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Leaf senescence and reactive oxygen species metabolism of broomcorn millet (*Panicum miliaceum* L.) under drought condition

Hui-Ping Dai[1], Pan-Pan Zhang[2], Chao Lu[2], Gen-Liang Jia[3], Hui Song[2], Xue-Min Ren[2], Jia Chen[2], An-Zhi Wei[4*], Bai-Li Feng[2*] and She-Qi Zhang[3]

[1]College of Life Science, Northwest A&F University, Yangling, Shaanxi 712100, P.R. China
[2]State Key Laboratory of Crop Stress Biology on Drought Regions, Northwest A&F University, Yangling Shaanxi 712100, P.R. China;
[3]College of Science, Northwest A&F University, Yangling, Shaanxi 712100, P.R. China
[4]College of Forestry, Northwest A&F University, Yangling, Shaanxi 712100, P.R. China

* Corresponding authors: daihp72@yahoo.com.cn or 7012766@163.com

Abstract

From anthesis to mature stage, changes in the contents of chlorophyll and soluble protein, malondialdehyde (MDA) and the activities of antioxidative enzymes: superoxide dismutase (SOD, EC1.15.1.1), catalase (CAT, EC 1.11.1.6) and peroxidase (POD,EC 1.11.1.7) in the leaves of three broomcorn millet varieties (Ning-Mi 13,Nei-Mi 5 and Yu-Mi 3) were studied. The results showed that the contents of chlorophyll and soluble protein and the activities of SOD, CAT and POD in leaves of three broomcorn millet varieties rose and then declined with the progression of leaf senescence, the content of activities of antioxidative enzymes of flag leaf was high and declined slowly, and the content of MDA was low and rose gradually with senescence. Although the leaves of the three varieties generally presented a similar tendency in their senescence, their senescence progresses significantly differed. Among the varieties, the activities of SOD, CAT and POD reduced slowly in Ning-Mi 13, followed by Nei-Mi 5 and Yu-Mi 3. These changes were observed significantly in the middle and late stage of grain filling. Taken together, the results implicated a vitally important role played by leaf senescence alleviation in promoting grain filling and enhancing the yield and quality of Ning-Mi 13 in the rain-fed agriculture area.

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

Introduction

Drought stress limits plant growth and crop productivity significantly. However in certain tolerant/adaptable crop plants morphological and metabolic changes occur in response to drought, which contribute towards adaptation to such unavoidable environmental constraints. Previously Siddique et al., (2000) had shown that drought stress significantly decreased RWC (relative water content) and this in turn had a pronounced effect on the photosynthetic rate. They suggested that an increase in leaf and canopy temperature, due to drought stress, resulted from an increase in respiration and a decrease in transpiration due to stomata closure. Broomcorn millets (*Panicum miliaceum* L.) are major food in arid and semi-arid regions. Broomcorn millet are rich in B vitamins, especially abundant in niacin, B6, folic acid, calcium, iron, potassium, magnesium, and zinc. However, broomcorn millets are also a mild thyroid peroxidase inhibitor. Broomcorn millet porridge serves as a traditional food in Russia, Germany and China, and it is generally grown in spring season, on marginal lands with less or no irrigation water. Moreover, the crop can survive with sub-optimal rate of fertilizer and without organic manures (Dai et al., 2008; Dai et al., 2009). Water and nutrients are the limiting factors for getting good harvest. Little information is known about efficient water management in combination with nutrient management (Dai et al., 2008). In previous studies, remote sensing application was employed to monitor crop growing conditions and estimate crop yields. Crop yield integrates the accumulated effects of many spatially-variable factors such as soil properties, fertilization, topography, and the infestations of weeds, insects, and diseases; therefore, the yield is one of the most important information for precision farming (Mandal et al., 2010; Dai et al., 2011). Not only does it help identify within-field spatial variability for variable rate applications, but also enables people to evaluate the economic returns of different farming management strategies (Jia et al., 2009; Dai et al., 2009; Dai et al.,2011). In recent years, leaf senescence represents an endogenously controlled degenerative process that ultimately leads to organ death. It progresses in an age-dependent manner, but is also affected by a complex interaction of developmental age with other internal and external factors (Noodén, 1988; Smart, 1994; Dai et al., 2009). The external factors include temperature,

Table 1. Plant height and yield components with correlation analysis of yield factors in broomcorn millet.

