 mg/mL
Taoyuan 2	Control	0.2 b	1.0 b	2.4 AB
	Drought	0.6 b	0.7 b	2.6 A
	Flooding	0.5 b	0.5 b	2.0 B
Sushu 18	Control	1.0 ab	1.7 a	2.4 AB
	Drought	1.2 ab	2.0 a	2.9 A
	Flooding	0.9 ab	1.6 a	2.3 B
Simon 1	Control	1.4 a	2.0 a	2.4 AB
	Drought	1.0 a	1.7 a	2.8 A
	Flooding	1.0 a	1.7 a	2.0 B

Among three varieties, means with the same small letters were not significantly different by the least significant difference (LSD) at $P \leqq 0.05$ with completely randomized design.
Among three water conditions, means with the same capital letters were not significantly different by the least significant difference (LSD) at $P \leqq 0.05$ with completely randomized design.
NS: non-significant difference; Each value was the mean of four replicate analyses.

Simon 1 at 0.5 mg/mL of the extract (Table 5). Moreover, significantly higher percentages of scavenging superoxide anion were detected under normal water conditions compared to flooding stress at both 0.5 and 1 mg/mL of the extract. Additionally, significantly different results among genotype were observed at 0.5 mg/mL of the extract only. It is noteworthy that Taoyuan 2 under normal water treatment (33.5%) displayed a threefold increase over Simon 1 exposed to flooding stress (10.1%) at 0.5 mg/mL of the extract. Hence, the various water treatments showed significantly different levels of scavenging superoxide anions in the leaves of sweet potatoes. Either flooding or water-deficit stress showed a significantly higher conjugated dienes inhibition percentage than normal water conditions (Table 5). The conjugated dienes inhibition percentage appears to be involved in imparting tolerance against both flooding and drought stresses.

DISCUSSION

The determination of the function of an observed response is one of the most complex issues in plant stress physiology. In trying to understand responses to stresses involving drought and a waterlogged component, many enzymes and metabolites induced by periods of water depletion and flooding have been identified and characterized (Price et al., 1991; Ushimaro et al., 1992). Nevertheless, studies of the response of the antioxidative system of sweet potato in regard to its ability and capacity to survive water-deficit and flooding stresses are scarce. Enhancement in the production, ability and capacity of antioxidants may play an important role in metabolic stress tolerance. The effects of drought and flooding stresses on the antioxidant system of sweet potato leaf were thus examined in this study. The involvement of the antioxidative system in the regulation of free-radical metabolism was examined by measuring changes in antioxidant content, capacity, and ability under normal, flooding, and drought conditions. Tables 2 to 5 illustrate that the different varieties may prepare for oxidative damage by up-regulating their antioxidant contents. Porphyrins, polyphenol, flavonoids (Table 2), and DPPH radical scavenging at 4, 6, and 8 mg/mL (Table 3), reduction power at 2 and 4 mg/mL (Table 4) and superoxide anion scavenging at 0.5 mg/mL (Table 5) were involved in this process. Among the three varieties, Sushu 18 exhibited significantly higher levels of porphyrins and flavonoids (Table 2) than Simon 1 and Taoyuan 2 under control, drought and flooding conditions. On the other hands, polyphenol content for Taoyuan 2 and Simon 1 (Table 2), scavenging DPPH radicals percentage (4, 6 and 8 mg/mL) for Simon 1 (Table 3), reduction power (2 and 4 mg/mL) for Sushu 18 and Simon 1 (Table 4), and scavenging superoxide anion percentage (0.5 mg/mL) for Taoyuan 2 (Table 5) were significantly higher than for other varieties. These results imply that genotypes exhibited different abilities and specificities in their antioxidative systems. Past studies (Li and Staden, 1998; Sairam et al., 1998; Hwang et al., 1999; Herbinger et al., 2002) have demonstrated that drought or flooding sensitive species and cultivars have a lower antioxidant capacity than do tolerant species and cultivars. In our study, Chinese drought-tolerant entry ‘Sushu 18’ exhibited higher reduction power in 4 mg/mL extract probably due to higher flavonoids content.

The higher extract concentration, the higher percentage of scavenging DPPH radicals were found among the varieties (Table 3). An opposite trend in scavenging superoxide anion percentage can be observed in Table 5. Superoxide is a biologically important substance that

Table 5. The effect of drought and flooding treatment on the scavenging superoxide anions and conjugated dienes inhibition of sweet potato leaves.

