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Research Paper

Physiological responses of sweet potato seedlings under drought-stress conditions with selenium applications

Chongping Huang[1, 2*] • Mingyan Yu[1], Li Sun[1] • Nannan Qin[1] • Lai Wei[1]

[1]Department of Agronomy, College of Agriculture and Biotechnology, Zhejiang University, 866 Yu-Hang-Tang Road, Hangzhou 310058, China.
[2]Agricultural Experiment Station of Zhejiang University, 866 Yu-Hang-Tang Road, Hangzhou 310058, China.

*Corresponding author. E-mail: hcping@zju.edu.cn. Tel: +86-571-8820-8490.

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Abstract. Drought often occurs during sweet potato growth and development, and selenium (Se) has beneficial roles in alleviating biotic and abiotic stresses. Here, we investigated the changes in antioxidative enzyme activities in leaves and fibrous roots, photosynthetic rates and chlorophyll fluorescence levels in seedlings of two sweet potato cultivars 'Xinxiang' and 'Zheshu 77' under drought conditions after Se applications. Drought stress significantly changed the photochemical parameters and decreased the photosynthetic capability, as well as leaf and root antioxidant enzyme activities, in both cultivars. The Se applications reversed the negative effects of drought stress. Furthermore, applications of appropriate Se concentrations (0.5 to 1.0 $mg \cdot L^{-1}$) significantly decreased the malondialdehyde level, while increasing the superoxide dismutase and catalase activities in leaves and fibrous roots. The leaf relative water contents and the root vigor levels in the drought-stressed plants of both cultivars also significantly increased. The visual chlorophyll fluorescence images provided corroborating evidence. Thus, Se appears to enhance gas exchange, improve the leaf water status and alleviate drought-induced oxidative stress by regulating the sweet potato seedlings' antioxidant defense systems in the chloroplasts and root cells. Thus, roots and leaves may simultaneously contribute to the increased photochemical efficiency of Photosystem II, which allows higher photosynthetic rates to be maintained. In addition, the chlorophyll fluorescence images can increase our understanding of the ameliorative mechanisms.

Keywords: Sweet potato, drought stress, photosynthetic parameters, chlorophyll fluorescence visualization, antioxidant enzymes, root vigor.

INTRODUCTION

Sweet potato, *Ipomoea batatas* (L.) Lam, is the seventh most consumed crop in the world and the fourth most significant crop in China. It provides calories, proteins, vitamins, edible fiber and minerals for humans. It has saved millions of people from starvation during periods of food shortage and is presently popular with city dwellers in southeastern coastal China for obesity prevention and weight reduction. Its wide adaptability and good growth in various environments are advantages of sweet potato that ensure food supply and food safety in developing countries and dietary diversity in rich areas. Although sweet potato has a high tolerance against water deficiency, the yield is reduced. Thus, identifying measures to alleviate the negative impacts of drought stress is important.

Drought is often encountered during sweet potato growth and development, which negatively affects plant photosynthesis, stomatal movement, membrane integrity, pigment content, osmotic adjustment and plant growth and yield (Thangasamy *et al*., 2019; Bandara *et al*., 2014). Selenium (Se) enhances the activities of antioxidant enzymes *in vivo* and alleviates oxidation caused by drought and other abiotic stresses. Foliar spray applications of appropriate Se concentrations down-regulate auxin and ethylene biosynthesis in rice seedlings to modify primary metabolism and root architecture (Rafael *et al*., 2019). Se protects rice and sorghum plants from water-deficiency stress (Nesrine et al., 2018; Fabrício *et al*., 2018), *Dendrobium officinale* and strawberry leaves from chilling stress (Yanyan *et al*., 2013; Chongping *et al*., 2018) and sorghum leaves from high-temperature stress (Djanaguiraman *et al*., 2010) through the regulation of antioxidant enzymes and osmoprotectants. Se mitigates cadmium toxicity in *Brassica juncea* L. by up-regulating the antioxidative system and secondary metabolite production (Parvaiz *et al*., 2016; Neha *et al*., 2017), and in sunflower (Ghader, 2017) and tomato seedling (Ming *et al*., 2014), it decreases salt-induced damages by stimulating antioxidant activity levels. Se can also regulate the subcellular distribution of antimony to reduce its toxicity in paddy rice (Yongzhen *et al*., 2015).

Although Se can protect plants from biotic and abiotic stresses through varied metabolic mechanisms, the complete mechanism is not clear. Moreover, there are rare reports regarding the synergistic effects of antioxidant enzymes in leaves and roots that promote the photosynthetic system. In this study, we elucidated the role of Se in the protection of chloroplast membranes and the regulation of the photosynthetic and chlorophyll fluorescence parameters, as well as in the regulation of antioxidant enzyme activities in sweet potato leaves and roots under drought-stress conditions. The results provide new insights that increase our understanding of how antioxidant metabolism is regulated by Se during drought stress.

MATERIALS AND METHODS

Plant materials and growing conditions

The experiment was performed at the Agricultural Experiment Station of Zhejiang University (AES-ZJU) from January to October 2017. The original sweet potato seedlings of ‘Xinxiang’ (‘XX’) and ‘Zheshu 77’ (‘ZS77’) were provided by the Institute of Crop and Nuclear Technology Utilization, Zhejiang Academy of Agricultural Sciences. The seedlings were transferred to plastic pots (top and bottom pot diameters of 16 and 12 cm, respectively; pot depth of 17 cm) containing 1.0 kg loam soil from the Huajiachi Campus’ experimental field of AES-ZJU. The soil pH was 6.8, and it contained 25.58 $g·kg^{-1}$ total soil organic matter, 1.56 $g·kg^{-1}$ total nitrogen, 45.1 $mg·kg^{-1}$ available phosphorus and 46.6 $mg·kg^{-1}$ available potassium. The soil total Se content was 0.031 $mg·kg^{-1}$, classifying it as Se-deficient soil. Every pot contained one seedling, which had three grown leaves and was 8-cm high at planting, and they were grown in a greenhouse at the Zijingang Campus of AES-ZJU with a temperature regime of 25/20°C day/night and natural sunlight before the drought treatment. The plants were irrigated once every 2 or 3 d to avoid water stress. Based on the field production, 1 g of compound fertilizer (20:20:20%; nitrogen: phosphorous: potassium, respectively; provided by AES-ZJU) was applied to each pot every month. After a new leaf was fully spread, healthy and uniform seedlings (with 4 to 4.5 leaves and 11-cm high) were selected for experiments.

Se treatment and drought stress

The sweet potato seedlings were placed into three climate chambers (AGCM-113DC01, Hangzhou, China) at 25/20°C day/night, having a 12-h photoperiod with a photosynthetic photon flux density of 360 $\mu mol·m^{-2}·s^{-1}$ and a 80 ± 5% relative humidity for 7 d as a pretreatment. In another preliminary experiment, we found that the sweet potato seedlings became withered when the soil water content was lower than 7%. Thus, after a 7-d pretreatment, watering was stopped and the humidity was regulated to 35 ± 5%. The soil water content was gradually reduced and maintained at 8 ± 0.5% for 3 d. The soil water content was determined twice daily at 8 a.m. and 5 p.m. using a HH2 water determiner (Delta-T Devices, Cambridge, UK). The water was carefully replenished if the soil water content was lower than 8%. After the soil water content in all the plots was maintained at 8 ± 0.5%, the seedlings underwent independent foliar spraying with one of four Se concentrations (0.25, 0.5, 1.0 and 2.0 $mg·L^{-1}$, abbreviated as C1, C2, C3 and C4, respectively). There was also a distilled water control (C0). Another drought treatment (DR) lacked the distilled water spray, and a control (CK) was maintained at the regular soil water content of 20 ± 2%. There were 30 trays of plants in every treatment set, with three replications of 10 trays per replication, and the trays were randomly arranged. Every treatment was sprayed one time with 20 ml of sodium selenite (Na_2O_4Se) hydroponic solution. The soil water content was maintained for 3 d after Se was sprayed for sampling. The third and fourth leaves from the tops of the stems were taken at 24, 48 and 72 h after Se spraying. The apical fibrous roots (less than 5 cm from top) were collected at 24 and 48 h after Se spraying. The sampled roots were washed several times with distilled water and dried using neutral filter paper. All the green leaves and roots from the 10 trays of plants for each treatment were taken, frozen in liquid nitrogen and stored at −70°C.

