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Leaf senescence and photosynthesis in foxtail millet [*Setaria italica* (L.) P. Beauv] varieties exposed to drought conditions

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Abstract

In this paper, we studied the changes of net photosynthetic rates (P_n), the contents of chlorophyll, soluble protein, malondialdehyde (MDA), superoxide radical ($O_2^{\cdot-}$), and hydrogen peroxide (H_2O_2), and the activities of superoxide dismutase (SOD; EC1.15.1.1) and catalase (CAT; EC 1.11.1.6) in the leaves of two foxtail millet varieties (05-61 and Jingu3) under drought stress from 14 days after anthesis to maturity. P_N, the contents of chlorophyll and soluble protein and the activities of SOD and CAT increased and then declined as the plants aged, whereas the accumulation of MDA, $O_2^{\cdot-}$ and H_2O_2 gradually increased with senescence. Although the leaves of two varieties generally shared a similar pattern of senescence, their rates of aging differed. The activities of SOD and CAT in leaves of 05-61 declined more quickly than Jingu3, particularly in the late stages of grain filling. Taken together, the results implicated that the alleviation of leaf senescence played an important role in promoting grain filling and enhancing the yield and quality of 05-61 in the rain-fed agriculture area.

Keywords: foxtail millet; leaf senescence; chlorophyll content; antioxidative enzyme activity.
Abbreviation: PVC- Polyvinyl chloride; ROS- reactive oxygen species; SOD- superoxide dismutase; CAT- catalase; POD- peroxidase; MDA- malondialdehyde.

Introduction

Drought has a serious effect on plant growth and yield in many regions of the world. Environmental and endogenous factors interact to trigger the onset and progression of leaf senescence. Environmental factors may accelerate leaf senescence by affecting endogenous factors, including physiological aging (Noodén, 1988; Smart, 1994; Dai et al., 2009; Dai et al., 2011a, b), reproductive development, and hormone levels. Whereas, these endogenous factors may, in turn, affect the capacity of the plant to induce leaf senescence under drought stress. Leaf senescence occurs in an orderly manner and involves the catabolism of compounds associated with nutrient relocation to the developing grains (Dai et al., 2011b). The timing of senescence is important in agriculture. During plant aging, drought stress usually induces the accumulation of reactive oxygen species (ROS) (Chaves and Oliveira, 2004; Foyer and Noctor, 2005; Turkan et al., 2005). If not effectively and rapidly removed from plants, ROS can damage a wide range of cellular macromolecules, such as lipids, enzymes and DNA. Plants combat oxidative damage by synthesizing antioxidative enzymes, such as catalase (CAT, EC 1.11.1.6), ascorbate peroxidase (APX, EC 1.11.1.11), superoxide dismutase (SOD, EC 1.15.1.1), and glutathione reductase, as well as nonenzymatic compounds, such as glutathione and ascorbate (Mittler, 2002). Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acid hydroperoxides, can result in oxidative damage and has frequently been used as a biomarker for lipid peroxidation (Turkan et al., 2005). Furthermore, the inactivation of photosynthesis is closely related with loss of the reaction center complexes during leaf senescence (Kura-Hotta et al., 1987). Disruption of ROS metabolism is a key elicitor of early senescence in leaves, and results in chlorophyll degradation, loss of photosynthesis-related enzyme activity, and decline in photosynthetic competency. Foxtail millet (*Setaria italica* (L.) P. Beauv) has been an important cereal since ancient times and has contributed greatly to human civilization in both Asia and Europe (Li et al., 1996; Lu et al., 2009). Apart from being rich in a variety of amino acids and nutritional minerals, foxtail millet exhibits high photosynthetic efficiency and drought tolerance (Dai et al., 2008; Dai et al., 2011a). Furthermore, foxtail millet porridge is a traditional food in Asia, Europe, North America, Australia, and North Africa. Moreover, foxtail millet is generally grown on marginal lands with little or no irrigation water and can survive with little fertilizer and without organic manures (Dai et al., 2009; Dai et al., 2011a, b).