Cultivars	Plant height	Plant number (plant/hm^{-2})	Number of seeds/ grain	1000-grain weight (g)	Seeds yield (hm^{-2})	Harvest Index
Ning-Mi 13	106.76	540000	743.2±4.7**	7.8±0.2**	4317±289*	56.31
Nei-Mi 5	110.19	540000	468.8±2.0*	5.8±0.1**	5123±321*	43.19
Yu-Mi 3	121.3	540000	365±1.6**	5.1±0.2**	1531.9±6.2*	39.3

Note: Each point represents the mean of six biological replicates ±S.D. (significance levels: *p < 0.05).

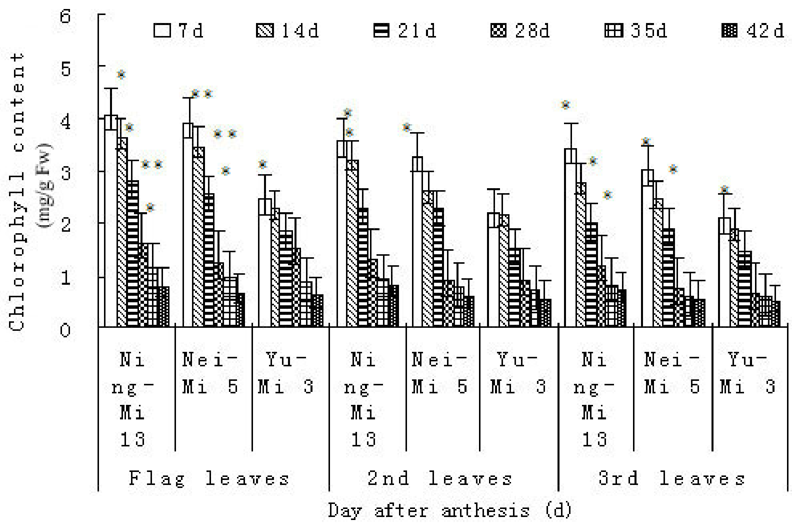

Fig 1. Changes of chlorophyll content of among different broomcorn millet. ** Significance at 0.01 probability level, * at 0.05 probability level among difference broomcorn millet varieties in the same day, according to Duncan's multiple comparison. Sample variability is given as the standard deviation (S.D.) of the mean.

drought, nutrient deficiency, shading, wounding, and pathogen infection. The internal factors include developmental stages and phytohormone levels (Hensel et al., 1993). The timing of senescence is very important in agriculture. During the process of plant aging, reactive oxygen species (ROS) injury was the primary factor leading to leaf senescence. Plant can generate ROS through multiple ways, while a host of ways existed to eliminate the ROS in living cell. Among them, ROS scavenger system played a pivotal role, and superoxide dismutase (SOD),catalase (CAT) and peroxidase (POD) activities were essential protective enzymes in the system which can effectively hamper intracellular ROS from increasing, such as $O_2^{\cdot-}$, H_2O_2,$\cdot OH$ and $^1O_2$, prevent lipid peroxidation, defer leaf senescence and thus maintain the normal growth and development in plant. These ROS then can result in injury to plant tissues (Foyer and Noctor, 2005; Turkan et al., 2005). Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids hydroperoxides, can result in oxidative damage and has been frequently used as a biomarker for lipid peroxidation (Turkan et al., 2005). Also, the inactivation of photosynthesis is closely related with loss of the reaction center complexes during leaf senescence in rice seedlings (Kura-Hotta et al., 1987). The disorder of ROS metabolism served as a key elicitor promoting early senescence in leaf, rendering chlorophyll degradation, the activity of photosynthesis-related enzymes decline and photosynthetic competency compromised. To date, most studies are focused in wheat, corn and rice, yet little is known about what happens during leaf senescence in broomcorn millet.