Determination of the chlorophyll content and photosynthetic parameters

The method of chlorophyll content determination was proposed by Hartmut (1987) and described previously (Chongping *et al*., 2018). The photosynthetic parameters of net photosynthetic rate (Pn), leaf stomatal conductance (Gs), intracellular CO_2 concentration (Ci) and transpiration rate (Tr) were determined in the treated plants using LI-6400 portable photosynthetic equipment (LI-COR, USA). The air temperature, relative humidity, CO_2 concentration and photosynthetic photon flux density were maintained at 25°C, 85%, 380 $\mu mol \cdot mol^{-1}$ and 1,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$, respectively.

Chlorophyll fluorescence parameters and visualization

Chlorophyll fluorescence parameters and images were collected using a portable chlorophyll fluorometer (IMAGINGPAM, Walz, Germany) from 10:00 to 11:00 a.m. at 1 d after the Se treatment. The third or fourth full-grown leaf from the seedling tip was chosen for the determination. The leaves were maintained in the dark for 30 min before the measurement. The actinic pulse was 400 $\mu mol \cdot m^{-2} \cdot s^{-1}$, and the saturated pulse was 8,000 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The calculation parameters, the maximum chlorophyll fluorescence (Fv/Fm), which represents the maximal photochemical efficiency, the non-photochemical quenching coefficient (qN) and photochemical quenching coefficient (qP) and the effective photochemical quantum yield of Photosystem II (PSII), Y(II), also expressed as ΦPSII, were the same as described by Alexander *et al*. (2017) and Jie *et al*. (2012). The images of Fv/Fm and Y(II) were acquired from the data in each experiment and normalized to a false scale bar, ranging from 0.0 (black) to 1.0 (purple), as described by Murchie and Lawson (2013).

Determination of relative water content (RWC) and malondialdehyde (MDA) content

Similar or same leaves after chlorophyll determination were used in the RWC assay. The fresh weights (FWs), FWs at full turgor (TWs) and dry weights (DWs) of sample leaves were measured to determine the RWC [RWC (%) = (FW − DW/TW − DW) × 100]. The MDA content determination method was based on Feibo *et al*. (2003) and described previously (Chongping *et al*., 2016).

Determination of the antioxidant enzyme activities and root vigor

The total superoxide dismutase (SOD) activity was determined as described by Dagmar *et al.* (2001) and as provided in our previous studies (Chongping *et al*., 2016, 2018). The methods to determine catalase (CAT) and peroxidase (POD) activity levels were described previously (Chongping *et al*., 2018). The root viability was determined using the TTC (2, 3, 5-triphenyltetrazolium chloride) method as described by Qi (1993) and Zhihuan *et al*. (2020), and it was expressed as the capacity ($mg \cdot g^{-1} \cdot FW \cdot h^{-1}$) of TTC (from colorless) to reduce to formazan (red) in viable cells.

Determination of the Se content

The root tuber samples were washed in distilled water, cut to pieces and then placed in an oven to dry. Dried root tuber samples (0.2 g) were ground, transferred to digestion tubes, to which 10 ml of 5:2 7 M nitric acid:hypochloric acid (v/v) and incubated for 12 h. The contents were then heated in a furnace at 210°C for 4 h. After the solutions were cooled and diluted, measurements were carried out using graphite furnace atomic absorption spectrometry according to Djanaguiraman *et al*. (2010). Each sample was analyzed at least in triplicate. The Se concentration was expressed in $\mu g \cdot g^{-1}$ DW.

Statistical analyses

A statistical analysis was performed using a one-way analysis of variance. Comparisons between the treatment means were performed using a least significant difference test at the $P \leq 0.05$ level.

RESULTS

Effects of Se applications on the chlorophyll content and photosynthetic parameters

Chlorophyll plays an important role in crop photosynthesis. As shown in Tables 1 and 2, when grown in water-deficient soil, the total chlorophyll contents of sweet potato plants decreased. The total chlorophyll content decreased 23.72, 25.98 and 32.43% in 'XX' and 11.17, 20.84 and 26.68% in 'ZS77' receiving the DR treatment after 24, 48 and 72 h of drought stress, respectively, compared with CK. The impacts were alleviated using exogenous Se. The chlorophyll contents of seedling leaves of varieties 'XX' and 'ZS77' significantly increased after receiving different exogenous Se concentrations from C1(0.25 $mg \cdot L^{-1}$) to C2 (0.5 $mg \cdot L^{-1}$) or C3 (1.0 $mg \cdot L^{-1}$) and then decreased after C4 (2.0 $mg \cdot L^{-1}$), independent of the drought time. The chlorophyll content increased 37.16, 35.57 and 34.34% in 'XX' under C3-treatment conditions and 14.33, 20.88 and 23.05% in 'ZS77' under C2-treatment conditions after 24, 48 and 72 h of drought stress, respectively, compared with C0.

The net photosynthetic rate (Pn) of sweet potato was greatly affected by soil water content and significantly decreased during drought stress (Table 3). The spraying of an appropriate Se concentration alleviated the impacts

Table 1. Effects of drought stress on total chlorophyll content（$mg \cdot g^{-1}FW$）in sweet-potato leaves

	Treatment time under drought conditions (h)					
	XX			ZS 77		
	24	48	72	24	48	72
CK	3.33±0.10a	3.31±0.15a	3.33±0.14a	3.31±0.12a	3.3±0.04a	3.26±0.06a
DR	2.54±0.11a	2.45±0.13a	2.25±0.15b	2.94±0.08a	2.62±0.12b	2.39±0.10c

Note: different letters in the same line indicate significant difference at 0.05 level (Duncan).

Table 2. Effects of exogenous selenium on total chlorophyll content（$mg \cdot g^{-1}FW$） in sweet-potato leaves under drought stress

Cultivars	Duration(h)	Exogenous selenium concentration($mg \cdot L^{-1}$)				
		C0	C1	C2	C3	C4
		0.00	0.25	0.50	1.00	2.00
XX	24	2.61±0.14d	3.02±0.06c	3.17±0.02bc	3.58±0.05a	3.27±0.04b
	48	2.53±0.08d	2.93±0.18c	3.13±0.02b	3.43±0.09a	3.05±0.13bc
	72	2.30±0.07d	2.62±0.04c	2.92±0.05b	3.09±0.04a	2.88±0.02b
ZS 77	24	3.07±0.11c	3.30±0.06b	3.51±0.10a	3.26±0.09bc	2.82±0.14d
	48	2.73±0.10c	3.17±0.09ab	3.30±0.05a	3.02±0.12b	2.43±0.10d
	72	2.43±0.07d	2.86±0.14bc	2.99±0.04a	2.75±0.09b	2.38±0.06d

Note: different letters in the same line indicate significant difference at 0.05 level (Duncan).

of drought stress. As shown in Figure 1A and B, the Pn increased by 36.85 and 72.41% at 24 h after C2 and C3 treatments, respectively, in variety 'XX'. Even when the treatment time was prolonged to 72 h, the mitigating effects of the C2 and C3 treatments on the Pn decline were still significant, increasing by 94.64 and 76.79%, respectively, compared with C0. For 'ZS77', the mitigating effects of C2 and C3 treatments on the Pn decline were also significant. Compared with C0, the C2 treatment increased the Pn 107.0, 118.4 and 146.7% and the C3 treatment increased the Pn 77.3, 102.8 and 80.6% after 24, 48 and 72 h, respectively.