Table 1. Quantification of various parameters of different foxtail varieties grown under drought conditions. Values represent the means of six replicates ± standard deviations. * represents significant differences ($P \leq 0.05$).

Cultivars	Plant height (cm)	Number of grains/plant	Number of seeds/grain	1000-grain weight (g)	Seed yield (kg hm^{-2})	Harvest index (%)
05-61	116.76	271±13*	1329±212*	2.33±0.2*	4317±289*	56.31
Jingu 3	110.19	265±15*	1090±201*	3.05±0.2b*	5123±321*	43.19

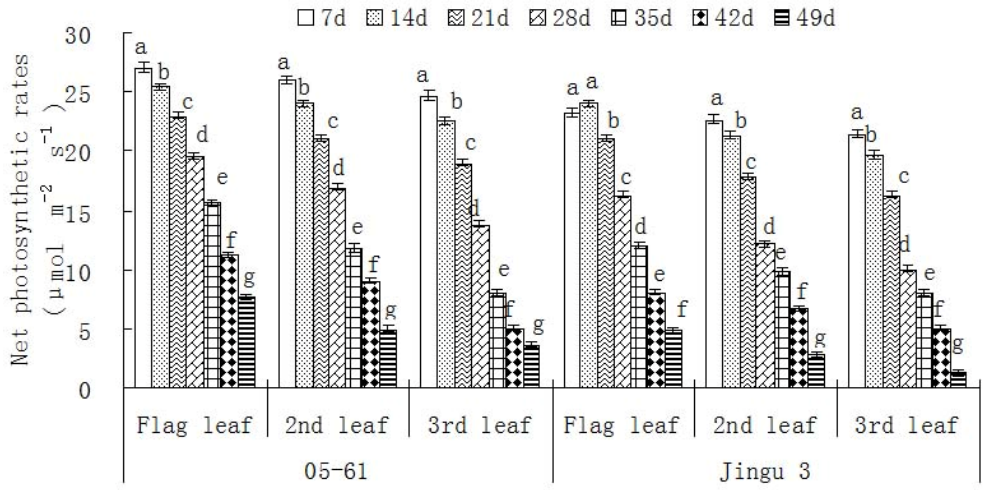

Fig 1. Changes in net photosynthetic rates at various stages of growth in different foxtail millet varieties grown under drought conditions. Values are the means of six replicates ± standard deviations. Different small alphabets are represent significant differences at $P \leq 0.05$.

However, little is known about the biochemical processes related with the drought tolerance of foxtail millet. To date, most studies of leaf senescence and reactive oxygen species metabolism under drought stress have focused on wheat, corn, and rice, but little is known about foxtail millet. Thus, it is interesting to study leaf senescence and reactive oxygen species metabolism of foxtail millet (*Setaria italica* (L.) P. Beauv) under drought conditions.

Results

Net photosynthetic rates in leaves

In Fig.1, the net photosynthetic rates of both varieties gradually declined from 14 days after the flowers reached maturity. 05-61 showed higher net photosynthetic rate than Jingu3. The flag leaves had higher net photosynthetic rate than the second and third leaves. As the plant matured, the net photosynthetic rate in foxtail millet leaves decreased gradually.

Chlorophyll content

Chlorophyll content in the leaves of foxtail millet exposed to drought gradually declined from 14 days after the varieties flowered (Fig.2A). Although leaf senescence of the two varieties proceeded in a similar manner, there was significant differences between them ($p \leq 0.05$). Interestingly, 05-61 had higher chlorophyll content than Jingu3, particularly in the flag leaves. The difference was smaller in the second and third leaves. As the plants matured, the chlorophyll content gradually decreased.

Antioxidant enzyme

The activities of SOD and CAT in the leaves gradually declined from 14 days after the varieties flowered (Fig. 2B and C). Although the changes in enzyme activity were similar in two varieties, there was also significant differences between them ($p \leq 0.05$). These results indicated that flag leaves might be able to alleviate the damage caused by active oxygen and the peroxidation of membrane lipids, and thereby delay leaf senescence.