Results

Chlorophyll and Soluble protein content

In Fig.1 and Fig. 2, the contents of chlorophyll and soluble protein gradually declined from 14 days after the varieties flowered to maturing.. Although the leaves of the three varieties generally presented a similar tendency in their senescence, their senescence progresses significantly differed. Among the varieties, Ning-Mi 13 showed the highest chlorophyll and soluble protein contents, followed by Nei-Mi 5 and Yu-Mi 3. Chlorophyll and soluble protein contents were considerably higher in the flag leaves but less than 2nd and 3rd leaves. With the advancement of maturity in plant, the content of chlorophyll in broomcorn millet leaf decreased gradually.

Antioxidant enzyme

In Fig 3, Fig 4, and Fig 5, the SOD, CAT and POD activities gradually declined from 14 days after the varieties flowered to maturing. Among the varieties, although the leaves of the three varieties generally presented a similar tendency in their senescence, their senescence progresses significantly differed, the Ning-Mi 13 was considerably the highest in the flag leav-

Table 2. Correlation coefficient between physiological indicators of drought tolerance and yield per plant at late growth stage of broomcorn millet.

Characters	Yield per/plant	Chlorophyll content	Soluble protein	SOD activity	CAT activity	POD activity
Chlorophyll content	0.9226*					
Soluble protein	0.9035*	0.9436*				
SOD activity	0.9595**	0.9763*	0.5883			
CAT activity	0.9549*	0.9516**	0.6735	0.7032		
POD activity	0.9245*	0.9051*	0.6125	0.6023	0.5872	
MDA content	–0.7765*	–0.8573*	–0.9011*	–0.8433*	0.7767*	–0.7765*

Note: Each point represents the mean of six biological replicates ±S.D. (significance levels: *p < 0.05).

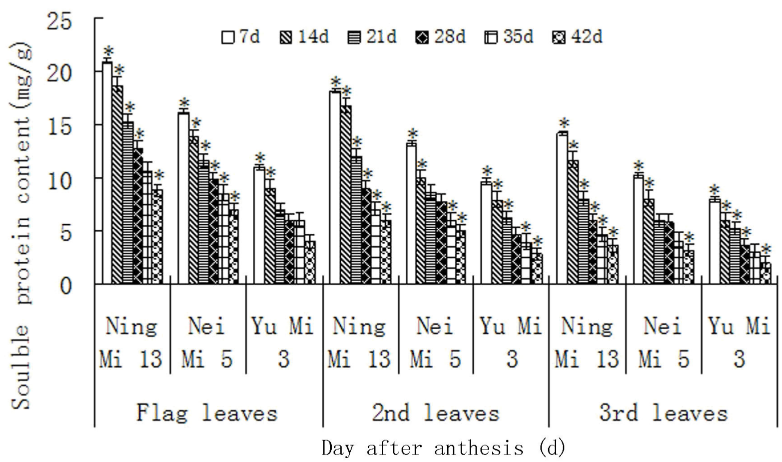

Fig 2. Changes of soluble protein content of among different broomcorn millet. ** Significance at 0.01 probability level, * at 0.05 probability level among difference broomcorn millet varieties in the same day, according to Duncan's multiple comparison. Sample variability is given as the standard deviation (S.D.) of the mean.

es but less than 2nd and 3rd leaves. The results indicated that flag leaves might be able to efficiently alleviate the damages to membranes caused by active oxygen and the peroxidation of membrane lipids, thereby delaying leaf senescence.

Lipid peroxidation

In Fig. 6, lipid peroxidation level in the leaves of the three broomcorn millet varieties, and the contents of MDA were low and rose gradually with plant ageing. Among the varieties, the Ning-Mi 13 showed the lowest MDA accumulation followed by Nei-Mi 5 and Yu-Mi 3, flag leaves of Nei-Mi 5 presented the lower declining rate, while Yu-Mi 3 flag leaf exhibited the highest declining rate. Our studies suggest leaf senescence might play a potent role in anabolism and affect yield in the late stage.

