

Antioxidant Enzymes and Photosynthetic Responses to Drought Stress of Three *Canna edulis* Cultivars

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Abstract. Edible canna is a productive starch source in some tropical and semitropical regions. In these regions, water deficit stress is one of factors that limit the crop yield. In the present study, we investigated seven physiological indexes and photosynthetic responses of three edible canna (*Canna edulis* Ker.) cultivars ('PLRF', 'Xingyu-1', and 'Xingyu-2') under 35 days drought stress. Our results indicated that drought treatment caused visible wilting symptoms in all cultivars, especially in 'Xingyu-1'. Coupled with the increase of wilting symptoms, relative water content (RWC) and chlorophyll content decreased progressively, malondialdehyde (MDA) content gradually increased, and key antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD), catalase (CAT) activities increased first and then decreased in all three cultivars. The effect of water stress was more pronounced in 'Xingyu-1' than in 'PLRF' and 'Xingyu-2', and in lower leaves than in upper leaves. In addition, 35 days drought stress also significantly reduced the photosynthetic capacity. Consistent with antioxidant parameters, photosynthetic changes of 'Xingyu-2' were less than those of the other cultivars under water deficit stress. Drought stress caused a significant increase of water use efficiency (WUE) in 'Xingyu-2', but little in 'PLRF', and obvious decrease in 'Xingyu-1'. These results indicated that 'Xingyu-2' was more tolerant to drought stress than 'PLRF' and 'Xingyu-1' by maintaining lower lipid peroxidation and higher antioxidant enzyme activities.

Additional key words: catalase, edible canna, malondialdehyde, peroxidase, superoxide dismutase, water deficit

Introduction

Edible canna (*Canna edulis* Ker.) is one of tuber crop food sources, grown in some tropical and semitropical regions where cereal crops are not suitable for growing due to climatic and agronomic reasons. It has been an useful starch source not only for food but also for industry materials (Pérez et al., 1997). In recent years, edible canna is also used as an important source of bio-fuel, since it has high starch content in the rhizome of about 60-70 percent of its dry weight and a high yield of more than 60-75 tons per hectare by fresh weights (Sriroth et al., 2001; Wu et al., 2005). In production, water is essential for plant growth and metabolism (Boyer, 1982; Jones and Corlett, 1992). In tropical and semitropical regions, plants are often exposed to water deficit stress, also known as drought stress, which greatly limits

the crop yield by decreasing photosynthesis (Gobin, 2012; Mafakheri et al., 2010). Drought stress may lead to production of excessive photon energy in chloroplasts which damages photosynthetic apparatuses, and this in turn causes a decrease in the photochemical efficiency. Photosynthesis inhibition is one of the primary physiological consequences of drought stress (Chaves, 1991; Cornic, 1994; Lawlor, 1995). The reduction of intercellular CO₂ concentration, as a result of stomatal closure, is one of the main causes of photosynthesis decrease under water stress conditions (Chaves and Oliveira, 2004; Ennahli and Earl, 2005; Grassi and Magnani, 2005). On the other hand, under drought stress circumstance, the decrease of chlorophyll content also leads to reduction of the photosynthesis efficiency (Loggini et al., 1999).

In addition to that, drought stress can cause the formation of reactive oxygen species (ROS) as a result of water potential

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※ Received 18 March 2013; Revised 1 July 2013; Accepted 3 July 2013. This work was supported by the Social Welfare Program of Forestry Administration in China (200704009), Scientific and technological project in Shaanxi Province (2009K01-11) and the Natural Science Foundation of Guizhou Province ([2007]2055). The authors thank Technical Bureau of Xingyi, Guizhou Province for assistance to obtain the edible canna bulbs and Prof. Feng Chen (Department of Plant Sciences, University of Tennessee) for proofreading the manuscript.

alteration in the plant cells (Sanchez-Rodrigues et al., 2010; Türkan et al., 2005). The ROS is potentially harmful to cell membranes, resulting in oxidative degradation of membrane lipids called lipid peroxidation (Foyer et al., 1994; Zhang et al., 2011). Malondialdehyde (MDA) is one of the breakdown products of lipid peroxidation, directly reflect the degree of plant cell damage, and has been frequently used as an indicator of lipid peroxidation in vivo (Li et al., 2013).

Plants have developed different scavenging mechanisms to protect against oxidative stress by controlling the level of ROS by interacting network of antioxidant systems, including nonenzymatic antioxidants and antioxidative enzymes, such as ascorbic acid (AsA), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and peroxidase (POD) (Habibi, 2012; Ozkur et al., 2009; Xue and Liu, 2008). The SOD can convert superoxide anion to O₂ and hydrogen peroxide (H₂O₂), which can be scavenged by CAT and a variety of peroxidases through the oxidation of co-substrates such as AsA or other antioxidants (Basu et al., 2010). Detailed information on drought-tolerance patterns is important not only for successful cultivation, but also for understanding the antioxidant defense mechanism of tolerant cultivars to abiotic factors and their dynamics in dry lands.

A great deal of work on edible canna has been done in the field of underlying photosynthetic capacity (Kato and Imai, 1996), starch characterization (Lii and Chang, 1991; Moorthy et al., 2002; Pérez et al., 1997, 1998; Thitipraphunkul et al., 2003a, 2003b), assessment of gelatinization parameters (Lares and Pérez, 2006; Wootton and Bamunuarachchi, 1978) and starch nutrition (Pérez and Lares, 2005; Yun et al., 2004). Nevertheless, only little is known about drought tolerance of the edible canna during starch accumulation stage, especially the antioxidative enzymes responsible for drought stress. This study was conducted to (i) investigate antioxidant enzymes defense mechanism of three edible canna cultivars, *Canna edulis* Ker. 'PLRF', 'Xingyu-1', and 'Xingyu-2' under drought conditions, (ii) characterize the difference in response to drought stress of three edible canna cultivars at a starch accumulation stage, and (iii) understand the relationship between antioxidant defense mechanism and drought stress. It will provide valuable information for cultivation, breeding for drought-tolerant plants, and deciphering its molecular mechanisms of drought tolerance in arid and semi-arid areas.