As shown in Table 3, under drought-stress conditions, leaf stomatal conductance (Gs) values of both cultivars decreased significantly, stomatal resistance increased, and as the stress duration increased, so did the degree of Gs decline. As shown in Figure 1C and D, exogenous Se treatments alleviated the decline in leaf Gs of both varieties, and the alleviation trend was generally consistent with that of the Pn. The mitigating effects differed with the Se solution's concentration and the sweet potato variety. After spraying Se for 24, 48 and 72 h, the C3 treatment effects were more obvious than those of the other three Se concentration treatments (C1, C2 and C4), which increased by 61.76, 57.59 and 57.14%, respectively, compared with C0. There were similar results obtained in variety 'ZS77' and among the four exogenous Se concentrations, C2 had the greatest mitigating effect. The differences between C0 and C3 (for 'XX') and C2 (for 'ZS77') were significant at the $P < 0.05$ level.

The intracellular CO_2 concentration (Ci) significantly decreased in drought treatments compared with CK (Table 3). However, the Ci decreased further after most of the Se treatments as shown in Figure 1E and F. For the

Table 3. Effects of drought stress on Pn, Gs, Ci and Tr in sweet potato leaves

		Treatment time under drought conditions (h)					
		XX			ZS 77		
		24	48	72	24	48	72
Pn	CK	13.02±0.82a	13.24±0.28a	13.47±0.61a	15.42±0.15a	15.71±0.32a	15.38±1.02a
	DR	3.12±0.13a	2.18±0.21b	1.90±0.3b	3.67±0.17a	3.06±0.27b	2.27±0.40c
Gs	CK	0.145±0.004a	0.150±0.004a	0.147±0.002a	0.233±0.017a	0.240±0.022a	0.238±0.004a
	DR	0.023±0.002a	0.017±0.001b	0.012±0.002c	0.024±0.003a	0.019±0.001b	0.015±0.002c
Ci	CK	241.7±6.2a	239.1±3.8a	243.4±4.1a	282.4±3.9a	281.7±3.9a	285.1±2.3a
	DR	154.3±7.6b	218.7±7.8a	234.3±7.4a	159.3±4.9b	177.3±5.7a	193.3±9.6a
Tr	CK	3.20±0.16a	3.24±0.23a	3.31±0.56a	6.05±0.21a	5.87±0.17a	6.10±0.32a
	DR	0.69±0.07a	0.51±0.02b	0.40±0.02c	0.82±0.07a	0.64±0.04ab	0.57±0.07b

Note: different letters in the same line indicate significant difference at 0.05 level (Duncan).

variety ‘XX’ at 24, 48 and 72 h after treatment C3, the Ci values of treated leaves decreased by 39.74, 20.94 and 13.80%, respectively, compared with C0. For ‘ZS77’, the C2 treatments effect on the Ci decrease was greater than those of the other treatments.

As shown in Table 3, under drought-stress conditions, the transpiration rate (Tr) of the leaves of the two sweet potato varieties decreased significantly compared with CK. As shown in Figure 1G and H, the decrease in Tr was alleviated to some extent by spraying Se. At 24, 48 and 72 h after the C3 treatment, the Tr value of ‘XX’ significantly increased by 46.92, 56.98 and 31.74%, respectively, compared with C0. For ‘ZS77’, the mitigating effect of the C2 treatment was greater than those of the other treatments. Compared with the C0 treatment, the Tr values significantly increased by 115.1, 137.6 and 95.9% at 24, 48 and 72 h, respectively, after the C2 treatment.

Effects of Se applications on chlorophyll fluorescence images and parameters

The effects of biotic and abiotic environmental factors on photosynthesis are reflected by changes in chlorophyll fluorescence. The Fv/Fm ratio reflects the original light energy conversion efficiency of PSII. The different Fv/Fm, YII, qN and qP values in the treated leaves were directly obtained from the measuring instrument, as shown in Table 4 and Figure 2. The images in Figure 2 provide detailed information on the fluorescence parameters distributed throughout the whole leaf under different treatment conditions. From the detail observations of Y(II), we found evidence of invisible damage to the leaves. In a spatial distribution, the damage levels to PSII in variety ‘XX’ after DR and C0 treatments were more serious along the leaf tip than the other leaf parts, while in variety ‘ZS77’ the damage levels were more severe near the petiole. Foliar spraying of distilled water did not alleviate the damage. From these converted images, we found that C2 and C3 treatments protected the chloroplasts, and there was no damage observed in the CK images. Additionally, the instrument provides overall data for the whole-leaf fluorescence parameters, which are the averages of the sums of the observations per pixel point.

As shown in Figure 3A, spraying a certain exogenous Se concentration alleviated the decrease in Fv/Fm, which was generally significantly different from the value of the C0 treatment at the $P < 0.05$ level. The Fv/Fm values of the two sweet potato varieties first increased and then decreased as the exogenous Se concentration increased. For ‘XX’, Fv/Fm increased by 1.78, 4.11, 5.64 and 2.15% under C1–C4-treatment conditions, respectively, with the C3 treatment producing the greatest value. For ‘ZS77’, the C1 treatment produced the greatest value.

As shown in Table 4 and Figure 3B, the Y(II) values of the two varieties of sweet potato were significantly reduced

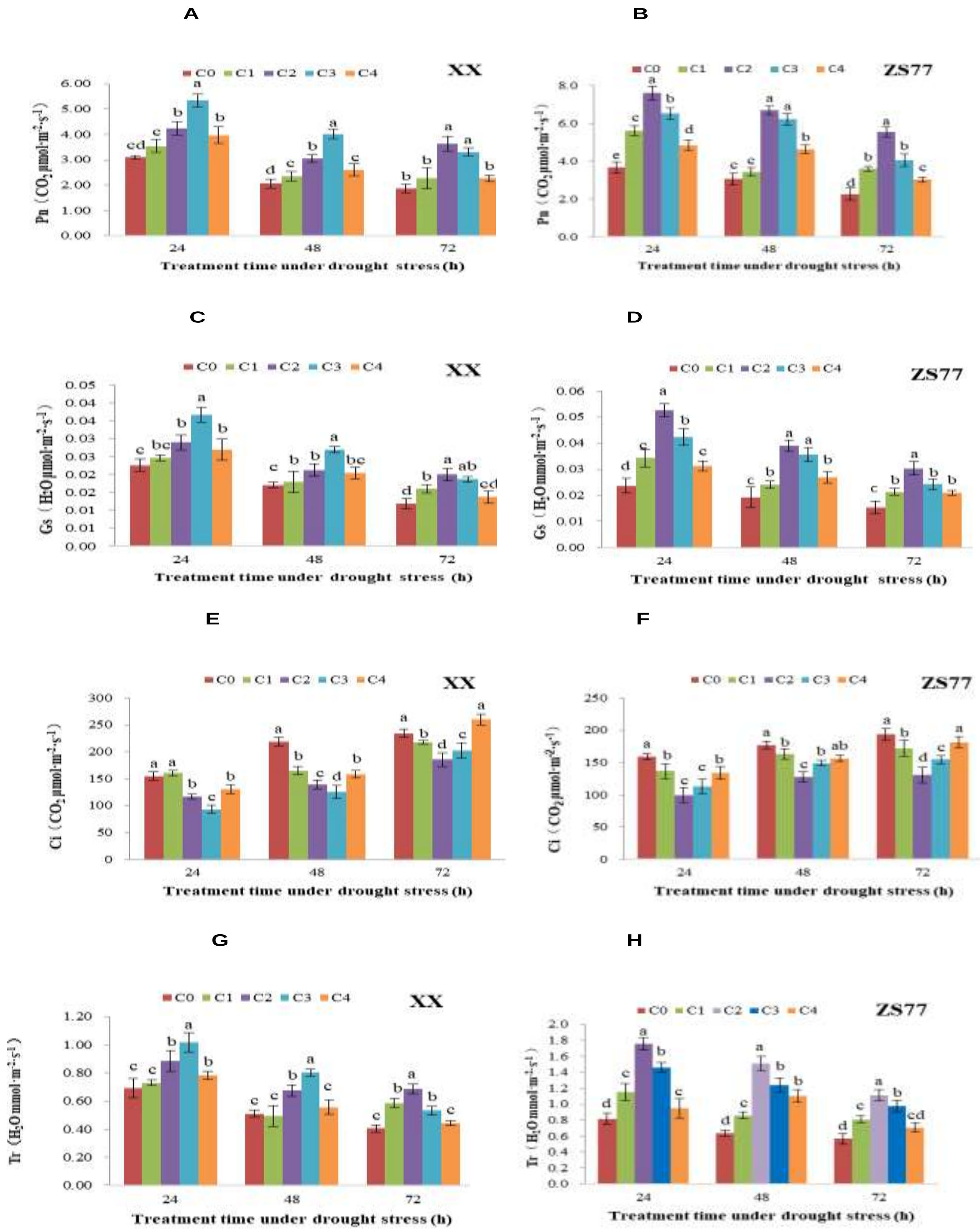

Figure 1. Effects of exogenous selenium on Pn (A, B), Gs (C, D), Ci (E, F) and Tr (G, H) in sweet-potato leaves under drought stress. The Se concentrations are C0:0.00; C1:0.25; C2;0.5; C3: 1.0; C4:2.0 mg·L^{-1}. The different letters indicate significant differences among treatments at 0.05 level (Duncan).