Plant height and seed yield

As indicated in the table1, among the varieties, Ning-Mi 13 showed the highest seed number per plant, 1000 grains dry weight and harvest index, followed by Nei-Mi 5 and Yu-Mi 3. However, the yield of Ning-Mi 13 was significantly higher than that of Nei-Mi 5 and Yu-Mi 3. Furthermore, the broomcorn millet varieties plant height at harvest index, number of grains/plant, number of seeds, 1,000-seed weight and seed yield was significant (P<0.01).

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, and the results indicated (table2), chlorophyll and soluble protein, CAT activity and yield per plant exhibited significant positive correlation, and their corresponding correlation coefficient r amounted to 0.9226; 0.9035 and 0.9549, respectively; there was an extremely significant positive correlation between SOD dismutase activity and yield per plant, and the coefficient r = 0. 9595; POD activity and yield per plant presented a highly significant correlation, whose coefficient r =0. 9745; MDA and yield per plant revealed an insignificant negative correlation with the coefficient r =0.7765.

Discussion

During a drought period, plants face the dilemma 'to lose water to gain carbon' (Chaves et al., 2003). Under water stress conditions, increased stomatal closure and reduced mesophyll conductance are the main causes of decreased photosynthesis rates, by reducing CO_2 diffusion from the atmosphere to the carboxylation sites (Chaves et al., 2003; Flexas et al., 2006). However, the drought-induced regulation of assimilation is relatively well known. Furthermore, senescence and oxidative stress syndromes share a number of

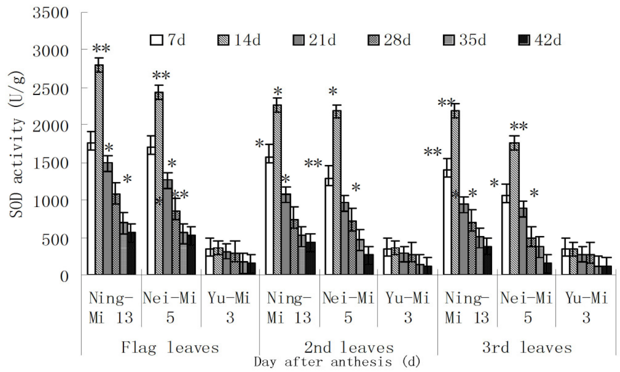

Fig 3. Changes of SOD activity of among different broomcorn millet. ** Significance at 0.01 probability level, * at 0.05 probability level among difference broomcorn millet varieties in the same day, according to Duncan's multiple comparison. Sample variability is given as the standard deviation (S.D.) of the mean.

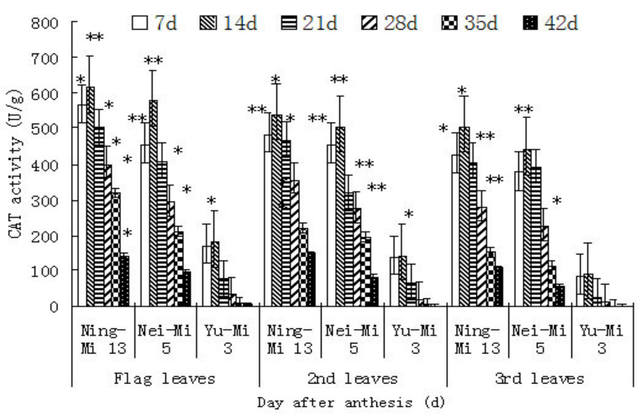

Fig 4. Changes of CAT activity of among different broomcorn millet. ** Significance at 0.01 probability level, * at 0.05 probability level among difference broomcorn millet varieties in the same day, according to Duncan's multiple comparison. Sample variability is given as the standard deviation (S.D.) of the mean.