Materials and Methods

Study Site and Plant Materials

The study was conducted at the Northwest A & F University (34°28'3" N, 108°06'7" E, 560 m altitude), which is situated in the center part of Kuan-chung Plain in Shaanxi Province,

China. Belonging to an arid and semi-arid region, the mean annual precipitation in this area is about 500 to 800 mm, 60% of which falls from June to September, with a marked dry season from October to May. In March 2010, bulbs of three edible canna (*Canna edulis* Ker.) 'PLRF', 'Xingyu-1', and 'Xingyu-2' were obtained from Technical Bureau of Xingyi, Guizhou Province in China. The bulbs were planted in the university nursery. When five euphylla were fully expanded at the latter part of May, the young plants were transplanted into plastic pots (45 cm diameter × 30 cm height) that contained 20 kg soil (yellow loam), screened by 1cm sieve. A young plant was transplanted per pot. The transplanted plants were regularly weeded and irrigated about 2-3 times per week until September in a greenhouse, where the mean temperature was 23-30/16-19°C (day/night), and relative humidity (RH) was between 50 and 70% (from morning to late afternoon) during the experiment. On September 6 (100 days after transplanting), 36 pots with similar plant size were selected and irrigated to the field capacity. From September 6 to October 11, the plant materials were treated under two irrigation regimes: the regular irrigation (control) and 35 days of continuous drought treatment. The soil water potential was monitored using a tensiometer (Tension Meter System, SoilSpec, Australia). The soil water potential of the control was continuously monitored to maintain the range from 10 to 30 Kpa, which was suitable for the growth of most crops (Adhanom et al., 2012). The soil water potential under drought stress was continuously controlled to maintain above 50 Kpa, which is the range, known as drought to most crops (Adhanom et al., 2012). Six potted plants under each irrigation regime per cultivar were used for analysis of physiological measurements. The physiological measurements were taken weekly (7, 14, 21, 28, and 35 days). Samples of every measurement time point were harvested from the same ten leaves in upper and lower positions and pooled for determination of parameters. The plant was divided into two parts (upper and lower part) from the intermediate portion. Each treatment had three replicates and each replicate included 2 pots with one plant in a pot.

Determination of Physiological Parameters

Relative water content: Leaf water status was estimated in the fully developed leaves by measuring the relative water content (RWC). Ten discs with the diameter about 1 cm were cut from ten leaves using a hole puncher (3 replicates), and the fresh weight (FW) was immediately taken after harvest. Then the leaf discs were placed in a petri dish (90 mm in diameter) filled with MilliQ distilled water overnight at room temperature and the turgid fresh weight (TW) for each replicate was measured. The samples were dried in an

oven at 80°C for 48 h to measure their dry weights (DW). The relative water content was calculated as Weatherley's method (Weatherley, 1950): $RWC (\%) = (FW - DW) / (TW - DW) \times 100$.

Determination of photosynthetic gas exchange parameters:

Before harvest, the net photosynthetic rate (Pn), stomatal conductance (Cond), intercellular carbon dioxide concentration (Ci), and transpiration rate (Tr) were measured on October 11 using a photosynthetic system (Li-6400, Li-Cor, Inc., Nebraska, USA). The measurements were taken from three most recently matured leaves in each replicate plant during the period from 9:00 to 11:00 in the morning, under photosynthetic flux density (PPFD) of $1,000 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$ provided by a LED light source. The air humidity in the leaf chamber was about 50%, with CO₂ concentration of $420\text{--}450 \mu\text{mol} \cdot \text{mol}^{-1}$, ambient air temperature of $24 \pm 2^\circ\text{C}$, and flow rate of $500 \mu\text{mol} \cdot \text{s}^{-1}$. Water use efficiency (WUE) was calculated as previously described by Pan et al. (2011): $WUE = Pn/Tr$.

Pigments analysis: The 0.02 g ($d = 0.0001$) of shredded leaves were randomly taken from ten upper or lower leaves of plants (3 replicates). Chlorophyll a (Chl.a) and chlorophyll b (Chl.b) of the leaves were extracted in 5 mL 80% (v/v) acetone, and the contents of pigment were determined by measuring absorbance at 663 nm and 645 nm, using a UV-Vis spectro-photometer (UV-1700, Shimadzu, Kyoto, Japan). The Chl.a and Chl.b contents were calculated according to Lichtenthaler and Wellburn (1983).

Assays of SOD, CAT, POD activities and MDA contents:

For extraction of enzymes, leaf samples (0.5 g) were homogenized over ice in 8 mL ice-cold sodium phosphate buffer (PBS, 50 mM, pH 7.8) containing 1% (w/v) polyvinyl polypyrrolidone (PVP). The homogenate was centrifuged at 15,000 g for 20 min at 4°C, and the supernatant was divided into 500 μL aliquots and stored at -70°C as crude extract for enzyme activity and MDA content assay.

