academicJournals

Vol. 8(29), pp. 4025-4032, 1 August, 2013
DOI: 10.5897/AJAR11.1827
ISSN 1991-637X ©2013 Academic Journals
http://www.academicjournals.org/AJAR

African Journal of Agricultural Research

Full Length Research Paper

Leaf senescence and physiological characters in different adzuki bean (*Vigna angularis*) cultivars (lines)

Hui Song[1], Xiaoli Gao[1], Baili Feng[1]*, Huiping Dai[2], Panpan Zhang[1], Jinfeng Gao[1], Pengke Wang[1] and Yan Chai[1]

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

Accepted 5 August, 2013

The aim of the study is to examine the relation of leaf senescence and its physiological characters at different flower internodes from flowering to maturing, to explore the aging mechanisms of adzuki bean leaf, to find out the intrinsic yield-forming mechanisms and to provide a theoretical basis for high-yield breeding and production of adzuki beans. A field experiment was conducted, the high-yielding (2000-75 and JiHong 9218) and the low-yield varieties (HongBao1 and WanXuan1), all adopted in the summer planting ecological region of China, were grown in 2008 and 2009, and their leaf physiological characters, such as chlorophyll contents, net photosynthetic rates (Pn), superoxide dismutase (SOD), catalase (CAT), peroxidase (POD) activities and malondialdehyde (MDA) contents were determined. The results indicated that the chlorophyll contents, P_n, SOD and CAT activities gradually decreased from 15 days after the varieties flowered to maturing, but their POD activities and MDA contents gradually increased when leaves senescence started. Leaf senescence initiated from the low internodes and gradually moved toward to the upper internodes after the plants flowered. Compared with low-yielding varieties, high-yielding varieties maintained higher contents of chlorophyll contents, P_n and SOD, CAT in the late stages, and thus resulting significantly higher grain yields. The overall data indicated that yield is positively correlated with leaf chlorophyll and P_n, as well as SOD and CAT activities and negatively associated with POD activities and MDA accumulations at the late growth stage. Therefore, effective inhibition of leaf senescence or prolonging the functional period of leaves at the late growing stage plays an important role in raising yield.

Key words: Adzuki bean, leaf senescence, chlorophyll content, seed yield.

INTRODUCTION

Adzuki bean (*Vigna angularis* (Willd.) Ohwi & H. Ohashi), one of the major food legumes of China, has a short growth period and is tolerant to drought, poor soil fertility and salinity (Lin et al., 2002). In traditional Chinese medication, adzuki is commonly used for many purposes including diuretics and antidotes, and symptom alleviations of dropsy and beriberi (Itoh and Furuichi, 2009). Recent studies indicate that adzuki bean is widely planted in the northeast and northern parts of China. Strong interests in planting adzuki bean have aroused in China because of its high quality.

Leaf senescence, which occurs at the final leaf development stage, is a critical process for plants because the process exerts influence on their fitness and

*Corresponding author. E-mail: songhui@nwsuaf.edu.cn, 7012766@163.com. Tel: +86-29-87082889. Fax: +86 - 29- 87082889.

leaf nutrient relocation into their seeds. Leaf senescence involves a coordinated process under the control of the highly regulated genetic programs at cellular, tissue, organ, and organism levels (Nooden, 1988). Theoretically, delayed leaf senescence after flowering and increased photosynthesis at the seed filling stage can improve seed dry matter accumulation and then resulting in yield increase (Hayati et al., 1995). The onset of leaf senescence is typically characterized as an orderly procedure which involves catabolism of chlorophyll, proteins, lipids and nucleic acids along with nutrient remobilization to developing grains, and ended with plant death (Breusegem and Dat, 2006). According to the free radical theory, leaf senescence is that if reactive oxygen species are metabolically imbalanced, their reactive oxygen species accumulation substantially increases the product (MDA) content of lipid peroxidation, and the damage caused by oxygen free radicals exerts direct influence on the progress of aging. Superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD), which play their roles coherently, can eliminate excessive reactive oxygen species, maintain their balance and protect membrane structure, delay the onset of aging (Kukavica and Velijovic, 2004). In the recent years, researches on leaf senescence, which mainly focus on crops, such as wheat (Zhang et al., 2006; Feng et al., 2009), maize (Pommela et al., 2006; Efeoğlu et al., 2009), soybean (Kaschuk et al., 2010), mung bean (Batish et al., 2006; Gao et al., 2007), indicate that one of the main reasons for leaf senescence is that plants produce reactive oxygen species, which can lead to leaf damage and even leaf death.

However, there are few research reports on morphological and physiological changes of adzuki bean. The study investigated the dynamic changes of the chlorophyll contents, P_n and antioxidative enzymes (SOD, CAT, and POD) activities and MDA contents in the leaves of four adzuki bean varieties (two high-yield versus two low-yield varieties) from flowering to ripening. The objectives of the study were to reveal the leaf senescence mechanism of adzuki bean and the relationship between the metabolic properties and yield of the bean, and to provide theoretical guidance for effectively improving field managements for the adzuki bean.