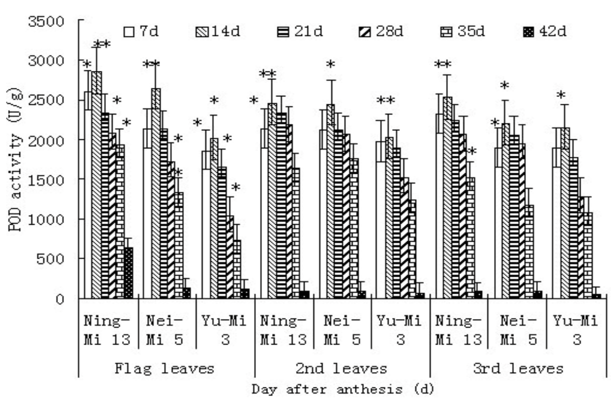

Fig 5. Changes of POD activity of among different broomcorn millet. ** Significance at 0.01 probability level, * at 0.05 probability level among difference broomcorn millet varieties in the same day, according to Duncan's multiple comparison. Sample variability is given as the standard deviation (S.D.) of the mean.

Common symptoms such as net loss of chloroplastic pigments and proteins, lipid peroxidation, and membrane alterations (Dai et al., 2011) leading to a progressive decrease in photosynthetic capacity. The results showed that soluble protein contents, antioxidative enzymes (SOD, CAT and POD) activities gradually declined from 14 days after the varieties flowered to maturing, while the chlorophyll and soluble protein contents shared a similar trend with antioxidative enzymes activities during the same period, but the MDA contents were low and rose gradually with plant ageing. Among the varieties, the leaves of Ning-Mi 13 declining slowly in SOD, CAT and POD activities, thus prolonging their function durations and keeping their net photosynthetic rates relatively higher during late growth. Taken together, the yield of Ning-Mi 13 was significantly higher than that of Nei-Mi 5 and Yu-Mi 3. It is possible to delay leaf senescence by senescence-specific cytokinin production as pioneered by Gan and Amasino (1995) in *Arabidopsis*. This strategy is of particular relevance to forage crops and is being increasingly applied (Hu et al., 2005). Leaf losses are closely related to a reduced penetrance of the light in the lowest layers of the canopy in Ning-Mi 13, therefore increased leaf longevity as reported in this paper can reduce yield losses and also improve forage quality (Calderini et al., 2007; Jia et al., 2009; Mandal et al., 2010; Dai et al., 2011). Leaf senescence is an evolutionarily acquired process, thus plants evolved in different ecological settings will exhibit distinct senescence pattern. Thus, it would be of great interest to do a comparative study utilizing the information obtained from broomcorn millet. In our experiment, compared with tested (Ning-Mi 13, Nei-Mi 5 and Yu-Mi 3) varieties under drought condition. From 14 days after anthesis to maturity, the Ning-Mi 13 showed the higher antioxidative enzymes activities, chlorophyll and soluble protein contents, and less accumulation of MDA, followed by Nei-Mi 5 and Yu-Mi 3. Taken together, it is an undeniable fact that leaf senescence can greatly reduce crop yield and other characteristics such as decreased longevity, yet the knowledge obtained so far have been poorly understood. Meanwhile, exogenous material, combined with fertilizer, water management and other effective control measures are used to increase grain yield. Considering the potential food shortage in the future and its use as a source of bioenergy, improving broomcorn millet productivity should be a top priority.

Materials and methods

Materials

Three broomcorn millet varieties were chosen for the experiments (2007-2008 years). These materials are Ning-Mi 13, Nei-Mi 5 and Yu-Mi 3. The growth periods of the three broomcorn millet varieties are consistent with each other.