Lipid peroxidation

The malondialdehyde (MDA) content was low in the leaves of two varieties and increased gradually as the plants aged (Fig. 2D). 05-61 accumulated less MDA than Jingu3, and the contents of MDA in the flag leaves of 05-61 declined more gradually than Jingu3. Our studies suggested that leaf senescence played an important role in anabolism and affected yield in the late stage

Superoxide radicals ($O_2^{-\cdot}$) and hydrogen peroxide (H_2O_2) content

Levels of $O_2^{-\cdot}$ and H_2O_2 were low and gradually increased as the plants aged (Fig. 3A and B). Although the levels of these compounds in the leaves of two varieties changed in a similar manner, there was significant difference between them ($p \leq 0.05$). 05-61 accumulated less $O_2^{-\cdot}$ and H_2O_2 than Jingu3, and levels of $O_2^{-\cdot}$ and H_2O_2 in the flag leaves of 05-61 declined more gradually than Jingu3.

Table 2. Correlation coefficient between various physiological indicators of drought tolerance and yield per plant at the late growth stage of foxtail millet. Values are the means of six replicates ± standard deviations. ** and * represent significant differences at $P \leq 0.01$ and $P \leq 0.05$, respectively.

Parameter	Yield per/plant	P_n	Chl	SOD	CAT	MDA	0_2^-
*P*n	0.9726**						
Chl	0.9623**	0.9586**					
SOD	0.9121*	0.9269*	0.9538*				
CAT	0.8682*	0.9231*	0.9356*	0.9618*			
MDA	-0.8376*	0.9338*	-0.8715	-0.8293*	0.7157*		
0_2^-	-0.8236*	0.9013*	-0.8571*	-0.8913*	-0.9265	0.8837	
H_2O_2	-0.8166	0.8369*	-0.8923*	-0.8479	-0.7879	0.7661	0.7553

Plant height and seed yield

As indicated in Table 1, 05-61 exhibited a higher number of seeds per grain than Jingu3, 1000-grain weight and harvest index. However, the yield of 05-61 was significantly higher than Jingu3. Furthermore, there were significantly differences ($P<0.01$) in the plant height at harvest, the number of grains per plant, and the seed yield between two varieties.

Correlation analysis between yield and biomedical and physiological factors

Average of each biomedical and physiological index during the functional leaf late growth stage and yield per plant were selected to conduct correlation analysis (table2).The results indicated that net photosynthesis rate, chlorophyll content, and yield per plant exhibited significant positive correlation, and their corresponding correlation coefficient r amounted to 0.9726 and 0.9623, respectively. The net photosynthesis rate and chlorophyll content exhibited a significant positive correlation with yield per plant, and the corresponding correlation coefficient values were 0.9726 and 0.9623, respectively. The activities of CAT and SOD both positively correlated with yield per plant, and the corresponding correlation coefficient values were 0.9121 and 0.8682, respectively. Levels of MDA, 0_2^-, and H_2O_2 were negatively correlated with yield per plant, and the corresponding correlation coefficient values were -0.8376, -0.8236 and -0. 8166, respectively.