MATERIALS AND METHODS

Experimental design

The study was conducted in No.1 Agricultural Experiment Station of the Northwest A&F University, Yangling, Shaanxi, China. Located in the Loess Plateau (108°E and 34°N). The station is covered by a sub-humid warm temperate climate of which the average annual rainfall is 660 mm (mainly distributing from July to September), and the elevation above sea level is 520 m. Two high-yield cultivars (2000-75 and JiHong 9218), and two low- yield cultivars (HongBao1 and WanXuan1) of adzuki bean were planted in 2008 and 2009. The P-K fertilizers were separately applied at 225 and 30 kg hm^{-2} days before the sowing date of June 12. The four adzuki bean cultivars were cultivars participating in the State Regional Bean cultivar Test of China and their yield components are presented in Table 1. The areas of the plots were 2 m × 5 m and in each plot there were six rows of bean planted of which the spaces were 0.4 m. The field management practices for the study were the same as required by the State Regional Adzuki Bean Test of China. The design of the study was a Randomized complete block design with three replications.

Leaf sampling was done within 8:00 to 9:00 am. Every 7 days at the full-blooming stage and sample Leaves were chosen from the 5th, 6th, 7th, and 8th flowering nodes of the stems (the 5th leaf grew on the first flowing node). The middle leaflets of the sample ternate leaves were viewed as the reference for screening leaf-growing nodes for leaf sampling. After collected, the samples were placed in a cooler box, brought to lab, cleaned with water, and blotted with filter paper.

Measurement of chlorophyll content

The sample leaf chlorophyll were extracted by the 80% acetone-soaking extraction (Heath and Packer, 1968) and measured with UVIKON810 spectrophotometer (Kontron Instruments, Zurich, Switzerland).

Measurement of net photosynthetic rate

The photosynthetic parameters were measured with a portable LI-6400 photosynthesis system (LI-COR Inc., USA), and while the system was used to measure the parameters, its leaf temperatures and flow rate were set separately at 27°C and 500 μmolm^{-2} s^{-1}, and its photosynthetically active radiation provided with a red-blue light source was set at 1,000 μmolm^{-2} s^{-1}.

Antioxidative enzyme extractions and activity assays

0.500 g frozen leaves were homogenized in 50 mM potassium phosphate buffer (pH 7.8) containing 1 mM ethylene diamine tetraacetic acid, 3 mM 2-mercaptoethanol, and 2% (w/v) polyvinylpyrrolidone in a chilled mortar and pestle. The homogenate was centrifuged at 20,000 g for 20 min at 4°C and the supernatant was used for enzyme assays.

Superoxide dismutase (SOD) activity was assayed by the nitroblue tetrazolium method (Dhindsa et al., 1981). The 3 ml reaction mixture contained 50 mM Na–phosphate buffer (pH 7.3), 13 mM methionine, 75 mM NBT, 0.1 mM EDTA, 4 mM riboflavin, and 0.2 ml of enzyme extract. This reaction was started by the addition of riboflavin, and the glass test tubes were shaken and placed under fluorescent lamps (160 μmol m^{-2} s^{-1}). The reaction proceeded for 5 min and was then stopped by switching off the light. Absorbance was measured at 560 nm. Blanks or controls were run in the same manner but without illumination or enzyme, respectively. One unit of SOD was defined as the amount of enzyme that produced 50% inhibition of NBT reduction under assay conditions.

Activities of peroxidase (POD) and catalase (CAT) were determined by modified the method of Chance and Maehly (1955). Samples were homogenized with acetone on ice. The POD reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.8), 25 mM guaiacol, 200 mM H_2O_2, and 0.5 ml of enzyme extract. Changes in absorbance of the reaction solution at 470 nm were determined every 30 s. One unit of POD activity was defined as an absorbance change of 0.01 unit·min^{-1}. The CAT reaction solution (3 ml) contained 50 mM phosphate buffer (pH 7.0), 200 Mm H_2O_2, and

Table 1. Yield components of different Adzuki bean varieties.

Variety	Plants /ha	Pods /plant	Seeds/pod	1000-grain weight (g)	Seeds yield (kg/ha)
2000-75	100000	28.52	6.33	159.3	1579.3
JiHong 9218	100000	27.91	6.54	158.4	1558.0
HongBao 1	100000	24.82	6.12	152.1	1337.7
WanXuan 1	100000	22.31	6.08	146.5	1316.9

Each point represents the mean of six biological replicates.