Table 4. Changes of chlorophyll fluorescence parameters of sweet-potato leaves in drought stress

Cultivars	Treatments	Fv/Em	qN	qP	Y(Ⅱ)
XX	CK	0.813±0.007	0.761±0.002	0.671±0.017	0.402±0.015
	DR	0.762±0.003	0.858±0.004	0.443±0.032	0.229±0.009
ZS 77	CK	0.817±0.005	0.764±0.003	0.538±0.021	0.316±0.012
	DR	0.785±0.002	0.841±0.007	0.312±0.034	0.178±0.014

Note: The drought treatment time is 24 hours.

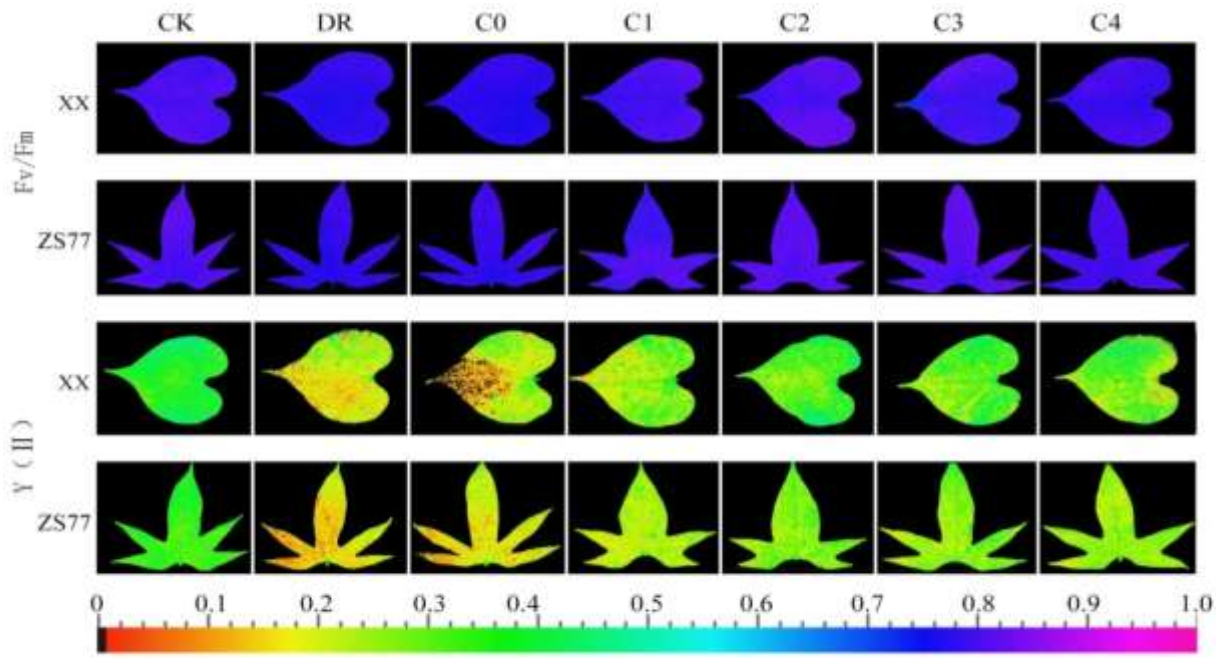

Figure 2. Effects of exogenous selenium on chlorophyll fluorescence imaging of sweet-potato leaves under drought stress.

under drought-stress conditions. Variety 'XX' and 'ZS77' were reduced by 75.55 and 77.53%, respectively, compared with CK under DR-treatment conditions. All four treatments alleviated the decrease of Y(II) in the two sweet potato varieties. Among the treatments, the effects of C2 and C3 were greater, and the Y(II) values of 'XX' increased by 31.60 and 31.31%, respectively, while those of 'ZS77' increased by 50.70% and 54.19%, respectively, compared with the C0 treatment. The differences were significant at the $P < 0.05$ level.

The drought treatment significantly increased the qN values of both varieties (Table 4). Applications of Se solutions alleviated the increases in the qN values of the leaves of sweet potato seedlings. At 24 h after the C1 and C2 treatments, the qN values of 'XX' significantly decreased by 3.64 and 3.17%, respectively, while those of 'ZS77' significantly decreased by 3.46 and 2.83%, respectively, compared with C0 (Figure 3C).

The qP after the DR treatment was significantly lower than in the CK (Table 4). As shown in Figure 3D, the exogenous C2 and C3 Se treatments effectively alleviated the decreases in qP values, which were significantly increased by 37.80 and 53.19%, respectively, compared with C0. There were no significant differences among C1, C4 and C0. For 'ZS77', the C1, C2 and C3 treatments all significantly increased the qP value.