Activities of superoxide dismutase (SOD, EC 1.15.1.1) were determined by measuring its ability to inhibit the photo-reduction of nitroblue tetrazolium (NBT) (Giannopolities and Ries, 1977) based on the absorbance at 560 nm. Activities of catalase (CAT, EC 1.11.1.6) were assayed by measuring the decrease in absorption at 240 nm in 3 mL reaction medium containing 2.85 mL PBS (50 mM, pH 7.0), 50 μL H₂O₂ (10 mM) and 100 μL enzyme extract as described by Chance and Maehly (1955). For measurement peroxidase (POD, EC 1.11.1.7) activity, 50 μL of the supernatant was mixed with 2.95 mL PBS (100 mM, pH 6.8) containing 1.65 μL guaiacol and 0.56 μL 30% H₂O₂ according to Li et

al. (2009). Reaction started by addition of enzyme extract in phosphate buffer. The increase of the solution absorbance was recorded at 470 nm within 6 min by an UV-Vis spectrophotometer (UV-1700, Shimadzu, Kyoto, Japan). One unit (U) of SOD activity was defined as the amount of enzyme required to cause 50% inhibition of the NBT photo-reduction rate per hour. One CAT activity unit (U) was defined as absorbance at 240 nm decreasing 0.01 per minute and one POD unit activity was defined as absorbance at 470 nm increasing 0.1 per minute. The activities of SOD, CAT, and POD were expressed as units per gram of fresh weight.

The MDA contents were analyzed according to Heath and Packer (1968) with slight modification, for taking into account the possible influence of interfering compounds in the assay for thiobarbituric acid (TBA) reactive substances. 1 mL of supernatant and 2 mL of 10% (w/v) trichloroacetic acid (TCA) containing 0.6% (w/v) TBA were added. After heating at 100°C for 15 min, the mixture was quickly cooled in ice-water mixture and centrifuged at 4,000 g for 10 min. The absorbance of the supernatant was measured at 532 nm and the value for non specific absorption at 600 nm and 450 nm was subtracted. The contents of MDA were expressed as nmol MDA per gram fresh weight with an extinction coefficient of $155 \text{ mM} \cdot \text{cm}^{-1}$ and the coefficient at 450 nm according to Li et al. (2009) was 0.56: $\text{MDA concentration (nmol} \cdot \text{mL}^{-1}) = 6.45(A_{532} - A_{600}) - 0.56A_{450}$.

Statistical Analysis

The treatment means were compared according to Duncan's multiple range tests at 0.05 level using SAS 8.1 statistical software.

Results

Plant Growth

As shown in Figs. 1A to 1R, significant drought symptoms could be observed in all cultivars as yellowing, brown spot, and necrosis under drought stress. But the wilting symptoms were more severe and earlier in 'PLRF' (Figs. 1A to 1F) and 'Xingyu-1' (Figs. 1G to 1L) than in 'Xingyu-2' (Figs. 1M to 1R), and in lower leaves than in upper leaves. Wilting symptoms were not obvious in the upper leaves of three cultivars after 7 days of drought stress (Figs. 1A to 1B, 1G to 1H, and 1M to 1N), but a sharp increase of wilting symptom was observed in lower leaves of 'PLRF' and 'Xingyu-1' under 7 days drought stress (Figs. 1B and 1H). Since then, the wilting index continued to increase. 'Xingyu-1' (Figs. 1G to 1L) was more serious than 'PLRF' (Figs. 1A to 1F), when the drought stress continued to 35 days, the lower leaves of 'Xingyu-1' wilted seriously and was even unsuitable to

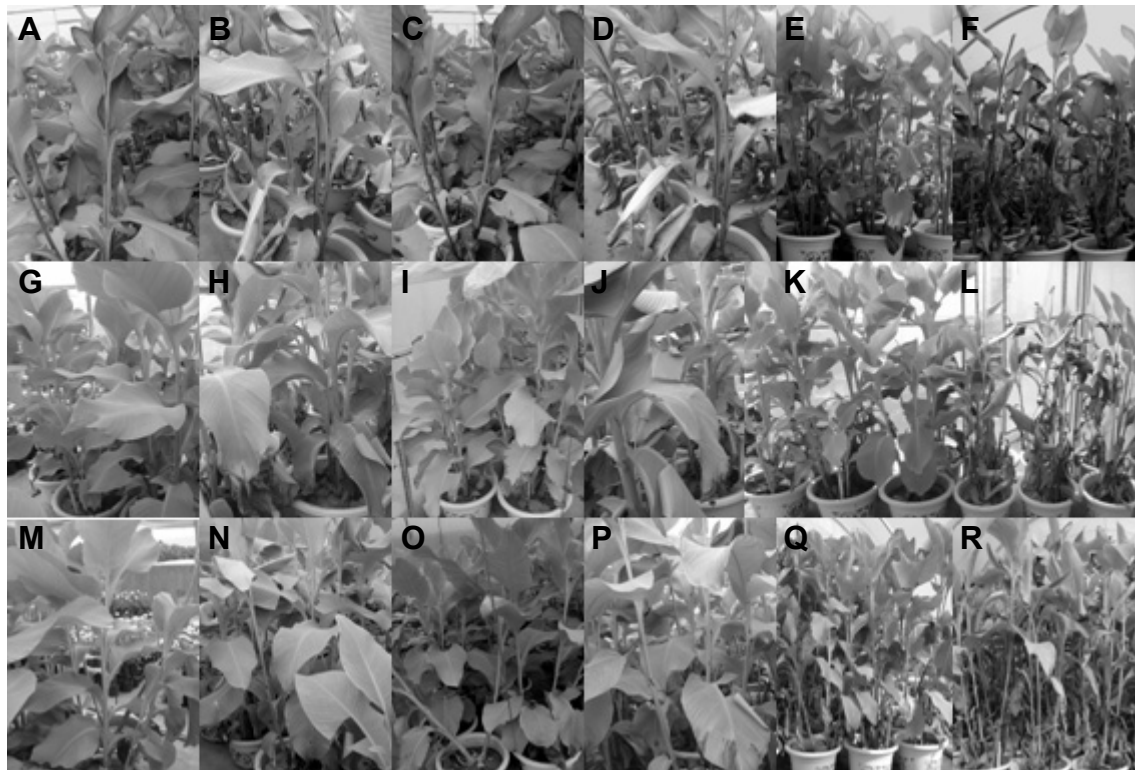


Fig. 1. Growth of edible canna 'PLRF' (A-F), 'Xingyu-1' (G-L), and 'Xingyu-2' (M-R) under drought stress for 14 (B, H, and N), 21 (D, J, and P), or 35 (F, L, and R) days or the control (A, C, E, G, I, K, M, O, and Q).