Discussion

The expansion of arid zones on our planet and the growing population of world will have a direct impact on water resources and water availability (Dai et al., 2009). Water scarcity and the concurrent high temperatures will significantly limit crop productivity. Here, we showed that the changes in metabolites under drought may reflect changes in photosynthesis. The rate of photosynthesis decreased mainly due to stomata closure (Kura-Hotta et al., 1987; Sigaud-Kutner et al., 2002). As the stress progressed, biochemical constraints might limit photosynthetic CO_2 fixation more directly (Dai et al., 2011a, b). As limitation of CO_2 assimilation preceded inactivation of electron transfer reactions, an excess of reducing power is frequently generated in water-stressed plants (Chaves et al., 2003). Thus, the over-reduction of the photosynthetic electron chain may result in the formation of active oxygen species (AOS) which then led to photoinhibitory and photooxidative damage. Furthermore, senescence and oxidative stress syndromes shared a number of symptoms, such as net loss of chloroplastic pigments and proteins, lipid peroxidation, and membrane alterations, which together led to a progressive decline in photosynthetic capacity (Dai et al., 2011a, b). Crops could protect themselves from active oxygen species by antioxidant systems. Our results indicated that the net photosynthetic rate, chlorophyll content, and the activities of SOD and CAT gradually decreased from 14 days after the varieties flowered, but the contents of MDA, H_2O_2, and $O_2^{\cdot-}$ gradually increased as leaves senesced. Although the leaves of two varieties generally exhibited a similar pattern of senescence, they displayed significant differences ($P \leq 0.05$). The activities of SOD and CAT in the leaves of 05-61 declined more gradually plants than Jingu3, thus prolonging their function durations and keeping their net photosynthetic rates relatively higher during late growth. Taken together, the yield of 05-61 was significantly higher than Jingu3. Above results indicated that effectively slowing down the senescence of leaves at the grain-filling stage and maintaining the physiological functions of leaves during late growth was important to the seed yield formation of foxtail millet.It is possible to delay leaf senescence by reducing senescence-specific cytokinin production, as pioneered by Gan and Amasino (1995) in *Arabidopsis*. This strategy is of particular relevance to forage crops and has been increasingly applied (Pang et al., 2005; Hu et al., 2005; Dai et al., 2011b). Leaf loss is related to the reduction in penetrance of light in the lowest layers of the canopy in 05-61. Therefore, the increase of leaf longevity reported in this paper could reduce yield losses and improve forage quality (Calderini et al., 2007; Mandal et al., 2010; Dai et al., 2011a, b). Since leaf senescence is an evolutionarily acquired process, plants evolved in different ecological environment will exhibit distinct senescence patterns (Dai et al., 2011b). Our results indicated that crops like foxtail millet cultivated in water deficit areas would increase their antioxidant metabolism. Taken together, the crops appeared to be highly regulated by secondary metabolites and components of antioxidative systems under drought stress. In our experiment, 05-61 genotypes with better drought tolerance maintained higher antioxidant enzyme activities and had lower oxidative damage from 14 days after anthesis to maturity. Resistance against water deficit-induced oxidative stress was therefore mainly dependent on the genetic potential (better antioxidant defence system) of the genotypes. Genotypic response to