Effects of Se applications on the RWC and MDA content

The leaf RWC is an important indicator of plant drought

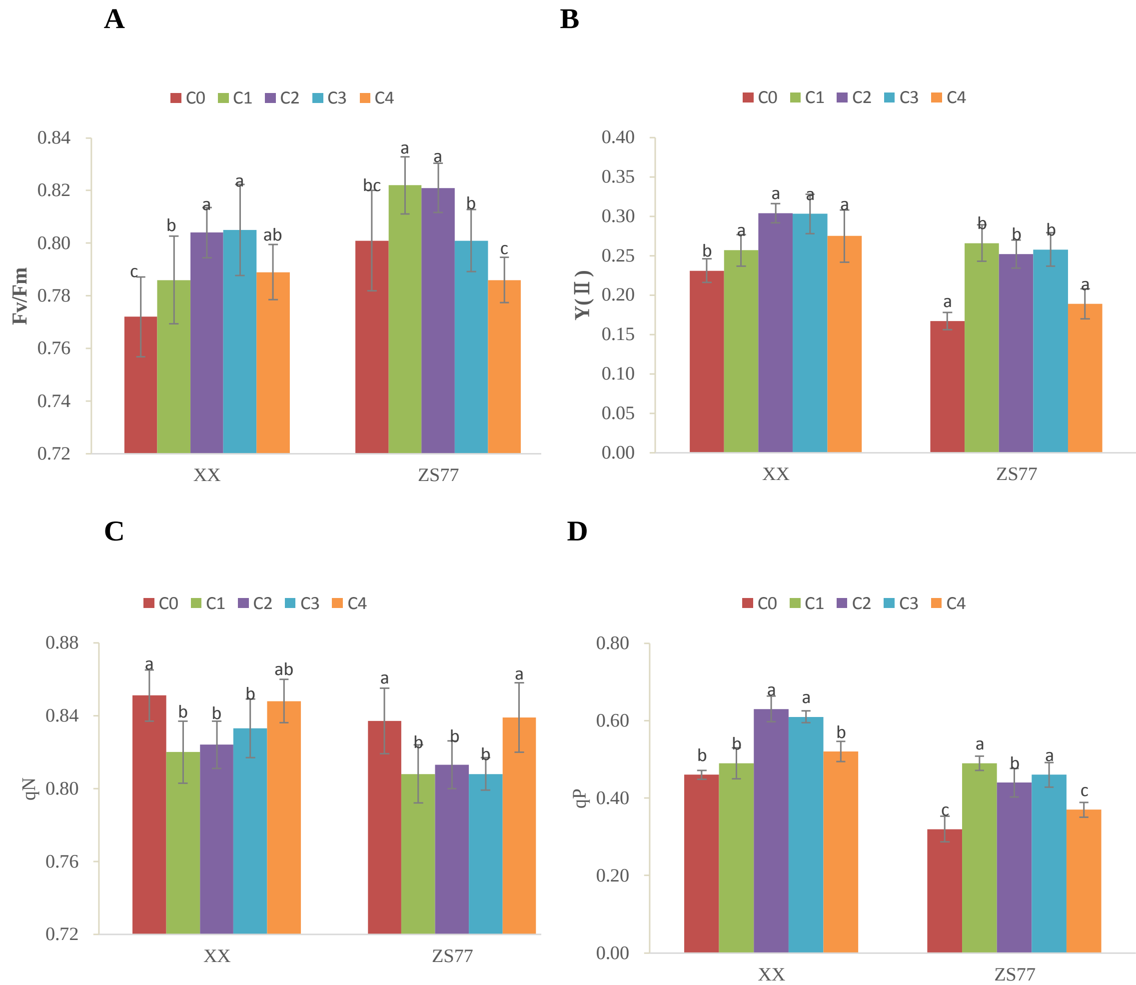

Figure 3. Effects of exogenous selenium on chlorophyll fluorescence parameters of sweet-potato leaves under drought stress. The different letters indicate significant differences among treatments at 0.05 level (Duncan).

Table 5. Effects of drought stress on RWC in sweet-potato leaves

Treatment		XX			ZS 77		
		24	48	72	24	48	72
RWC (%)	CK	92.8±1.3a	93.1±0.8a	92.9±1.0a	91.6±0.7a	92.1±1.2a	92.0±0.4a
	DR	82.3±0.5a	78.4±0.9b	72.4±0.7c	84.5±0.6a	82.7±0.6b	72.1±1.0c

Note: different letters in the same line indicate significant difference at 0.05 level (Duncan).

tolerance. As shown in Table 5, under drought-stress conditions, the RWC values of the two varieties decreased compared with CK, and the degree of decline increased along with the time under drought conditions. Appropriate exogenous Se concentrations (0.25 to 0.50 mg·L^{-1}) alleviated the decrease in the RWC to some extent (Figure 4). For ‘XX’ at 24, 48 and 72 h after the C2 treatment, the RWC increased 4.69, 8.35 and 3.20%, respectively, compared with C0, and the differences were significant at the P < 0.05 level. For ‘ZS77’, the mitigating effects of C1 and C2 were greater. However, the greater Se concentration did not alleviate the decline in RWC, and even exacerbated the decline, especially after the C4 treatment in both varieties.

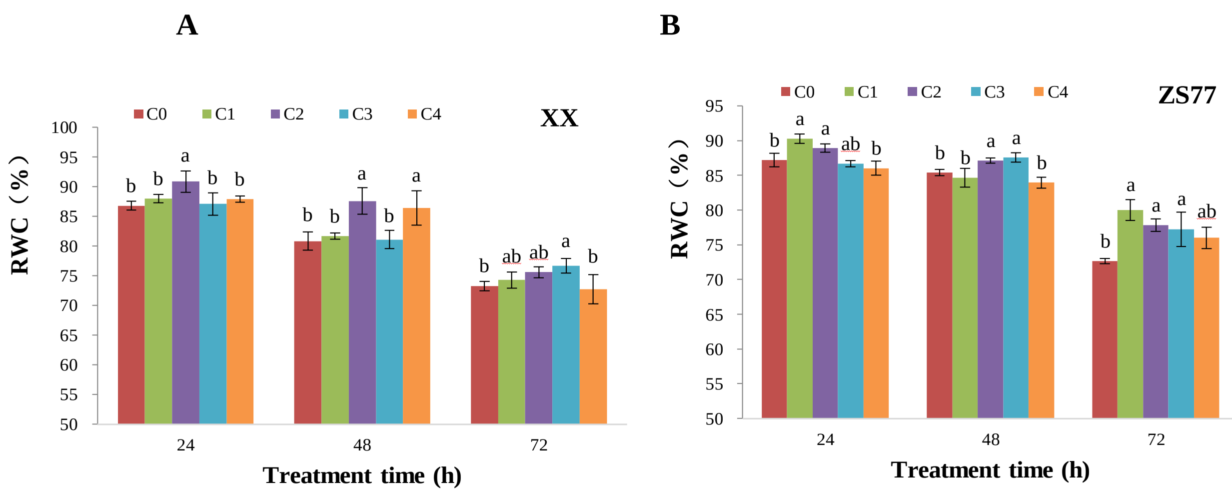

Figure 4. Effects of exogenous selenium on RWC of sweet-potato leaves under drought stress. The different letters indicate significant differences among treatments at 0.05 level (Duncan)

Table 6. Effects of Selenium on MDA contents (μmol·g^{-1}FW) of sweet potato leaves under drought stress

Cultivars	Duration(h)	Exogenous selenium concentration(mg·L^{-1})				
		C0	C1	C2	C3	C4
		0.00	0.25	0.50	1.00	2.00
XX	24	11.97±0.78a	10.57±0.42b	9.46±0.44cd	10.21±0.80c	11.73±0.41ab
	48	13.73±0.52a	12.26±0.26b	10.02±0.16c	11.41±0.48c	13.49±0.54a
	72	15.80±0.34a	13.13±0.55c	12.67±0.47c	10.74±0.27d	14.74±0.17b
ZS77	24	9.17±0.37a	8.28±0.28ab	7.17±0.57cd	8.01±0.27bc	9.02±0.52a
	48	10.10±0.49a	8.63±0.30b	7.69±0.49c	8.90±0.35b	10.60±0.58a
	72	12.23±0.37a	11.01±0.59b	8.95±0.37c	10.25±0.53b	12.41±0.50a

Note: different letters in the same line indicate significant difference at 0.05 level (Duncan).

The drought stress significantly increased the MDA contents in both varieties at 24, 48 and 72 h after treatment (Table 6). Applications of appropriate Se concentrations alleviated the increase in MDA. After 24 h of drought stress, the C1–C4 treatments decreased the MDA contents in 'XX' seedlings by 11.70, 20.95, 14.65 and 1.95%, respectively, compared with after the C0 treatment. For 'ZS77', the MDA contents decreased 9.73, 21.79, 12.62 and 1.64%, respectively. C1, C2 and C3 treatments produced significant differences compared with C0 at the $P < 0.05$ level. The C2 treatment produced the greatest effect, while the C4 treatment produced no significant mitigating effect in either variety. When the time after treatment was prolonged to 48 h, the mitigating effect of C2 was still good in both varieties. After 72 h, the C2 treatment's effects were greater in 'ZS77' while those of the C3 treatment were greater in 'XX'. The C4 treatment produced negative effects in both varieties.