take samples for assay (Fig. 1F). For 'Xingyu-2' (Fig. 1M to 1R), wilting index changed little in upper leaves from 7 days to 28 days drought stress, but it changed obviously from 28 days to 35 days. This trend was more obvious in the lower leaves. As experiment time was longer and this time was a transitional season from summer to autumn, environment temperature and light condition changed largely, leaves of the control were also yellowing, in particular in the lower leaves from 4 Oct to 11 Oct (Figs. 1A, 1C, 1E, 1G, 1I, 1K, 1M, 1O, and 1Q).

Relative Water Content

In our study, relative water content (RWC) of three canna cultivars progressively decreased under drought stress compared to control (Figs. 2A to 2F). The decrease of RWC was more serious in the lower leaves than in the upper leaves. Among three cultivars, the strongest and earliest decline in RWC was found in 'Xingyu-1' (Figs. 2B and 2E), followed by 'PLRF' (Figs. 2A and 2D), the changes of 'Xingyu-2' under drought stress was the least (Figs. 2C and 2F). Compared with the control, the RWC decreased significantly in the upper leaves after 28 days, 35 days and 35 days of drought stress in 'Xingyu-1' (Fig. 2B), 'PLRF' (Fig. 2A) and 'Xingyu-2' (Fig. 2C), while that of the lower leaves decreased significantly on 21 days in 'Xingyu-1' (Fig. 2E) and 'PLRF' (Fig. 2D),

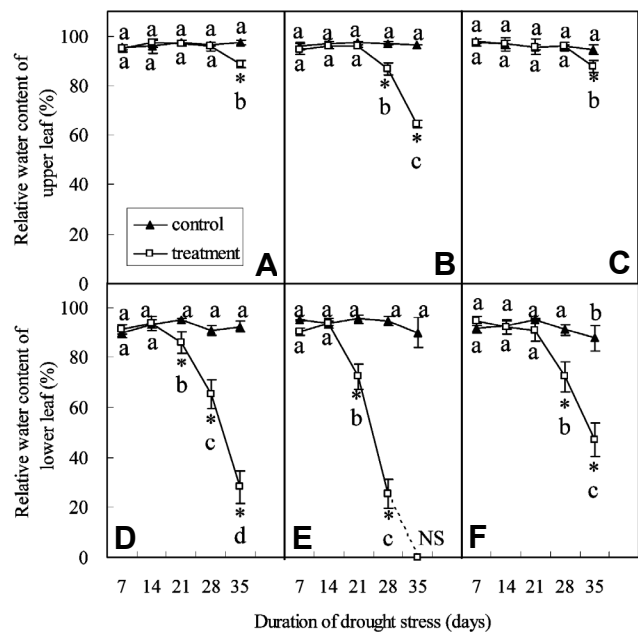


Fig. 2. Effects of drought stress on relative water content in upper leaf (A-C) and lower leaf (D-F) of edible canna 'PLRF' (AD), 'Xingyu-1' (BE), and 'Xingyu-2' (CF). Different letters on top of error bars represent significant differences among every measurement time point according to Duncan's multiple range tests and asterisks represent significant difference as compared to the control at $p < 0.05$ ($n = 3$). The NS represents unable to take samples at the time point. Vertical error bars represent SD.

and 28 days in 'Xingyu-2' (Fig. 2F), respectively. The lower leaves of 'Xingyu-1' wilted seriously on 28 days of drought stress, the RWC decreased to 25.36% at this point time, and the lower leaf of this cultivar completely shriveled on 35 days of drought stress, and was unsuitable to take samples (Fig. 1E).

Photosynthetic Parameters

As a consequence of decrease of RWC under water stress, a sharp decline in the rate of net photosynthesis (Pn), the value of stomatal conductance (Cond), intercellular carbon dioxide concentration (Ci), and transpiration rate (Tr) was observed in three cultivars, in addition to Ci of 'Xingyu-1' (Table 1). The decrease of Pn, Cond, Ci and Tr in 'PLRF' was stronger than in 'Xingyu-1' and 'Xingyu-2' after 35 days

of continuous drought stress. Compared to the control, the water use efficiency (WUE) decreased slightly in 'PLRF', decreased obviously in 'Xingyu-1', while increased significantly in 'Xingyu-2' under 35 days drought stress (Table 1).

Photosynthetic Pigments Content

In upper leaves, compared with the control, chlorophyll a (Chl.a) contents decreased significantly in 'PLRF' and 'Xingyu-1' after 35 days of drought stress (Figs. 3A and 3B), while Chl.a content of 'Xingyu-2' significantly increased at 7 days, 21 days and 28 days, and there were no significant difference at 14 days and 35 days between the control and drought stress (Fig. 3C). In lower leaves, compared with the control, drought stress obviously declined Chl.a content of 'PLRF' and 'Xingyu-1' from 7 days to 35 days, except