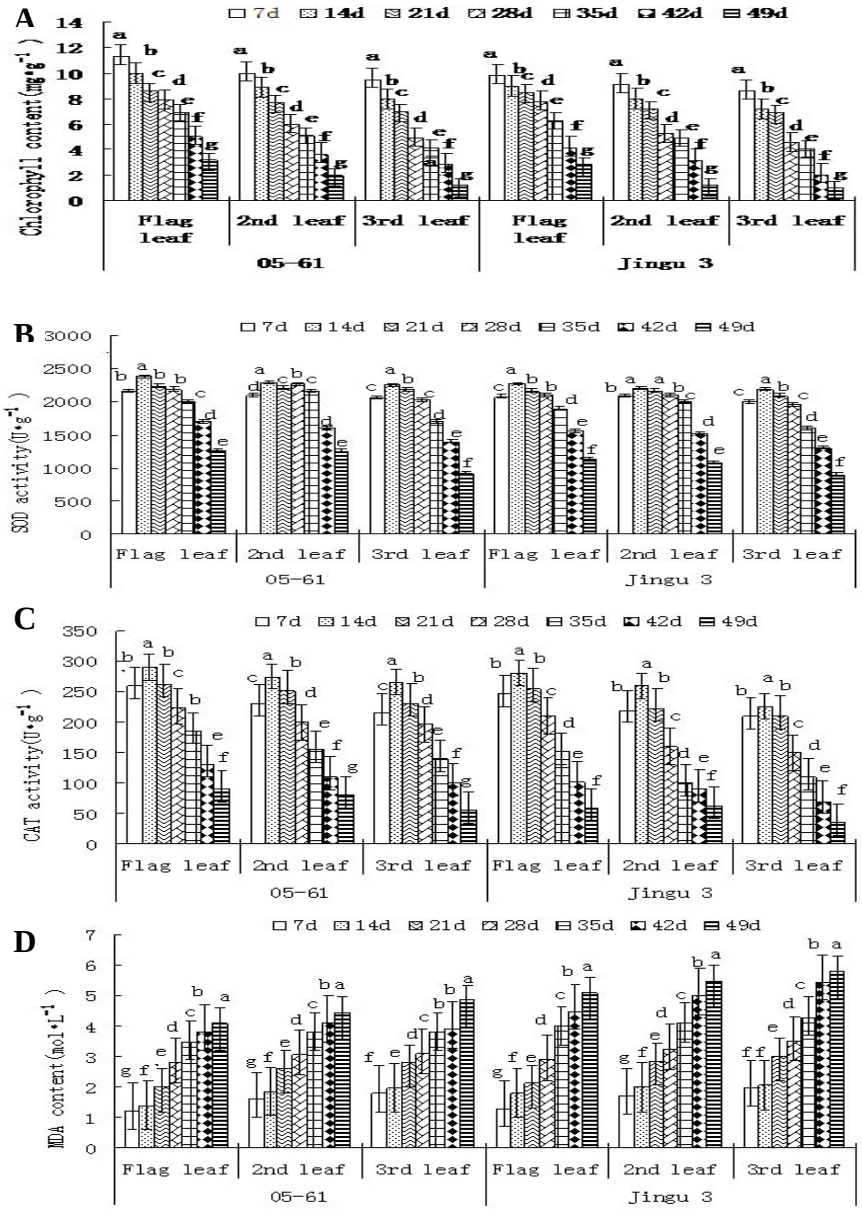

Fig 2. Changes in chlorophyll content (A), SOD activity (B), CAT activity (C), and MDA content (D) in leaves of the two foxtail millet varieties at various growth stages. Values are means of six replicates ± standard deviations. Different small alphabets are represent significant differences at $P \leq 0.05$.

water deficit was more prominent and clear under drought condition as compared to other stress levels. Antioxidant status and lipid peroxidation in flag leaves can be used as indices of water deficit tolerance in foxtail millet. Thus, it would be of great interest to utilize the information obtained from foxtail millet for the production of crops in the arid area.

Materials and Methods

Materials

Two foxtail millet varieties, 05-61 and Jingu3 were chosen for the experiment. The experiment was conducted in 2007 and 2008. The two varieties were grown under similar conditions.

Experimental Design

The experiment was carried out at the No 1 Agricultural Experiment Station in Northwest A&F University, Yangling, China. The station is located in the southern, sub-humid area of the Loess Plateau (108°04′E，34°20′N), the altitude of which is 505 m, with the average precipitation of 18.8 mm (May), 53.1 mm (June), 55.6 mm (July), 131 mm (August), and 71 mm (September), respectively. Field experiment was conducted in the summer of 2007 and 2008 (from 11 May to 1 September). Loam soil was used in the experiment. The