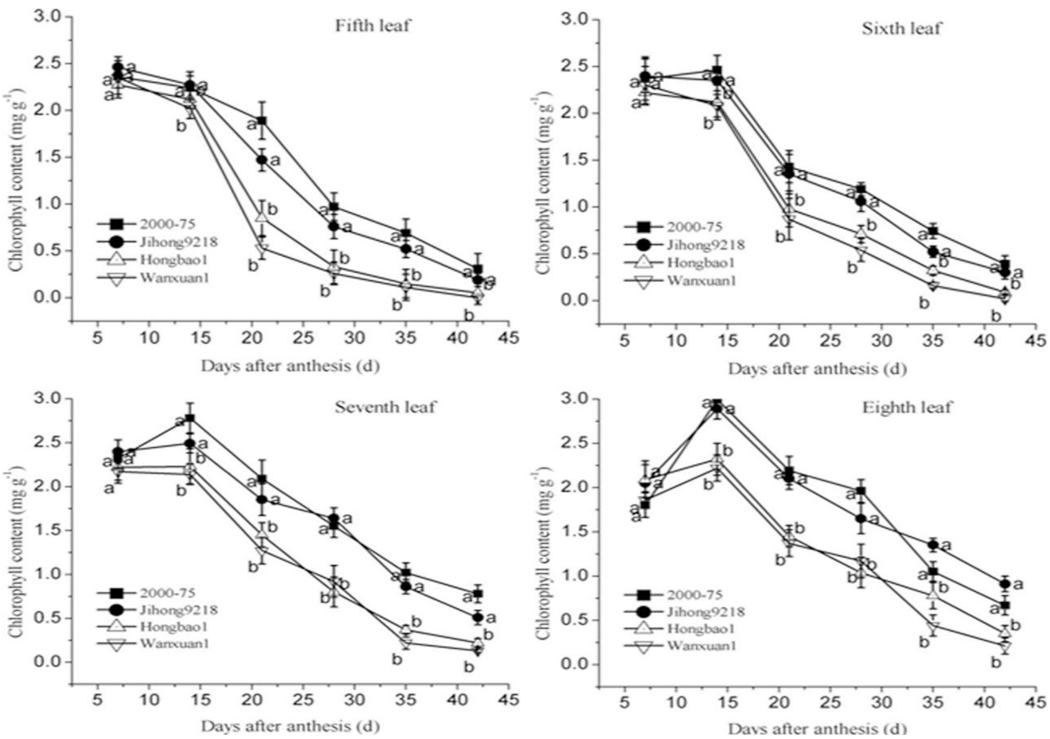

Figure 1. Trend of chlorophyll content in the leaves of four adzuki bean varieties at different flowering nodes. Data were the average ± SD in two years (2008 and 2009); a and b, the significant levels in net photosynthetic rate at the same day after anthesis between four varieties and relative ab controls at $p < 0.05$.

50 ml of enzyme extract. The reaction was initiated by adding the extract. Changes in absorbance of the reaction solution at 240 nm were read every 30 s. One unit of CAT activity was defined as an absorbance change of 0.01 units·min^{-1}.

Measurement of MDA content

The MDA contents were determined by modified TBA method (Heath and Packer, 1968). Generally, the crude enzyme extracts will present colors while when TBA reacts with MDA. The MDA contents were calculated depending on the absorbance subtraction at the wavelengths of 532 and 600 nm.

Statistical analysis

The data were processed using SAS software with the figures accomplishing by Origin 8. The difference significances among the various agronomic traits of the cultivars were tested by analyses of variance (ANOVA) for the various agronomic variables using SAS (SAS Institute, 2003) PROC MIXED procedure followed by Tukey multiple comparison tests.

RESULTS

Leaf chlorophyll contents

Figure 1 shows that the leaf Chl contents of the different cultivars tended to decline at the flowering and fruiting stages, and the leaf senescence of the cultivars initiated at their lower nodes and gradually moved up to the upper nodes after their flowering. The leaf Chl contents of 2000-75 and JiHong 9218 were higher than those of HongBao1 and WanXuan1. When HongBao1 and WanXuan1