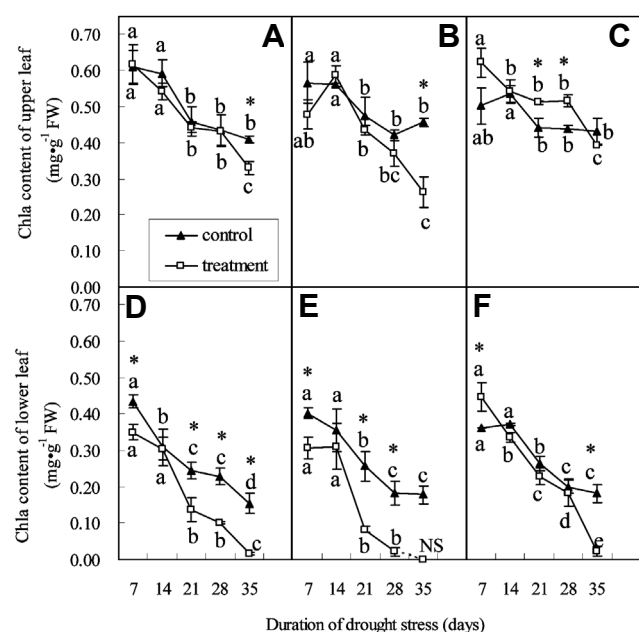


Fig. 3. Effects of drought stress on Chl.a content in upper leaf (A-C) and lower leaf (D-F) of edible canna 'PLRF' (AD), 'Xingyu-1' (BE), and 'Xingyu-2' (CF).

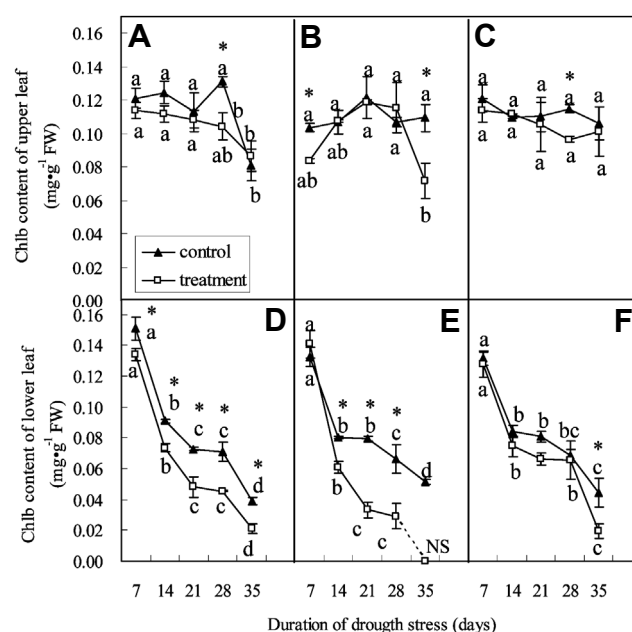


Fig. 4. Effects of drought stress on Chl.b content in upper leaf (A-C) and lower leaf (D-F) of edible canna 'PLRF' (AD), 'Xingyu-1' (BE), and 'Xingyu-2' (CF).

Table 1. Net photosynthetic rate (Pn), stomatal conductance (Cond), intercellular CO₂ concentration (Ci), transpiration rate (Tr), and water use efficiency (WUE) in edible canna 'PLRF', 'Xingyu-1', and 'Xingyu-2' under 35 days of drought stress.

Cultivar	Treatment	Pn ($\mu\text{mol CO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Cond ($\text{mol HO}_2 \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	Ci ($\mu\text{mol CO}_2 \cdot \text{mol}^{-1}$)	Tr ($\text{mmol H}_2\text{O} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$)	WUE ($\mu\text{mol CO}_2 \cdot \text{mmol}^{-1} \text{H}_2\text{O}$)
PLRF	Control	9.54 ± 2.02 a ²	0.099 ± 0.005 a	217.19 ± 9.98 a	2.107 ± 0.443 a	4.53 ± 0.006 a
	Drought	0.74 ± 0.24 b	0.007 ± 0.002 b	157.13 ± 10.95 b	0.203 ± 0.059 b	3.96 ± 0.356 a
Xingyu-1	Control	8.89 ± 0.75 a	0.118 ± 0.004 a	292.64 ± 6.95 a	1.823 ± 0.1597 a	4.82 ± 0.409 a
	Drought	1.54 ± 0.41 b	0.019 ± 0.007 b	274.04 ± 14.00 a	0.463 ± 0.06 b	3.45 ± 0.417 b
Xingyu-2	Control	11.96 ± 2.04 a	0.145 ± 0.020 a	243.77 ± 16.45 a	2.933 ± 0.198 a	4.06 ± 0.489 b
	Drought	2.35 ± 0.03 b	0.011 ± 0.002 b	51.99 ± 10.00 b	0.247 ± 0.035 b	9.90 ± 0.357 a

²Data are mean of three replicates ± SD. Different letters indicate statistical differences between drought treatment and the control in the same cultivar at $p < 0.05$ ($n = 3$) according to Duncan's multiple range tests.

14 days (Figs. 3D and 3E). But Chl.a contents of 'Xingyu-2' increased obviously at 7 days of drought stress, and there was no significant difference between the control and drought stress from 14 days to 28 days, the Chl.a content decreased obviously only after 35 days of drought stress (Fig. 3F).

In upper leaves, no significant difference of chlorophyll b (Chl.b) contents was detected in 'PLRF' and 'Xingyu-2' between the control and drought stress from 7 days to 35 days, except for 28 days (Figs. 4A and 4C), but drought stress obviously decreased Chl.b contents of 'Xingyu-1' at 7 days and 35 days (Figs. 4B). In lower leaves, the Chl.b contents of 'PLRF' and 'Xingyu-1' remarkably declined under drought stress from 7 days to 35 days when compared with the control, except 'Xingyu-1' at 7 days (Figs. 4D and 4E). However, Chl.b contents of 'Xingyu-2' in lower leaves sharply reduced by 55.36% only at 35 days of drought stress (Fig. 4F).