Effects of Se applications on antioxidant enzyme activities in leaves and roots

Applications of appropriate Se concentrations significantly

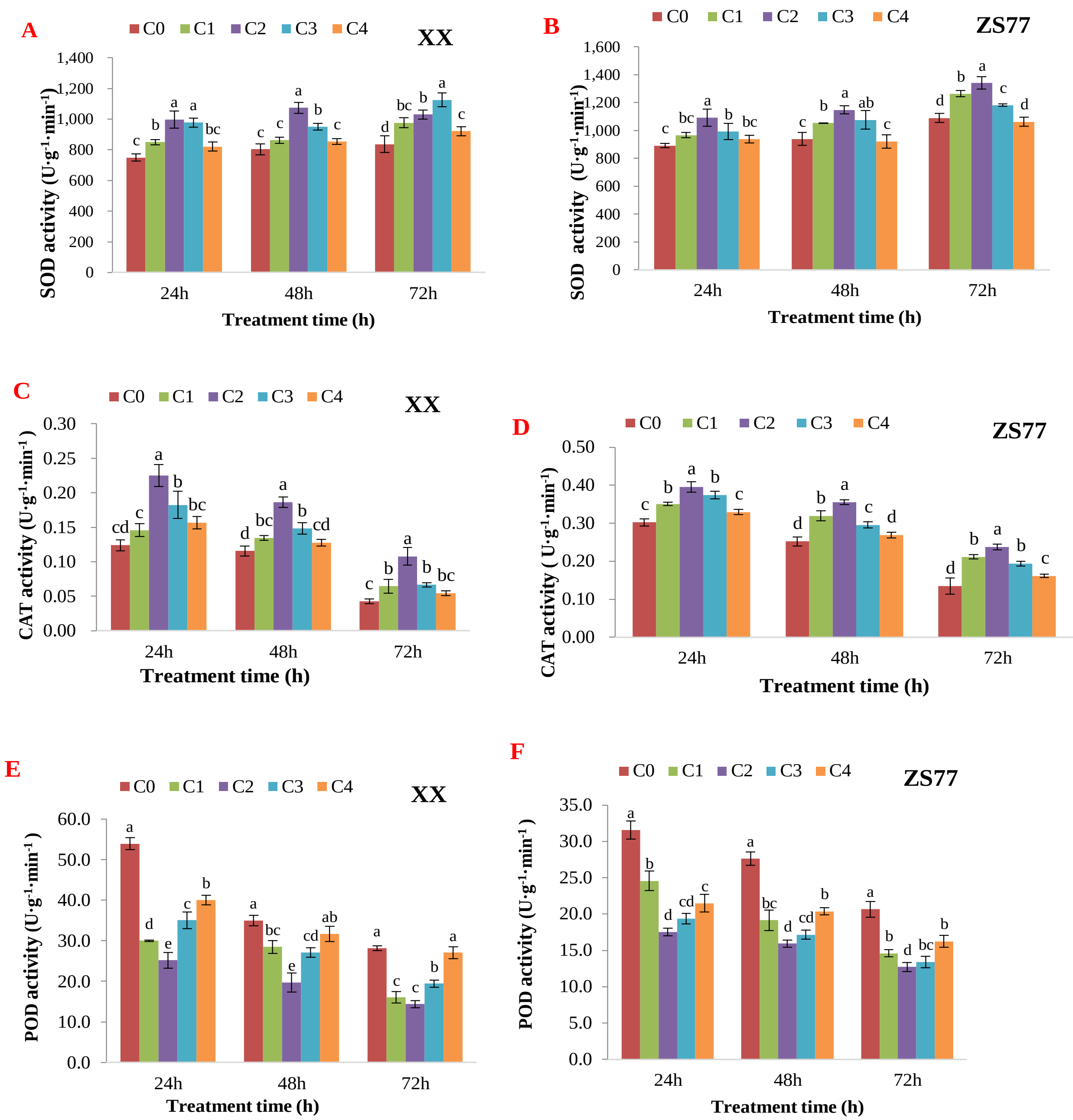

Figure 5. Effects of exogenous selenium on SOD (A, B), CAT (C, D), POD (E, F) activity in sweet-potato leaves under drought stress. The different letters indicate significant differences among treatments at 0.05 level (Duncan).

increased the SOD activity levels in the leaves of the two sweet potato varieties (Figure 5A, B). The SOD activities in 'XX' increased by 13.30, 32.94, 30.36 and 30.36% at 24 h after the C1–C4 treatments, respectively, compared with after the C0 treatment. There were significant differences ($P < 0.05$) between the C2, C3 and C4 treatments and the C0 treatment. At 48 h after treatment, the promotive effects of C2 and C3 on SOD in 'XX' remained good, increasing significantly by 33.61 and 18.18%, respectively, compared with C0, while the effects of C1 and C4 were not significant. After 72 h, the effects of the C2 and C3 treatments were still significantly greater than those of the other treatments. There was a similar change trend in variety 'ZS77'. However, the higher dose of Se (> 2.00 mg) inhibited the SOD activity of 'XX'.

The CAT activity levels in the leaves of the two cultivars decreased significantly as the time under drought conditions increased (Figure 5C, D). As shown in Figure 6, after spraying exogenous Se, the CAT activity levels of sweet potato seedlings effectively increased. At 24 h after treatments with C1–C4, the CAT activity levels in leaves of ‘XX’ increased by 17.74, 81.66, 47.25 and 26.55%, respectively, compared with after the C0 treatment. There were significant differences between the C2, C3 and C4 treatments and the C0 treatment. The four treatments in ‘ZS77’ increased the CAT levels by 16.11, 30.88, 23.90 and 9.08%, respectively, which were significantly different from that after the C0 treatment. After 48 h, the promotive effects of the C2 and C3 treatments on ‘XX’ remained positive, increasing by 60.98 and 28.13%, respectively, compared with after the C0 treatment. The results in ‘ZS77’ were similar to those in ‘XX’. After 72 h, the CAT activity trend was similar to that at 48 h after treatment.

Thus, the C2 and C3 treatments significantly increased CAT activity levels in sweet potato seedlings under drought-stress conditions.

After spraying exogenous Se, POD activity levels in the leaves of the seedlings generally decreased compared with the C0 treatment (Figure 5E, F). The POD activity levels in ‘XX’ at 24 h after receiving C1–C4 treatments were significantly reduced by 44.41, 53.26, 35.26 and 40.00%, respectively, compared with after the C0 treatment, and they were decreased by 22.18, 44.48, 38.68 and 31.98% in ‘ZS77’, respectively. There were significant differences among the different treatments. At 48 and 72 h after treatment, POD activity levels in leaves of sweet potato seedlings treated with C1–C3 were still significantly lower than after the C0 treatment, and the inhibitory effect of the C2 treatment was still the most significant. When the Se concentration increased to 2.00 $mg \cdot L^{-1}$ (C4 treatment), the inhibitory effect on ‘ZS77’ was still significant. The POD activity in ‘XX’ at 24 h after receiving the C4 treatment was also significantly lower than in leaves of plants receiving the C0 treatment, but there were limited differences in the POD activity levels at 48 and 72 h after C0 and C4 treatments.

The root antioxidant enzyme activities play important roles in sweet potato development. Foliar spraying of the appropriate Se concentration significantly increased the SOD activity levels in the fibrous roots of the two sweet potato varieties (Figure 6A, B). The SOD activities in ‘ZS77’ fibrous roots increased by 22.39 and 11.38% at 24 h after the C2 and C3 treatments, respectively, compared with after the C0 treatment. There were significant differences ($P < 0.05$) between the C2 and C3 treatments and the C0 treatment. Although the rate of increase declined at 48 h after treatment, the SOD activities in ‘ZS77’ fibrous roots after C2 and C3 treatments increased significantly by 22.16 and 14.55%, respectively, compared with C0. There was a similar change trend in variety ‘XX’, and the rate of increase was higher in ‘XX’ than in ‘ZS77’.

The drought stress significantly decreased the CAT activities of sweet potato fibrous roots, and the Se application reversed the decrease. As shown in Figure 6(C, D), after spraying exogenous Se, the CAT activity levels of sweet potato roots effectively increased. At 24 h after C2 and C3 treatments, the CAT activity levels in fibrous roots of ‘ZS77’ significantly increased by 40.22 and 16.4%, respectively, compared with after the C0 treatment. At 48 h after the C2 and C3 treatments, the CAT levels in ‘ZS77’ increased by 75.86 and 45.10%, respectively, which were significantly different from after the C0 treatment. The results in ‘XX’ were similar to those in ‘ZS77’.

As in leaves, Se applications decreased the POD activity levels in the fibrous roots of the seedlings compared with the C0 treatment (Figure 6 E, F). The POD activity levels in ‘XX’ at 24 h after receiving C2 and C3 treatments were significantly reduced by 53.09 and 34.88%, respectively, compared with after the C0 treatment, and they were decreased by 36.22 and 40.81% in ‘ZS77’, respectively. At 48 h after C2 and C3 treatments, POD activity levels in ‘XX’ decreased by 44.86 and 23.63%, respectively, compared with after the C0 treatment, and they decreased by 42.16 and 37.95% in ‘ZS77’, respectively. There were significant differences between the C2 and C3 treatments and the C0 treatment.