Variety	Water treatment	Scavenging superoxide anions %		Conjugated dienes inhibition %
		0.5 g/mL	1 mg/mL	0.3125 mg/mL
Taoyuan 2	Control	33.5 aA	20.8 A	54.1 B
	Drought	30.3 aA	19.7 A	61.4 A
	Flooding	23.8 aB	13.4 B	68.6 A
Sushu 18	Control	18.8 bA	23.4 A	56.9 B
	Drought	13.6 bB	13.7 B	65.1 A
	Flooding	11.0 bB	14.8 B	68.2 A
Simon 1	Control	16.2 bA	28.6 A	60.7 B
	Drought	18.5 bA	16.0 B	64.8 A
	Flooding	10.1 bB	17.2 B	68.4 A

Among three varieties, means with the same small letters were not significantly different by the least significant difference (LSD) at $P \leqq 0.05$ with completely randomized design.

Among three water conditions, means with the same capital letters were not significantly different by the least significant difference (LSD) at $P \leqq 0.05$ with completely randomized design.

NS: non-significant difference; Each value was the mean of four replicate analyses.

can be decomposed and form stronger oxidative species such as singlet oxygen and hydroxyl radicals (Korycka-Dahl and Richardson, 1978). The highly reactive hydroxyl radicals can cause oxidative damage to DNA, lipids, and proteins (Grootvled and Jain, 1989). Too much superoxide anion may also damage the cell membrane by the adverse effect of stress, which leads to a decrease in the scavenging superoxide anion percentage in plant, and fails to bring about water-depletion or flooding tolerance. Thus, different genotypes responded differently to scavenge DPPH radicals or superoxide anions under various water treatments, and the differential expression of each genotype was associated with flooding or drought stress response.

Tables 2, 4 and 5 demonstrate the impact of drought and flooding stresses on the antioxidative system of sweet potatoes. The polyphenol content and scavenging superoxide anion percentage of all plants were significantly reduced under flooding stress as compared to normal water conditions (Tables 2 and 5). Reduction power at the 8 mg/mL concentration of all plants under drought stress was significantly higher compared to those under flooding stress (Table 4). Under drought stress, Sushu 18 and Simon 1 exhibited a significantly lower percentage of scavenging superoxide anion at a 1 mg/mL concentration than normal water treatment (Table 5). In contrast, with all plants subjected to flooding or drought stress, the conjugated dienes inhibition percentage was significantly increased as compared to normal water conditions (Table 5). Antioxidant activity plays a major role in maintaining the balance between the production and elimination of free radicals. Presumably, the accumulation of a component amount of the antioxidative system and ROS formation favored drought tolerance. An increased level of ROS in the water-depleted plants could lead to an increased capacity of the scavenging system of ROS, particularly in reduction power ability (Table 4). The impact of water deficit on plant growth and development varies depending on the severity of the water limitation, the duration of the stress, and the plants' developmental stage.

Sweet potato production is limited to the hot and wet summer season in Taiwan. Flooding has been an important factor affecting summer sweet potato production. In the flooded soil, oxygen limitation is one of the primary threats to plants. Plants are able to maintain radical damage through their natural defense mechanisms. Anoxic stress is a major factor in flooding conditions. When roots are submerged this condition inhibits aerobic respiration and less energy is yielded. The roots translocate less nutrients to the leaves. The solutes entering the leaves via the transpiration stream may also decrease (Yan and Dai, 1996). As the plants encounter anoxic stress, a higher level oxidative system illustrates their superior tolerance mechanisms in ROS scavenging over the plants. Our results indicate that the percentage of conjugated dienes inhibition increased under flooding and drought stresses, and this can be considered as a mechanism for overcoming these stresses. This antioxidative ability may be useful in screening for flooding-tolerant and drought-tolerant plants. Heavy rainfall in summer results in loss of fresh market production of sweet potato. High light stress usually also exerts its effect on sweet potato following heavy rainfall during summer. Previously, we have found that reduced susceptibility to waterlogging together with high-light stress was related to increases of superoxide dismutase and catalase activities in the leaves of sweet potatoes (Hwang et al., 1999). Hence, different stress conditions might generate different antioxidative mechanisms for tolerance.