Malondialdehyde Content

For three cultivars, malondialdehyde (MDA) contents were progressively increased during experiment period in the test leaves (Figs. 5A and 5F). In the same cultivar, increase of MDA contents was stronger in the lower leaves than in the upper leaves. Compared with the control, continuous drought stress could considerably aggravate the membrane lipids peroxidation, the MDA contents of all cultivars in lower leaves increased significantly from 28 days to 35 days. The maximum increase rate of MDA contents was observed in the 'Xingyu-1', followed by 'PLRF', and then 'Xingyu-2'.

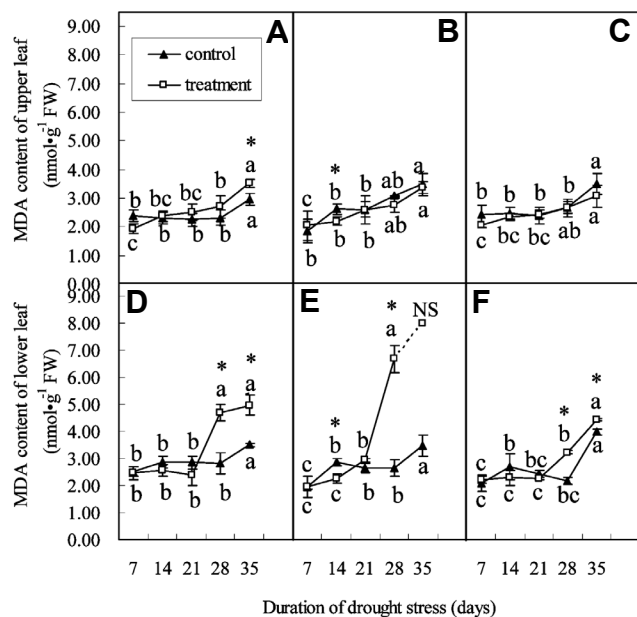


Fig. 5. Effects of drought stress on MDA content in upper leaf (A-C) and lower leaf (D-F) of edible canna 'PLRF' (AD), 'Xingyu-1' (BE), and 'Xingyu-2' (CF).

Antioxidant Enzyme Activities

Being exposed to drought stress, the antioxidant enzymes in three edible canna cultivars showed different responses (Figs. 6A to 6F). During whole experiment period, the activity of superoxide dismutase (SOD) increased progressively first and then changed little or decreased slightly in upper leaves of three cultivars (Figs. 6A to 6C). In upper leaves, the SOD activities of 'PLRF' under both treatments and 'Xingyu-2' in the control were peaked at 21 days, but these of 'Xingyu-1' under both treatments and 'Xingyu-2' under drought stress reached peak at 28 days (Figs. 6A to 6C). There was significant difference of SOD activity in lower leaves of three cultivars between the control and drought stress (Figs. 6D to 6F). Compared with the control, drought stress significantly depressed SOD activity of 'PLRF' from 7 days to 35 days, but these of 'Xingyu-1' and 'Xingyu-2' were no significant difference between the control and drought stress.

In upper leaves of 'PLRF' and 'Xingyu-2', catalase (CAT) activity showed little difference between both treatments from 7 days to 35 days, in addition to that of 'Xingyu-2' under 35 days of drought stress was obviously lower than that of the control (Figs. 7A and 7C), but drought stress significantly reduced CAT activity of 'Xingyu-1' in upper leaves from 7 days to 35 days compared with the control (Fig. 7B). In the lower leaves, in contrasted with the control, drought stress obviously enhanced CAT activity in all cultivars at 7 days (Figs. 7D to 7F). Since then, CAT activity of 'PLRF' declined sharply first and then kept stable (Fig. 7D), that of 'Xingyu-1'

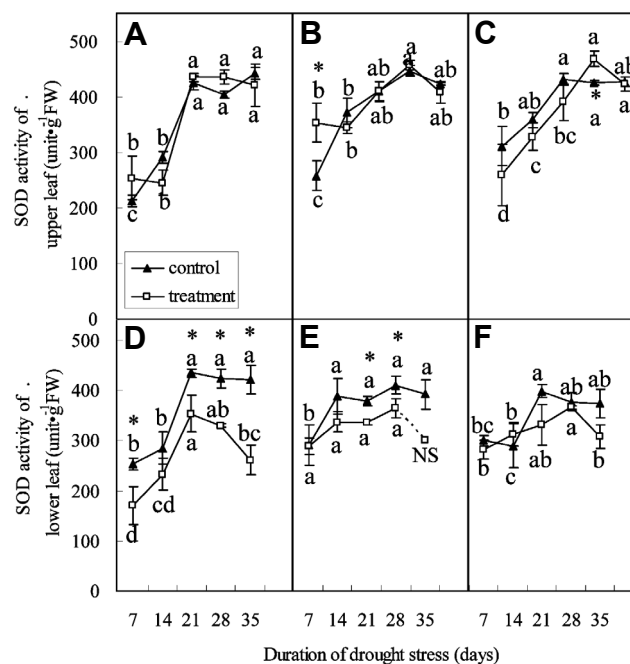


Fig. 6. Effects of drought stress on SOD activity in upper leaf (A-C) and lower leaf (D-F) of edible canna 'PLRF' (AD), 'Xingyu-1' (BE), and 'Xingyu-2' (CF).

progressively dropped (Fig. 7E), while that of 'Xingyu-2' kept stable first and then declined sharply (Fig. 7F). The CAT activities under drought stress were significantly lower than those of the control from 14 days to 35 days in 'PLRF', from 21 days to 35 days in 'Xingyu-1', and from 29 days to 35 days in 'Xingyu-2', respectively (Figs. 7D to 7F).