Effects of Se applications on root vigor

Exogenous SA treatments enhanced the root vigor significantly when compared with the CK (Figure 7). The root vigor levels in ‘XX’ at 24 h after receiving C2 and C3 treatments significantly increased by 17.28 and 25.29%, respectively, compared with after the C0 treatment, and they increased by 28.17 and 30.38% in ‘ZS77’, respectively. At 48 h after C2 and C3 treatments, root vigor levels in ‘XX’ increased by 29.15 and 35.28%, respectively, compared with after the C0 treatment, and they increased by 27.09 and 32.66% in ‘ZS77’, respectively. There were significant differences between the C2 and C3 treatments and the C0 treatment.

Effects of spraying Se on the contents of sweet potato tubers

As shown in Table 7, the Se contents of sweet potato tubers not receiving exogenous Se were ~7.37 to 8.28 $\mu g \cdot kg^{-1}$ DW, and the Se contents were similar in the two varieties. After Se applications, the Se contents in sweet potato tubers increased. After C1–C4 treatments, the Se contents in ‘XX’ potato tubers increased by 6.88, 26.57, 53.38 and 195.77% respectively compared with in the CK. For ‘ZS77’, the Se contents increased 26.66, 32.88, 48.36 and 131.76%, respectively. There were significant differences between both the C3 and C4 treatments and the CK at the $P < 0.05$ level, but no significant differences were found between DR, C0, C1 and C2 treatments and the CK.

DISCUSSION

When higher plants suffer from drought stress, growth

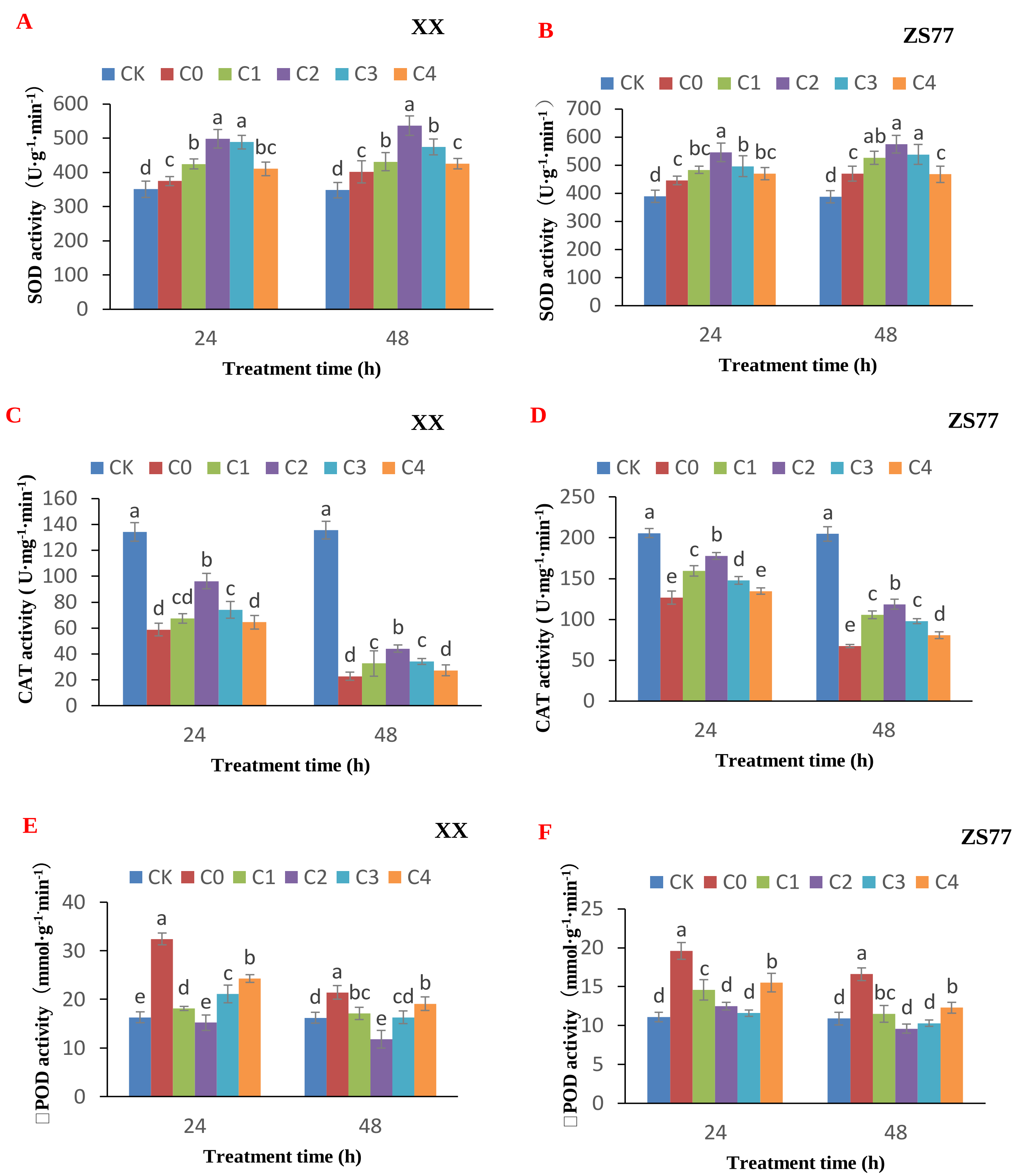

Figure 6. Effects of exogenous selenium on SOD (A, B), CAT (C, D), POD (E, F) activity in sweet-potato fibrous roots under drought stress. The Se concentrations are C0:0.00; C1:0.25; C2:0.5; C3:1.0; C4:2.0 mg·L^{-1} and CK with regular soil water content. The different letters indicate significant differences among treatments at 0.05 level (Duncan)

disorders, such as membrane damage, reactive oxygen species generation and toxic compound accumulation, normally occur. They can lead to reductions in the chlorophyll content and photosynthetic activity (Djanaguiraman *et al*., 2010). Sweet potato is considered a drought-tolerant plant; however, a soil water deficiency

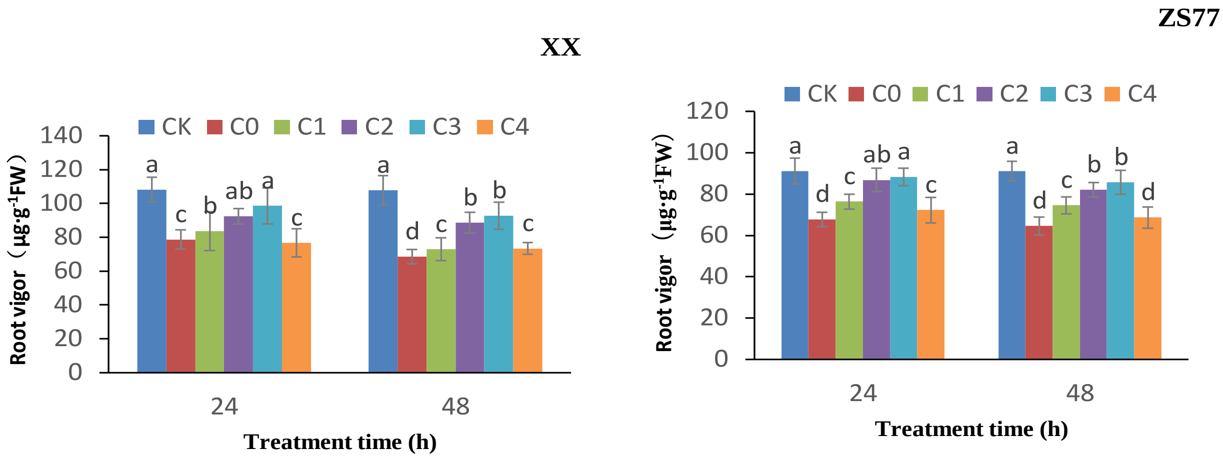

Figure 7. Effects of exogenous selenium on root activities under drought stress. The different letters indicate significant differences among treatments at 0.05 level (Duncan).