From our observations, the lower leaves of each variety looked epinastic and senescent after 3-days of flooding and 14-days of drought. However, under control conditions, most leaves appeared healthy and sported green throughout the duration of the experiment (photos not shown). Flooding and drought stresses had a harmful effect on the leaves of sweet potatoes, and some of the damage was irreversible once flooding or drought injury was done. Antioxidative activities changed were related to degree of reduced chlorosis of the plant leaves during flooding and drought. When significant flooding-injury or drought-injury in appearance occurred, oxy-radical production increased. The polyphenol content, reduction power, scavenging superoxide anion percentage, and conjugated dienes inhibition percentage in the leaves of sweet potatoes under flooding and drought stresses were significantly affected. The leaves of sweet potatoes became more tolerant to flooding or drought, and oxidative processes may be associated with this tolerance. The degree of flooding-injury or drought-injury seems to be a result of enhancement of conjugated dienes inhibition, and of decline in polyphenol and scavenging superoxide anion levels in sweet potatoes. Enhancement of conjugated dienes inhibition percentage under stresses may be an indicator of prooxidant production. Many polyphenols can exhibit antioxidant content as their extensive, conjugated electron systems allow ready donation of electrons or hydrogen atoms from the hydroxyl moieties to free radicals. Most polyphenols are very effective scavengers of hydroxyl and peroxyl radicals and can stabilize lipid oxidation. They are chelators of metals and inhibit the Fenton and Haber-Weiss reactions, which are important sources of ROS (Yamasaki et al., 1997; Chang et al., 2002; Debarry et al., 2005). In addition, polyphenols retain their free radical scavenging capacity after forming complexes with metal ions. Transition metal ions accelerate free-radical damage. Antioxidant defenses protect the plant against oxidative burst, but they are not 100% efficient, and so free-radical damage must be constantly repaired. ROS scavenging is important in imparting tolerance to environmental stress (Dixon and Paiva, 1995). The aforementioned antioxidative activity may be limiting the defense mechanisms of susceptible plants under waterlogged or drought conditions.

Our data show that the carotenoid content (Table 2)

of all varieties was not significantly affected by water conditions. Additionally, chelating Fe^{+2} ion percent of the three genotypes showed no significant difference among the various water treatments with different concentrations of the extract (data not shown). The Fe^{2+}-binding activity was measured by the decrease in the maximal absorbance of iron (II)–ferrozine complex (Dinis et al., 1994). Flooding and drought stresses could increase both parameters in all plants to a level equivalent to those of normal water conditions. On the other hand, it might be that the increases of other antioxidant abilities compensate for the need for carotenoids and chelating Fe^{+2} ions under stresses. Quercetin, kaempferol, and myricetin are the three most common flavonols that are also the most widely distributed flavonoids (Lee et al., 1995). The cultivar difference in antioxidative activity may due to pigments that possess effective antioxidative activity alone or synergistically. These antioxidative systems might influence a plant's ability to maintain a balance between the formation and scavenging of ROS, making leaves vulnerable to oxidative stress. Changes in intercellular redox might be a consequence of flooding and drought stresses. Some oxidative systems are stimulated by the oxidative burst in the sweet potato cells, but some are not. ROS are important modulators of the cellular signal of transduction events following flooding-stress or drought-stress injury. Plants are tuned to the absolute levels of ROS because a small amount of change can result in drastically different responses (Perata and Alpi, 1993). Thus, carotenoids and chelating Fe^{+2} ions are genotype- and environment-independent antioxidative systems which may stimulate the restoration of leaf oxidative damage.

CONCLUSION

In conclusion, different varieties of sweet potatoes responded differently to oxidative injury flooding and drought stresses according to various components of their antioxidative systems. Among three varieties, Sushu 18 proved more resistant to drought and flooding than Taoyuan 2 and Simon 1 under field conditions. Our results indicate that the contents of all antioxidative systems in sweet potatoes, except for carotenoids and chelating Fe^{2+} ions, were significantly affected by the stresses. The polyphenol content and scavenging superoxide anion percentage of sweet potato leaves decreased under flooding and drought. On the other hand, the percentage of conjugated dienes inhibition under flooding and drought increased when plants under stress generated ROS that may then have been removed by the above mentioned antioxidative system. Inhibiting conjugated dienes was related to antioxidative activity of leafy sweet potato under the stresses and can be an index to screen stress-tolerance plants. These findings may have greater significance for farming in frequently flooded areas or dry-lands. Our findings are also informative for further genetic and physiological studies on sweet potato flooding or drought-tolerance.

Acknowledgements. This research was supported by grants from the Council of Agriculture, Taiwan. The authors are grateful to Prof. Shin-Shinge Chang for assistance and Mr. M.Y. Hwang for collecting papers and rendering this manuscript.

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淹水與乾旱逆境對甘藷葉片抗氧化物組成份之影響

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環境逆境使植物產生活化氧族，並造成氧化逆境。本研究探討 3 個甘藷品種桃園 2 號、西蒙 1 號與徐藷 18 號受淹水與乾旱逆境時葉片之抗氧化系統，試驗採完全逢機裂區設計，取每株完全開展之嫩葉測定抗氧化活性，不同品種之吡啉、多酚與類黃酮含量、以及還原力、掃除二苯基-苦味基團 (DPPH) 游離基與超氧陰離子之能力各有不同，逆境下三品種之多酚含量與掃除超氧陰離子百分比皆顯著降低，而共軛雙烯 (conjugated dienes) 抑制百分比顯著升高，可做為甘藷淹水與乾旱耐受性之指標，並緩和淹水與乾旱效應。

關鍵詞：淹水逆境；乾旱逆境；抗氧化功效；甘藷。