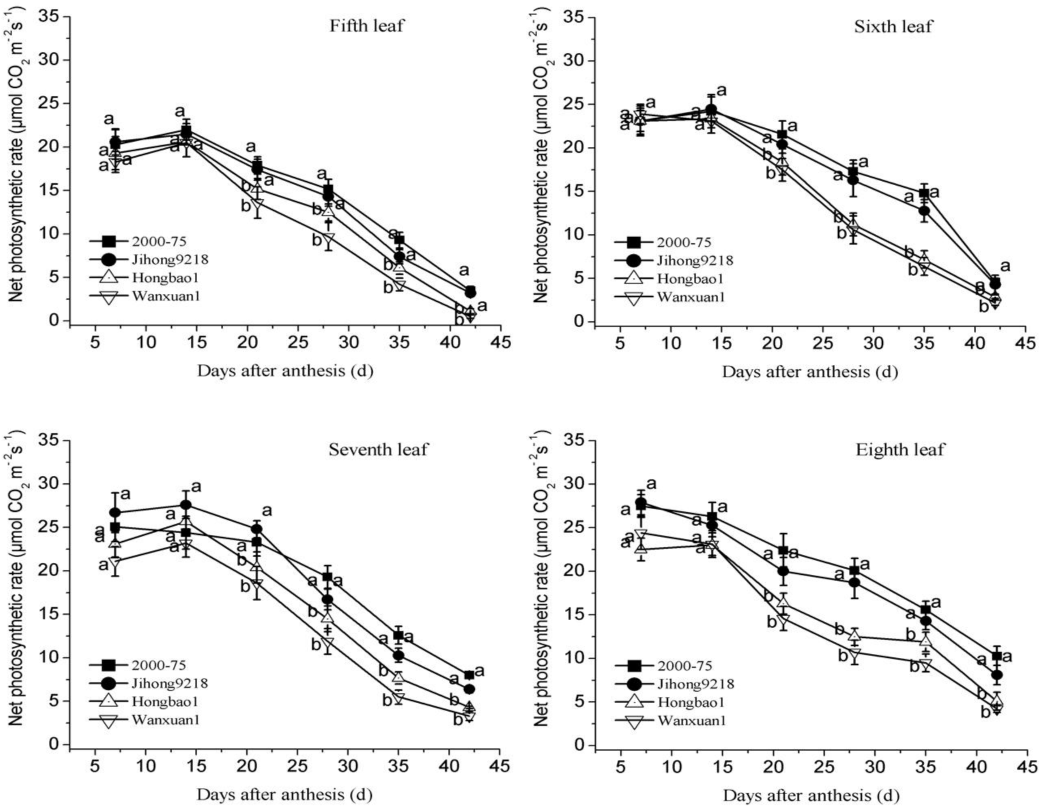

Figure 2. Trend of net photosynthetic rate in the leaves of four adzuki bean varieties at different flowering nodes.Data were the average ± SD in two years (2008 and 2009); a and b were the significant levels in net photosynthetic rate at the same day after anthesis between four varieties and relative a,b controls at $p < 0.05$.

approached to their maturity, their leaves became nearly dry and their leaf Chl content was hardly detectable.

Leaf net photosynthetic rates

The weighted average leaf P_n of the high-yield and low-yield cultivars at the different flowering nodes at the flowering and fruiting stages are shown in Figure 2. The P_n tended to decline at the flowering and fruiting stages. A the different flowering nodes, Te leaf photosynthetic capacities of the high-yield and low-yield cultivars decreased from the eighth leaf through seventh and sixth leaves to the fifth leaf. Compared with the two low-yield cultivars, 2000-75 and JiHong 9218 had considerably higher net leaf photosynthetic rate, indicating that they had higher photosynthetic efficiencies.

SOD and CAT activities

Figure 3 shows the leaf SOD activities of the adzuki bean cultivars after their anthesis. In general, the cultivars tended to present increasing SOD activities from their lower nodes to their upper nodes and initiate leaf senescence at their lower nodes, and their leaf SOD activities and leaf senescence initiations appeared significantly different, which was similar to what was shown on the CAT activities in Figure 4. High-yield2000-75 and JiHong 9218 showed higher leaf SOD and CAT activities than HongBao1 and WanXuan1. This indicated that the high-yield cultivars had stronger physiological capacities than the low-yield cultivars.

POD activities

Figure 5 shows that the POD activities of the different adzuki bean cultivars generally tended to increase at the flowering and fruiting stages. Comparison of the POD activity increments at the different flowering nodes showed that the POD activity increment of the fifth leaves appeared the largest, the POD activity increment of the eighth leaves appeared the lowest, and the POD activity increments of the seventh and sixth leaves stood between the former two. After their anthesis, the POD activities of the different cultivars generally tended to increase but differed significantly. High-yield 2000-75 and JiHong 9218 showed lower leaf POD activities than low-yield HongBao1 and WanXuan1.