Experimental Design

The trials were carried out at the No 1 Agricultural Experiment Station in Northwest A&F University, Yangling, China. The Station located in southern sub-humid and easily drought area of the Loess Plateau (108°04′, 34°20′), the altitude of which is 505m, with the average precipitation of 18.8 mm (May), 53.1 mm (June),55.6 mm (July),131mm (August), 71 mm (September) for the warm sub-humid climate. Field trials were conducted in the summer season of 2007-2008 (from 11 May to 1 September). The soil for tests is loam soil without preceding crops. Soil column method is

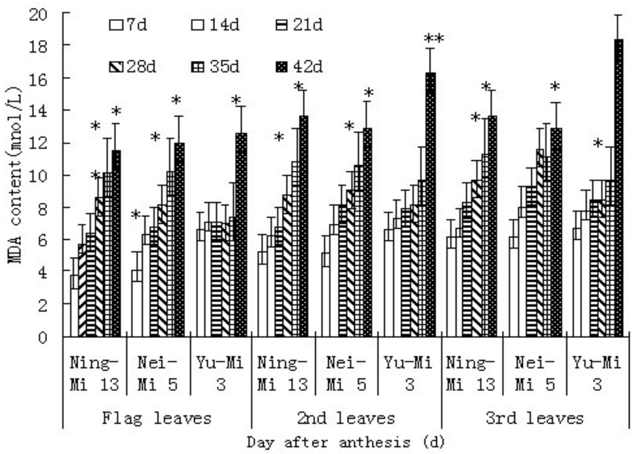

Fig 6. Changes of MDA activity of among different broomcorn millet. ** Significance at 0.01 probability level, * at 0.05 probability level among difference broomcorn millet varieties in the same day, according to Duncan's multiple comparison. Sample variability is given as the standard deviation (S.D.) of the mean.

used, and firstly digs a 100 cm deep hole then stack the soil together. After that, Polyvinyl chloride (PVC) pipes of 100cm long with the same diameter of 20 cm were put into the pits dug before, and then backfill the pits with the soil of the corresponding layers. Broomcorn millet was grown in an area that could be covered by a mobile rain shelter. The simulation experiment was performed using soil moisture treatments from third leaf stage to maturity by controlling irrigation. From 7 to 42 days after anthesis, the soil moisture was maintained as following: 22% (7day), 21% (14day), 21% (21day), 19% (28day), 18% (35 day), 16% (42day).

Biochemical analyses

Samples of the top three leaves for all genotypes in all plots were collected individually once every seven days from the anthesis to the kernel formation. The samples from the same leaf level of 5 individuals in one plot were mixed and measured. Leaf chlorophyll content was measured with a UVIKON220 spectrophotometer by the method of 80% acetone soaking extraction as described by Heath et al. (1968). Soluble protein content was measured with the Coomassie Brilliant Blue G-250 staining methods described by Wang (1987).

Assay of antioxidant enzymatic activity

The activity of SOD was determined following Stellmach (1992) by measuring its ability to inhibit the photoreduction of nitroblue tetrazolium (NBT).The reaction solution (3mL) contained 50mM NBT,1.3mM riboflavin,13mM methionine,75nM EDTA,50mM phosphate buffer (pH7.8)and 20–50ml 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 560nm was read using a spectrophotometer(UVIKON220).One unit of SOD activity was defined as the amount of enzyme that inhibited 50% of NBT photoreduction.Activities of CAT and POD were appraised using the method of Navabpour et al. (2003) with some modification. The CAT reaction solution (3mL) contained 50mM phosphate buffer (pH7.0), 5.9mM H_2O_2 and 0.1mL of enzyme extract.Then the reaction was initiated by adding the enzyme extract. Changes in absorbance of the reaction solution at 240nm were read every 20s.One unit CAT activity was defined as an absorbance change of 0.01 units per min. The POD reaction solution (3 mL) contained 50mM phosphate buffer (pH5.0), 20mM guaiacol, 40mM H_2O_2 and 0.1mL of enzyme extract. Changes in absorbance of the reaction solution at 470nm were determined every 20s.One unit POD activity was defined as an absorbance change of 0.01 units per min.The activity of each enzyme was expressed on protein basis.

Lipid peroxidation

Oxidative damage to lipids was estimated as the content of the total 2-thiobarbituric acid (TBA) reactive substance and expressed as equivalents of malondialdehyde (MDA) as described by 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 employed for assessing the significant differences among the mean values of different attributes. The values are means of six replications.

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