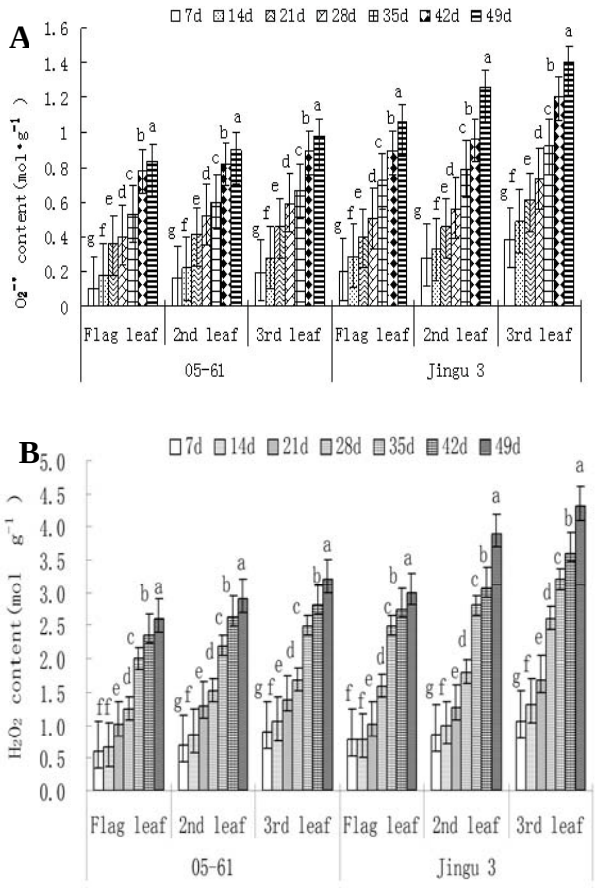

Fig 3. Changes in $O_2^{\cdot-}$(A) and H_2O_2(B) content at various growth stages in the leaves of two different foxtail millet varieties grown under drought conditions. Values are means of six replicates ± standard deviations. Different small alphabets are represent significant differences at $P \leq 0.01$.

soil column method was used. First, a 100-cm deep hole was dug and the soil was stacked together. After that, polyvinyl chloride (PVC) pipes (100 cm long) with a diameter of 20 cm were placed into the pits, and the pits were backfilled with the soil of the corresponding layers. Foxtail millet was grown in an area covered by a mobile rain shelter. The simulation experiment was performed by using soil moisture treatments from the third leaf stage to maturity by controlling irrigation. From 7 to 49 days after anthesis, the soil moisture was maintained as following: 22% (7 days), 21% (14 days), 21% (21 days), 19% (28 days), 18% (35 days), 16% (42 days), and 12% (49 days).

Biochemical analyses

Samples of the top three leaves for all genotypes in all plots were collected individually every seven days from anthesis to kernel formation. Samples from the same leaf level of five individuals in one plot were mixed and measured. Leaf extracts were soaked in 80% acetone, and chlorophyll content was measured by using a UVIKON220 spectrophotometer according to Heath et al. (1968).

Measurement of net photosynthetic rate

The net photosynthesis rate in leaves was measured by using a portable LI-6400 photosynthesis system (Li-Cor 6400, Li-Cor Inc., Lincoln, NE, USA) from 8:30 to 12:30 each day. The LI-6400 was operated as an open system. When the net photosynthesis rate (P_n) was performed, leaf temperature was set at 27℃, flow rate was set at 1000 μmol m^{-2} s^{-1}, and photosynthetically active radiation, provided by a red-blue light source, was set at 1,000 μmol m^{-2} s^{-1}.

Assay of antioxidant enzymatic activity

The SOD activity was determined according to Stellmach (1992) by measuring the ability of extracted enzyme to inhibit the photoreduction of nitroblue tetrazolium (NBT). The reaction solution (3 mL) contained 50 mM NBT, 1.3 mM riboflavin, 13 mM methionine, 75 nM EDTA, 50 mM phosphate buffer (pH7.8), and 20 to 50 ml of enzyme extract. The test tubes containing the reaction solution were irradiated under light (15 fluorescent lamps) at 78 mmolm-2 s-1 for 15min.The absorbance of the irradiated solution at 560 nm was read using a spectrophotometer (UVIKON220).One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction. The CAT activity was evaluated by using the method of Navabpour et al. (2003) with some modifications. The CAT reaction solution (3 mL) contained 50 mM phosphate buffer (pH7.0), 5.9 mM H_2O_2, and 0.1 mL of enzyme extract. The reaction was initiated by adding the enzyme extract. Changes in absorbance of the reaction solution at 240 nm were read every 20 s. One unit of CAT activity was defined as an absorbance change of 0.01 units per min. The absorbance rate at 410 nm was measured and the H_2O_2 concentration was calculated according to a standard curve. The generation rate of $O_2^{\cdot-}$ was determined following the method of Wang and Luo (1990).

Lipid peroxidation

Oxidative damage to lipids was estimated as the total amount of 2-thiobarbituric acid (TBA) reactive substance and expressed as equivalents of malondialdehyde (MDA) according to the method of Heath et al. (1968).

Statistical analysis

All statistical tests were performed with SPSS16.0. Data for each variable were subjected to one-way analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) at 5% probability was used to identify significant differences among the mean values of different parameters. Values are the means of six replicates.

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