During the experiment period, peroxidase (POD) activity progressively increased in upper leaves of 'PLRF' and 'Xingyu-2', and that of 'Xingyu-2' exhibited a more dramatic increase than in 'PLRF' (Figs. 8A and 8C), while POD activity of 'Xingyu-1' increased first and then decreased, drought stress obviously lowered POD activity at 21 days (Fig. 8B). Different responses of POD activity were seen in the lower leaves of edible canna cultivars (Figs. 8D to 8F). At early stage of drought stress, there existed little difference between the control and drought treatment in three cultivars, but after 28 days of drought stress, the POD activity decreased dramatically in 'PLRF' (Fig. 8D), changed little in 'Xingyu-1' (Fig. 8E),

but increased obviously in 'Xingyu-2' (Fig. 8F). When the treatment was up to 35 days, drought stress significantly decreased the POD activities in 'PLRF' and 'Xingyu-2' (Figs. 8D and 8F).

The Correlation between Parameters

The correlation analysis showed that the RWC showed significant positive correlation with Chl.a ($r = 0.811, p < 0.01$), Chl.b ($r = 0.720, p < 0.01$), and CAT ($r = 0.572, p < 0.01$), and significant negative correlation with MDA ($r = -0.917, p < 0.01$) (Table 2). The MDA showed significant negative correlation with Chl.a ($r = -0.720, p < 0.01$), Chl.b ($r = -0.616, p < 0.01$), and CAT ($r = -0.408, p < 0.05$). The Chl.a was positively correlated with Chl.b and CAT, the correlation coefficient was 0.806 ($p < 0.01$) and 0.531 ($p < 0.01$), respectively. There were obvious positive correlation between Chl.b and CAT ($r = 0.697, p < 0.01$), between SOD and CAT ($r = 0.399, p < 0.05$), and between SOD and POD

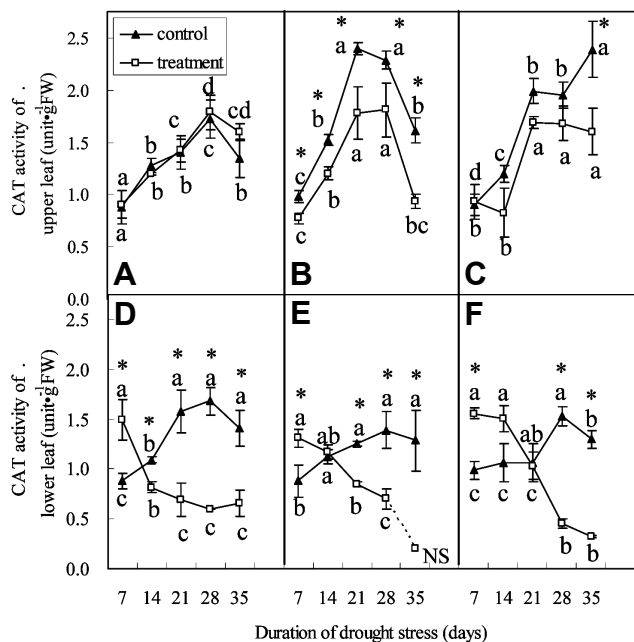


Fig. 7. Effects of drought stress on CAT activity in upper leaf (A-C) and lower leaf (D-F) of edible canna 'PLRF' (AD), 'Xingyu-1' (BE), and 'Xingyu-2' (CF).

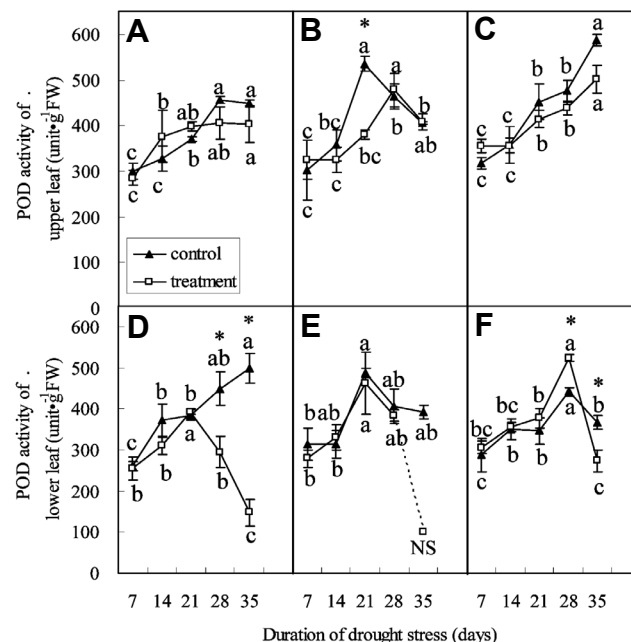


Fig. 8. Effects of drought stress on POD activity in upper leaf (A-C) and lower leaf (D-F) of edible canna 'PLRF' (AD), 'Xingyu-1' (BE), and 'Xingyu-2' (CF).

Table 2. Correlations of physiological index in leaves of edible canna 'PLRF', 'Xingyu-1', and 'Xingyu-2' under drought stress.

Index	RWC	MDA	Chl.a	Chl.b	CAT	POD
MDA	-0.917**					
Chl.a	0.811**	-0.720**				
Chl.b	0.720**	-0.616**	0.806**			
CAT	0.572**	-0.408*	0.531**	0.697**		
POD	0.242	-0.114	0.120	0.164	0.292	
SOD	0.055	0.102	0.039	-0.001	0.399*	0.693**

** Significant correlations at 5% and at 1% level (n = 29), respectively, using Pearson correlation coefficients.

($r = 0.693$, $p < 0.01$). These results indicated that drought stress directly led to leaf water loss, and then increased the MDA content and decreased Chl.a and Chl.b content, whereas CAT, SOD and POD presumably played major roles in response to drought stress.