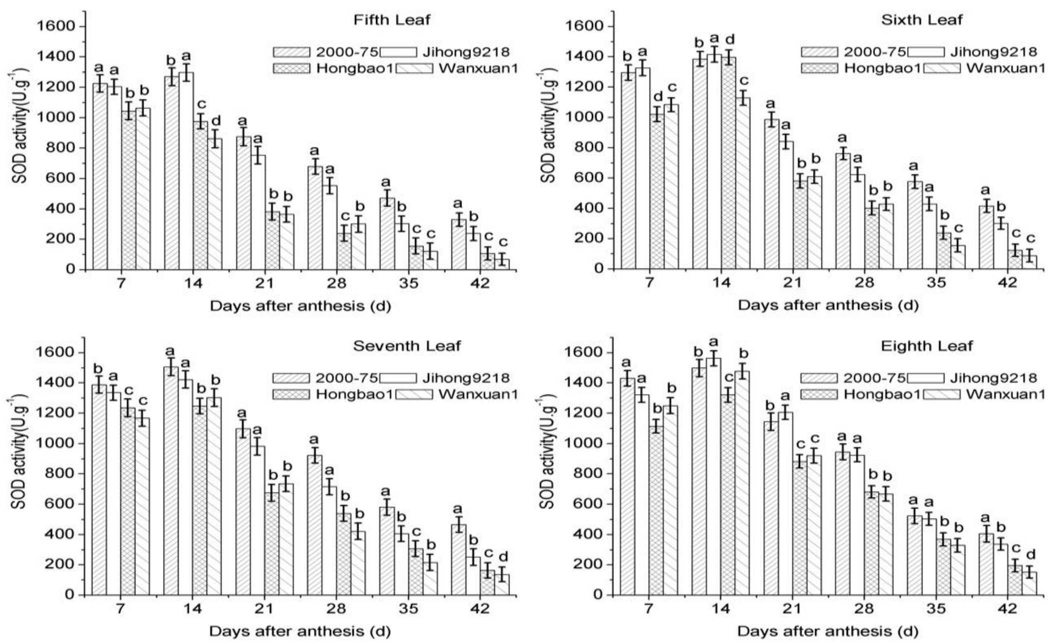

Figure 3. Trend of SOD activity in the leaves of four adzuki bean varieties at different flowering nodes. Date presented as mean of two years (2008 and 2009) ±SD. a, b and c represents significant difference in SOD activity at the same day after anthesis from control at $p < 0.05$.

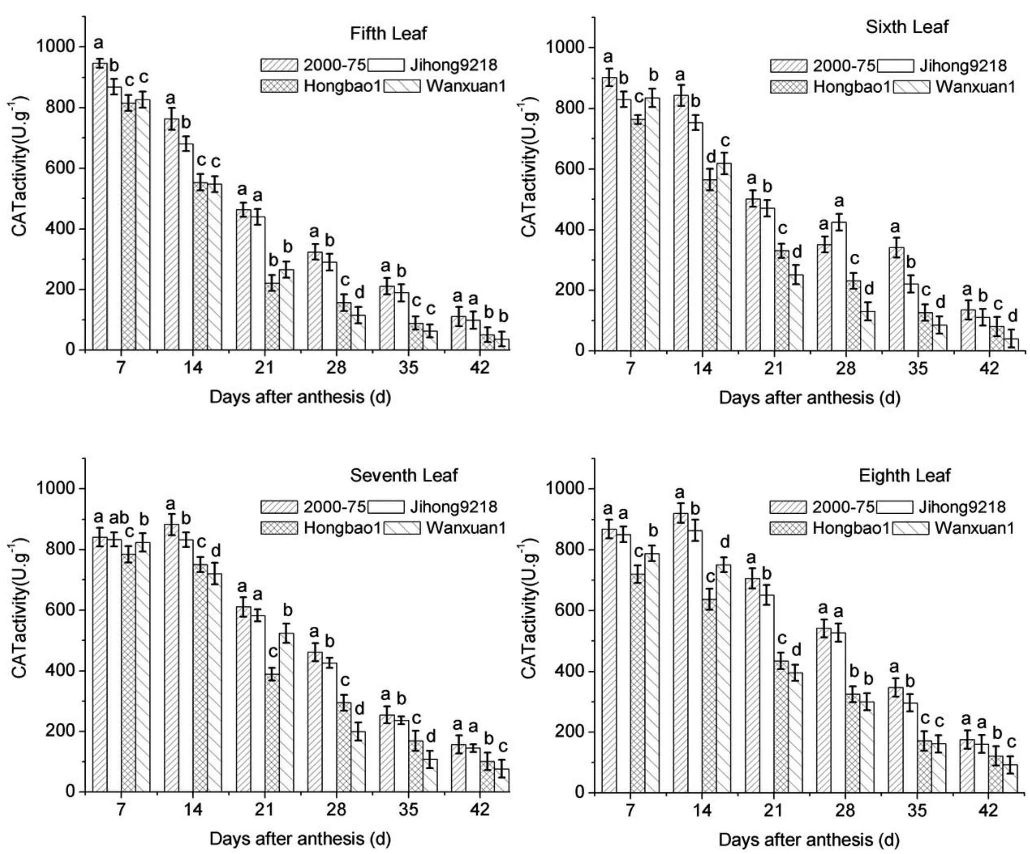

Figure 4. Trend of CAT activity in the leaves of four adzuki bean varieties at different flowering nodes. Date presented as mean of two years (2008 and 2009) ±SD. a, b and c represents significant difference in CAT activity at the same day after anthesis from control at $p < 0.05$.