Table7. Effects of selenium on Se contents (μg·kg^{-1}DW) of sweet-potato tuber under drought stress

Cultivars	Selenium concentration(mg·L^{-1})						
	CK	DR	C0	C1	C2	C3	C4
			0.00	0.25	0.50	1.00	2.00
XX	8.28±1.03bc	8.17±0.92bc	8.10±0.50bc	8.85±0.15bc	10.48±1.38bc	12.7±1.61b	24.49±2.41a
ZS77	7.65±0.60c	7.15±0.56c	7.37±1.28c	9.69±1.60bc	10.12±0.97bc	11.35±0.35b	17.73±1.25a

Note: different normal letters in the same line indicate significant difference at 0.05 level(Duncan).

decreases the leaf chlorophyll content. Here, negative effects of drought stress on the chlorophyll content in sweet potato leaves were alleviated by Se applications. The increase in the chlorophyll content under environmental drought stress can be attributed to enhanced chlorophyll synthesis and retarded chlorophyll degradation (Shikha and Meetu, 2016). There are rare reports regarding the simultaneous effects of Se applications on physiological responses in plant leaves and roots. In the soil drought experiment, the collected samples were limited and only SOD, CAT and POD activities, as well as vigor level, were determined in fibrous roots. In this study, the sweet potato roots' and leaves' SOD and CAT activities increased after Se applications, especially low concentrations, during drought stress, while the POD activity decreased. The root activities significantly increased after foliar spraying with an appropriate Se concentration, especially the C2 and C3 treatments (0.5 to 1.0 mg·L^{-1}) during drought stress. Thus, the antioxidant enzymes in fibrous roots may cooperate with those in leaves to contribute to the photosynthetic system during drought treatment and Se application.

A high photosynthetic yield is the basis of a high crop yield, and such a yield results from the collaboration of external foliar organs, such as stomata, and internal foliar organs, such as chloroplasts. Pn is a key indicator of plant photosynthesis, and it is affected by various environmental factors. The Pn markedly decreased in sweet potato seedlings as the time in a water-deficient soil environment increased, and a foliar Se spray significantly alleviated the negative effects of drought stress. The alleviating effect may result from the appropriate Se concentration enhancing the gas-exchange rate. Tr, closely related to Gs, reflects the ability of plant leaves to regulate their own water reserves, which is lost during drought stress. After exposure to drought, the Gs and Tr values following C3 treatments were significantly higher at 24, 48 and 72 h, but the Ci value decreased almost simultaneously. A low Ci when the Gs is high may result from the high efficiency of photosynthesis and result in a high Pn, as shown in Figure 1A and B. Se applications increased plant Pn during drought stress but this may occur not only because of beneficial Gs and Tr values, but also because of the protection afforded to chloroplast membranes, other photosynthetic apparatus and high root activities.

Chlorophyll fluorescence parameters represent the photochemical characteristics during photosynthesis. Under drought stress, the Fv/Fm, Y(II) and qP of sweet potato decreased significantly, but the qN increased markedly. Thus, the drought stress decreased the photochemical efficiency of PSII, and surplus light energy was absorbed by the chlorophyll, which dissipated in the form of heat to reduce damage to photosynthetic protein-related pigments of plant leaves. This damage includes the disintegration of chlorophyll and the decline of membrane functions caused by drought stress (Fabrício *et al*., 2018). Exogenous Se treatments in a certain concentration range (0.25 to 1.00 $mg \cdot L^{-1}$) significantly increased the Fv/Fm, Y(II) and qP, and significantly decreased qN, which was consistent with results in millet (Tingting *et al*., 2016). Thus, the light energy absorbed by PSII antennal pigments is more effectively used in photochemical reactions, the electron transfer activity of PSII is enhanced, and the heat loss is reduced (Jie *et al*., 2012; Murchie *et al*., 2013). Se applications promote the electron supply of photosynthetic carbon metabolism in sweet potato, which increased the transformation of the captured light energy into chemical energy. This may be related to the increases in some antioxidant enzyme activities in sweet potato leaves and the enhancement of the scavenging capacity for reactive oxygen species, which protect photosynthetic mechanisms. Higher root activity levels may also play supporting roles.

The chlorophyll fluorescence images vividly and comprehensively expressed the distribution of the PSII functional state, with countless points located in whole leaves under different treatment conditions (Céline *et al*., 2013; Huajin *et al*., 2019). Under normal water supply conditions, the Y(II) of the whole leaf results in a beautiful green color, and the maximum quantum yield is represented by a uniform purple color. However, during drought and C0 treatments, yellow colored spots near the leaf tips of 'XX' were obvious, while the yellow spots of 'ZS77' were closer to the petioles. This may result from differences in leaf morphology and structure between the two varieties. However, the distribution of yellow patches in Y(II) images and the changes among different treatments may also indicate that Se's protective function regarding PSII is not only related to the regulation of the internal and external structures and functions of leaves, such as stomatal regulation and chlorophyll protection, but is also related to the change or promotion of water transport and root function. Because of the limited numbers of samples, detailed investigations into the correlation between root activity and PSII function were not possible. However, it deserves further study.

MDA contents in higher plants increase under drought and other stress conditions (Mohammed *et al*., 2018). Here, spraying Se alleviated the seedlings' chlorophyll degradation and increased MDA accumulation during drought stress, which favored the stability of photosynthetic apparatus and membrane systems in plants, thereby, increasing the drought tolerance of sweet potato seedlings. The research of Djanaguiraman *et al*. (2010) on sorghum supports our results. The appropriate concentration (0.25 to 1.00 $mg \cdot L^{-1}$) of Se alleviated the increase in the MDA content, probably as a result of Se's promotion of antioxidant enzyme activities. However, a higher Se concentration (> 1.00 $mg \cdot L^{-1}$) had the opposite effect on the MDA content. This may result from Se being substituted for sulfur inside the plant and participating in sulfur-related protein metabolism, which disrupts protein synthesis along with its structure and function (Meetu and Shikha, 2017). Furthermore, a higher Se dose may disorder the plant's osmotic regulation during drought stress. The C1, C2 and C3 treatments increased the RWCs in sweet potato leaves and protected the photosynthetic apparatus, which had been shown previously (Yanyan *et al*., 2013; Mian *et al*., 2015; Mohammed *et al*., 2018).

The C3 and C4 treatments significantly increased the Se contents of sweet potato tubers. However, the Se contents were much lower than the Se-enriched tuber standard provided by Shanxi Province (Xu and Wenbin, 2017) and the U.S. Department of Agriculture's recommended dietary allowance of 50–70 µg Se d^{-1} for regular adults (Maria *et al*., 2017). Because, in China, ~72% of the counties are located in Se-deficient areas (Xu and Wenbin, 2017), there is no health risk in applying a Se (as sodium selenite) solution as a plant regulator, and it may even be helpful.

In conclusion, foliar Se spraying increased Gs and alleviated drought-induced oxidative stress by regulating the antioxidant defense systems in the leaves and roots of sweet potato seedlings. These systems are associated with the improved photochemical efficiency of PSII, thereby allowing the maintenance of a high photosynthetic rate.

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Abbreviations

CAT, Catalase; **Fv/Fm,** Maximum chlorophyll fluorescence; **MDA,** Malondialdehyde; **Pn,** Net photosynthetic rate; **POD,** Peroxide enzyme peroxidase; **ROS,** Reactive oxygen species; **SOD,** Superoxide dismutase; **XX,** Xinxiang - sweet potato cultivar; **Y(Ⅱ),** effective photochemical quantum yield of PSⅡ; **ZS77,**

Zheshu 77- sweet potatoes cultivar; **Gs,** leaf stomatal conductance; **Ci,** intracellular CO_2 concentration; **Tr,** transpiration rate; **qN,** non-photochemical quenching coefficient; **RWC,** relative water content.

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