Discussion

Drought, as an abiotic stress, is one of the major factors to limit the plant growth and ecosystem production around the world (Xiao et al., 2009). In our study, the growth of the edible canna was inhibited, and senescence was accelerated by drought stress, especially in the lower leaves (Fig. 1). Previous studies also documented that visible syndromes of plant exposed to drought were leaf wilting, decrease of plant height, number and area of leaves (Talebi, 2009). It is known that plants evolve to avoid the stress with the maintenance of high tissue water potential either by reducing water loss or by improving water uptake or both (Farooq et al., 2009). This may be a strategy for plant to avoid stress by supplying water first to the upper functional leaves to lessen the consequence of drought on the function leaves. In our study, it was indicated that water deficit showed up in lower leaves with sustaining of drought stress (Fig. 2) and the photosynthesis of upper leaves obviously declined due to water loss under 35 days of drought stress (Table 1). Reduction of photosynthesis is first triggered by stomatal closure, resulting in limitation of ambient CO₂ diffusion to the mesophyll and reduction of Ti (Ahmed et al., 2009; Grassi and Magnani, 2005). The WUE changed differently in edible canna when compared with the control, the WUE of 'Xingyu-2' showed a significant increase, 'PLRF' showed few change, while 'Xingyu-1' decreased obviously (Table 1). Drought-induced reductions of the photosynthetic pigment contents have been reported frequently (Elsheery and Cao, 2008; Loggini et al., 1999). In our study, in agreement with the plant morphological symptoms, photosynthetic pigment (Chl.a and Chl.b) contents decreased significantly under drought treatments in lower leaves of three cultivars (Figs. 3 and 4).

Drought stress is also known to enhance the production of reactive oxygen species (ROS), which could lead to the peroxidation of fatty acids in cell membranes (Foyer et al., 1994; Parkin et al., 1989). The Malondialdehyde (MDA) is the product of lipid peroxidation and usually used as a criterion to evaluate the stress-induced oxidative damage to membrane (Zhao and Tan, 2005). Low concentration of MDA has been associated with drought stress tolerance in some species, such as pea plants (Moran et al., 1994) and wheat (Sairam et al., 2000). In this study, the MDA contents increased progressively during our experiment period. Higher MDA contents were

observed in 'Xingyu-1' and in lower leaves (Figs. 5A to 5F) indicated 'Xingyu-1' was relatively more sensitive to drought than 'PLRF' and 'Xingyu-2', and the oxidative stress was more severe in the lower leaves than in the upper leaves. This result was accordance with the results of Zhang et al. (2013). The increase of MDA contents (Figs. 5A to 5F) and the rapid degradation of chlorophyll (Figs. 3 and 4) was observed in edible canna plants exposed to drought stress. There were significant negative correlation between chl. content and MDA content, significant positive correlation between Chl.a, Chl.b and RWC. These results suggested drought stress directly induced oxidative damage and led to the destruction of pigments synthesis, and finally decreased the photosynthesis efficiency (Tables 1 and 2).

Fortunately, plants have the capacity to eliminate ROS with an efficient ROS-scavenging system. Antioxidant enzymes, such as SOD, CAT, and POD, constitute the major part of the plant antioxidant defense system. Under drought stress, antioxidant enzymes are directly involved in scavenging ROS and protecting plant against ROS damage (Xue and Liu, 2008), they are crucial for determining the steady-state level of ROS (Habibi, 2012; Mittler, 2002; van Rensburg and Kruger, 1994; Yang et al., 2008). In this study, drought stress changed the activities of SOD, CAT, and POD in edible canna. In upper leaves, the SOD activity under drought stress was still as high as or even higher than the control in upper leaves of three cultivars (Figs. 6A to 6C), the CAT activity showed little difference between two treatments of 'PLRF' and 'Xingyu-2' from 7 days to 35 days (Figs. 7A to 7C), except 'Xingyu-2' at 35 days, but that of 'Xingyu-1' significantly decreased due to drought stress (Fig. 7B), and the POD activity of 'Xingyu-2' progressively increased and has no difference between two treatments (Fig. 8C), that of 'PLRF' also increased, but the increase rate was lower than 'Xingyu-2' (Fig. 8A), the POD activity of 'Xingyu-1' increased first and then decreased sharply in both treatments, and was obviously decreased by 21 days of drought stress (Fig. 8B). In lower leaves, the effect of water stress was more pronounced (Figs. 6 to 8). There were obvious linear trends between RWC, MDA, and CAT. So we could conclude that SOD, CAT and POD appeared to play an important role in resisting oxidative stress induced by water deficit, and associated with the better protection against oxidative damage in 'Xingyu-2' than in 'PLRF' and 'Xingyu-1'. These results indicated that 'Xingyu-2' was more drought tolerant than the 'PLRF' and 'Xingyu-1' by maintaining higher antioxidant enzymes. The similar results were observed in marigold (Tian et al., 2012), rice (Guo et al., 2006), cabbage (Singh et al., 2010), wheat (Simova-Stoilova et al., 2009) and white clover (Wang et al., 2008). The balance between SOD and CAT or POD

activities in cells is importance for ROS scavenging (Mathur et al., 2009). In our study, there were significant positive correlations between SOD, POD, and CAT. It was noted that the synergistic reactions of SOD-CAT-POD system effectively scavenged oxidative damage in edible canna under drought stress. This result was accordance with the results of Tian et al. (2012) and Han et al. (2010).

In conclusion, our results showed that ‘Xingyu-2’ appeared to be more resistant to drought stress because ‘Xingyu-2’ expressed lower wilting index at the same stress time and could tolerate more time under drought stress than ‘PLRF’ and ‘Xingyu-1’. In agreed with the wilting index and morphological symptoms, ‘Xingyu-2’ retained higher RWC and lower MDA content due to high antioxidant enzyme activities and higher WUE, especially in the lower leaves during early drought stress stage. These results could help us understand the response mechanism of edible canna genotypes to drought stress. It may contribute to select drought-resistant cultivars or germplasm based on these criteria, which will benefit cultivation and production of edible canna.

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