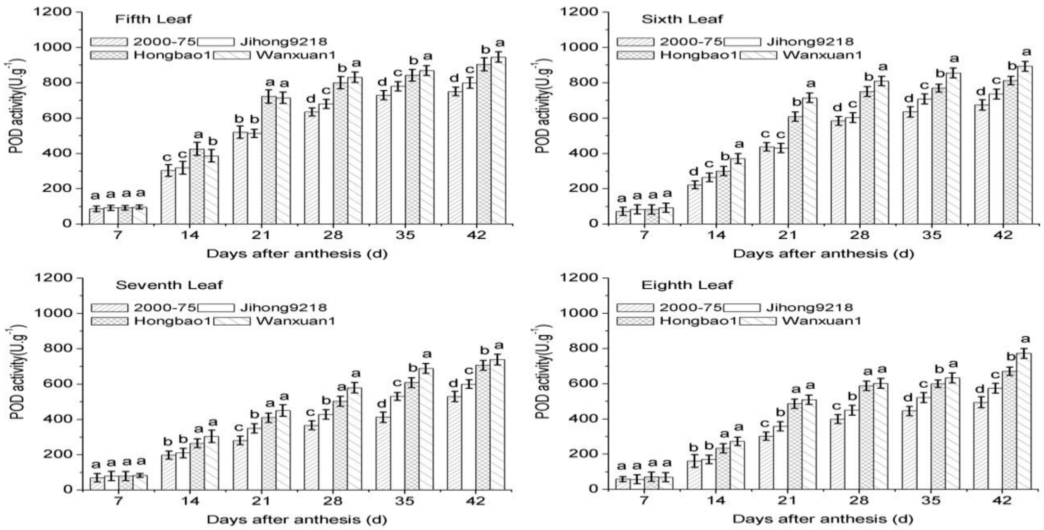

Figure 5. Trend of POD activity in the leaves of four adzuki bean varieties at different flowering nodes. Date presented as mean of two years (2008 and 2009) ±SD. a, b and c represents significant difference in POD activity at the same day after anthesis from control at $p < 0.05$.

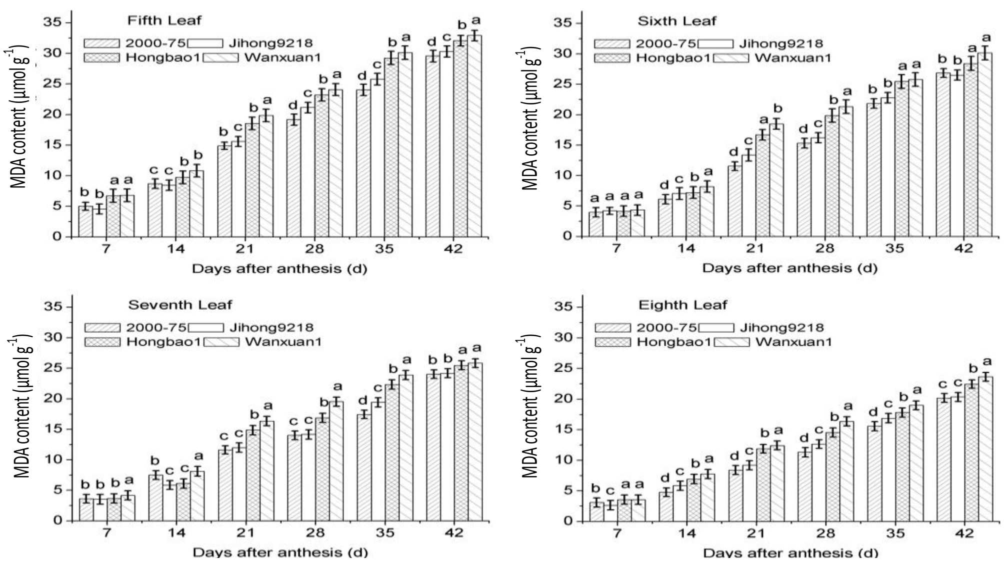

Figure 6. Trend of MDA content in the leaves of four adzuki bean varieties at different flowering nodes. Date presented as mean of two years ±SD (2008 and 2009). a, b and c represents significant difference in MDA content at the same day after anthesis from control at $p < 0.05$.

MDA contents

Figure 6 shows the weighted average MDA contents of the high-yield cultivars and low-yield cultivars at the different flowering nodes. The MDA contents of the different cultivars generally tended to rise as the cultivars approached to their maturities. It can be seen from Figure 6 that under the simulated conditions, the MDA contents of the cultivars increased from the lower nodes to the upper nodes after they flowered. The MDA contents of 2000-75 and JiHong9218 were considerably lower than those of the low-yield cultivars on most of the sampling dates.

Yield components

Table 1 presents four yield components of the cultivars,

Table 2. Correlation of seeds yield and physiological indicators of adzuki bean at the late growth stage.

Indicator	Seeds yield	Chlorophyll content	P_n	SOD	CAT	POD
Chl	0.932**					
P_n	0.966**	0.957**				
SOD	0.971**	0.916**	0.908**			
CAT	0.860*	0.893*	0.975**	0.834*		
POD	-0.824*	-0.870*	0.902*	-0.970**	-0.931**	
MDA	-0.863*	-0.955**	-0.842*	-0.892*	-0.950**	0.872*

Each point represents the mean of three biological replicates. Significance levels: *p < 0.05.

plant/ha, pod number per plant, seed number pod^{-1}, and 1000-seed weight. Compared to the yields of HongBao1 and WanXuan1, the yield of 2000-75 and JiHong9218 was significantly higher.

Correlations between the yields and physiological parameters

The correlations between the Physiological parameters of the functional leaves at the late growth stage and the yield per plant were analyzed (Table 2). The P_n activities of SOD and the yield per plant appeared significantly and positively correlated from the jointing stage to the maturing stage. In addition, appeared a negative correlation between the POD activities (r = -0.824) and MDA contents (r = -0.863) and the yield per plant.

DISCUSSION

Some researchers have indicated that the functioning durations of crop leaves and particularly the life spans of them after anthesis are crucially important for crop yield (Masulaux et al., 2001; Dai et al., 2011a). The study showed that the chlorophyll contents of the leaves of the cultivars peaked approximately 15 days after the varieties flowered, and the leaf Chl contents of 2000-75 and JiHong 9218 were higher than those of HongBao1 and WanXuan1. When HongBao1 and WanXuan1 approached to their maturity, their leaves became nearly dry and their leaf Chl content was hardly detectable. Therefore, it was important to seed yield formation of adzuki bean to effectively control or slow down the process of leaf chlorophyll degradation.

Photosynthesis is the primary process for crops to form their dry matters and grain yields. However, the photosynthetic carbon cycle will be compromised because the imbalance among vigorous metabolism, nutrition stress and energy metabolism causes free radicals to form (McDonald et al., 1969; Zelitch, 1982; Evans et al., 1984; Jiang et al., 1988).This study showed that the net photosynthetic rates of the cultivars tended to decline from flowering to ripening, and the net photosynthetic rates of the high-yield varieties. When the adzuki bean cultivars nearly reached their complete maturity, the leaves of the low-yield varieties (HongBao1 and WanXuan1) became completely dried up without showing any photosynthetic capacities. It follows that that the high-yield varieties had higher capacity of providing more carbohydrates to fill up their seeds.

Active oxygen species cause deterioration of membrane lipids, leading to increased leakage of solutes from membranes (Dai et al., 2011a). Lipid peroxidation which leads to impairment of membrane function is the system most easily ascribed to oxidative damage and also most frequently measured (Dai et al., 2011b). Malondialdehyde (MDA), a decomposition product of polyunsaturated fatty acids, has been utilized as a biomarker for lipid peroxidation that may occur in the presence of reactive oxygen species (Dai et al., 2011a, b). The leaf SOD and CAT activities of the cultivars first increased, then peaked and finally declined from the flowering stage to the maturing stage, and their leaf MDA contents substantially increased during the same period of time. Comparatively, the SOD and CAT activities of the low-yield varieties 2000-75 and JiHong9218 was high and presented lower decrements, and the MDA contents of them was lower and increased slower. Consequently, it needs further studies to understand how the international action mechanisms of different cultivars operate to minimize oxygen damage under variable environmental conditions.

POD has a dual function on protective enzyme systems and the damage factor of plant senescence (Zhang and Krikham, 1994; Srivallia and Khanna, 2001). The study found that the POD activities increased sharply when the leaf senescence started. Which was completely the same as what happened in Mung bean (Gao et al., 2008).

ACKNOWLEDGEMENTS

The study was supported by the Special Funds (200903007) for Scientific Research of Agriculture Ministry public-interest industry (agriculture), and Cyrus Tang Specific Plant Genetics and Breeding program of the Northwest A&F University (No. 50).

ABBREVIATIONS

CAT, Catalase; **Chl,** chlorophyll content; **MDA,** malondialdehyde; **POD,** peroxidase; $\boldsymbol{P}_{\mathbf{n}}$, net photosynthetic rate; **SOD,** superoxide dismutase.

REFERENCES

Batish DR, Singh HP, Setia N, Kaur S, Kohli RK (2006). 2-Benzoxazolinone (BOA) induced oxidative stress, lipid peroxidation and changes in some antioxidant enzyme activities in mung bean (*Phaseolus aureus*). Plant Physiol. Biochem. 44:819-827.

Breusegem FV, Dat JF (2006). Reactive oxygen species in plant cell death. Plant Physiol. 141:384-390.

Chance M, Maehly AC (1955) Assay of catalases and peroxidases. Methods Enzymol. 2:764-775

Dai HP, Jia GL, Lu C, Wei AZ, Feng B L, Zhang SQ (2011a). Studies of synergism between root system and leaves senescence in Broomcorn millet (*Panicum miliaceum* L.). J. Food Agric. Environ. 9:132-135.

Dai HP, Zhang PP, Lu C, Jia GL, Song H (2011b). Leaf senescence and reactive oxygen species metabolism of broomcorn millet (*Panicum miliaceum* L.) under drought condition. A .J.C.S. 5:1655-1660.

Dhindsa RS, Plumb-Dhindsa P, Throne TA (1981). Leaf senescence correlated with increased levels of membrane permeability and lipid peroxidation and decreased levels of superoxide dismutaseand catalase. J. Exp. Bot. 32:93-101

Efeoğlu B, Ekmekçi Y, Çiçek N (2009). Physiological responses of three maize cultivars to drought stress and recovery. S. Afr. J. Bot. 75:34–42.

Evans LT (1984). Morphological and physiological changes among rice varieties used in the Philippines over the seventy years. Field Crops Res. 8:105-125.

Feng BL, Yu H, Hu YG, Gao XL, Gao JF, Gao DL, Zhang SW (2009). The physiological characteristics of the low canopy temperature wheat (*Triticum aestivum L.*) genotypes under simulated drought condition. Acta Physiol. Plant 31:1229-1235.

Gao XL, Gao JF, Feng BL, Chai Y, Jia ZK (2007). Photosynthetic performance in the leaf of different mung bean Genotypes. Acta Agric. Sinica 33:1154-1161.

Gao XL, Sun JM Gao JF, Feng BL, Chai Y, Jia ZK (2008). Leaf senescence and reactive oxygen metabolism in different genotypes of mung bean. Sci. Agric. Sinica 41:2873-2880.

Hayati R, Egli DB, Crafts-Brandner SJ (1995). Carbon and nitrogen supply during seed filling and leaf senescence in soybean. Crop Sci. 35:1063-1069.

Heath RL, Packer L (1968). Photoperoxidation in isolated chloroplasts: 1. Kinetics and stoichiometry of fatty acid peroxidation. Arch. Biochem. Biophys. 125:189-198.

Itoh T, Furuichi Y (2009). Lowering serum cholesterol level by feeding a 40% ethanol-eluted fraction from HP-20 resin treated with hot water extract of adzuki beans to rats fed a high-fat cholesterol diet. Nutrition 25:318-321.

Jiang GZ (1988). Physiological and ecological characteristics of high yielding varieties in rice plants I. Yield and dry matter production. Crop Sci. 57:132-138.

Kaschuk G, Hungria M, Leffelaar PA, Giller KE, Kuyper TW (2010). Differences in photosynthetic behaviour and leaf senescence of soybean dependent on N_{-2} fixation or nitrate supply. Plant Biol. 12:60-69.

Kukavica B, Veljovic JS (2004). Senescence related changes in the antioxidant status of ginkgo and birch leaves during autumn yellowing. Physiol. Plants 122:321-327.

Lin RF, Chai Y, Liao Q, Sun SX (2002).Minor Grain Crops in China. Agricultural Science and Technology Press, Beijing, China. pp. 192-209.

Masulaux C, Quilleré I, Gallais A, Hirel B (2001). The challenge of remobilization in plant nitrogen economy. A survey of physio-agronomic and molecular approaches. Ann. Appl. Biol. 138:8-81.

McDonald DJ, Stansel JW, Gilmore EC (1969). Photosynthesis studies, Rice. J. 74:55.

Nooden LD (1988). whole plant senescence. In: Nooden LD. and Leopold AC (eds) Senescence and Aging in plants. Academic press, San Diego. pp. 391-439.

Pommela B, Gallais A, Coque M, Quilleré I, Hirel B, Prioul JL, Andrieu B, Floriot M (2006). Carbon and nitrogen allocation and grain filling in three maize hybrids differing in leaf senescence. Eur. J. Agron. 24:203-211.

Srivallia B, Khanna CR (2001). Induction of new is forms of superoxide dismutase and catalase enzymes in the leaf of wheat during monocarpic senescence. Biochem. Biophys. Res. Commun. 288:1037-1042.

Zelitch I (1982).The close relationship between net photosynthesis and crop yield. Biol. Sci. 32:796-802.

Zhang CJ, Chen GX, Gao XX, Chu CJ (2006). Photosynthetic decline in flag leaves of two field-grown spring wheat cultivars with different senescence properties maize. S. Afr. J. Bot. 72:15-23.

Zhang JX, Kirkham MB (1994). Drought stress induced changes in activities of superoxide dismutase, catalase, and peroxidase in wheat species. Plant Cell Physiol. 35:785-791.