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Gas Exchange, Chlorophyll Fluorescence and Antioxidant Enzymes in Leaves of Centipede Grass (*Eremochloa ophiuroides*) after Barley Stripe Mosaic Virus (BSMV) Infection

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We explored the gas exchange, chlorophyll fluorescence properties and antioxidant enzymes in centipede grass (*Eremochloa ophiuroides*) infected with barley stripe mosaic virus (BSMV). The results showed that with progress of virus infection, the net photosynthetic rate (Pn) and stomatal conductance (Gs) in centipede grass was dramatically decreased, while the intercellular CO_2 concentration (Ci) was gradually increased. Further studies showed that the maximum quantum yield of PSII photochemistry (Fv/Fm), the efficiency of energy conversion of open PSII (Fv' /Fm'), the actual efficiency of total PSII centers (Φ_{PSII}), and photochemical quenching (qP) were decreased, while the nonphotochemical quenching (NPQ) was increased after BSMV infection. Moreover, BSMV infection increased the antioxidant activities until 21 days of infection, and then was slightly decreased. The MDA contents were also increased after BSMV infection. There results suggested that the decreased Pn after BSMV infection was not resulted from the decrease of stomatal conductance, and the primary limitation of Pn was suppression of the number of open PSII reaction centers and the efficiency of light energy transformation by PSII reaction centers, thereby the electron transport were decreased. Additionally, the excessive excitation energy could not be dissipated efficiently, and reactive oxygen species (ROS) could not be removed efficiently, thereby resulting in membrane lipid peroxidation in centipede grass infected with BSMV.

Key words: Centipede grass, barley stripe mosaic virus, photosynthesis, antioxidant enzymes.

Barley stripe mosaic virus (BSMV) is a tripartite, positive-sense RNA virus that infects many agriculturally important monocot species including barley, oats, wheat and maize (Peng *et al.,* 1989, Sun *et al.,* 2008, 2010). On occasions it has been found in several grass species. Infections appear as chlorotic mottling with spots or stripes of a yellowish color. And infected plants may be stunted and may mature later than healthy plants (Peng *et al.,* 1989). Symptoms may vary with the virulence of the BSMV strain and time of[1] infection.

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Centipede grass is native to China and parts of Southeast

Asia. It is a slow growing creeping grass and has short stems growing upward (Li *et al.,* 2010). BSMV is one of the major viruses infecting turf grass. The BSMV infections have occurred in *Eremochloa ophiuroides* in China. Virus infection usually leads to the changes of physiological function in host plants, particularly in changes of photosynthesis and respiration.

The plant virus genome biology research and viral replication has made great progress in the last 20 years. In recent years, many studies began to focus on the relationship between the host plant and pathogen. In recent years, some researchers reported that the effects of broad bean wilt virus 2 (Li *et al.,* 2006), cucumber mosaic virus

(Wang *et al.,* 2000), turnip mosaic virus (Fu *et al.,* 2004, Guo *et al.,* 2005), potato virus Y (Guo *et al.,* 2000, Peng *et al.,* 2004) infection on photosynthesis of the host plant. With in-depth understanding of the relationship between fungi, bacteria and host, fungal and bacterial diseases have been able to chemical control. However, due to the complexity of the effects of virus infection on the plant, the prevention of viral disease is still difficult in the agricultural production. Photosynthesis is the most important metabolic processes in the green plants. Inhibition of photosynthesis by virus infection has been studied in many plant species. Naidu *et al* (Naidu *et al.,* 1984) showed that the reduced electron transport caused by peanut green mosaic virus infection is partly due to the reduction in chlorophyll levels, particularly chlorophyll a but also to direct inhibition of photosystem II, mainly at the plastoquinone level. Reinero and Beachy (Reinero *et al.,* 1986) reported that coat protein (CP) of tobacco mosaic virus (TMV) accumulates in chloroplasts of systemically infected leaves. The large accumulation of TMV-CP inside chloroplasts could affect photosynthesis in virus-infected plants by inhibiting photosystem II activity (Reinero *et al.,* 1989).

Although virus infection on plant physiological characteristics has been reported in the past few decades, exactly how virus infection affects the physiological mechanism remains a matter of debate. In this study, we examined the response of gas exchange, chlorophyll fluorescence and antioxidant metabolism to BSMV infection in the leaves of centipede grass. To our knowledge, there is no study to examine time course of BSMV infection and its effect on photosynthesis.

MATERIALS AND METHODS

Plant materials and treatments

The study was conducted with centipede grasses (*Eremochloa ophiuroides*) grown for 50 days from seed on plastic pots filled with a 1:2 (v/v) mixture of coarse sand and soil in a greenhouse with temperature of 28/20 °C (day/night) under natural light. Plants were watered daily and were given fertilized water once per week with a half-strength Hoagland solution. Before treatment, plants were randomly separated into the two groups of 15 plants each. Inoculation was performed by placing 0.5 ml of inoculum of a field BSMV isolate on one newly expanded leaf in the first group by a procedure as described by Guo *et al.,* (Guo *et al.,* 2005). The other group was not inoculated throughout the experimental period as the non-infected control.

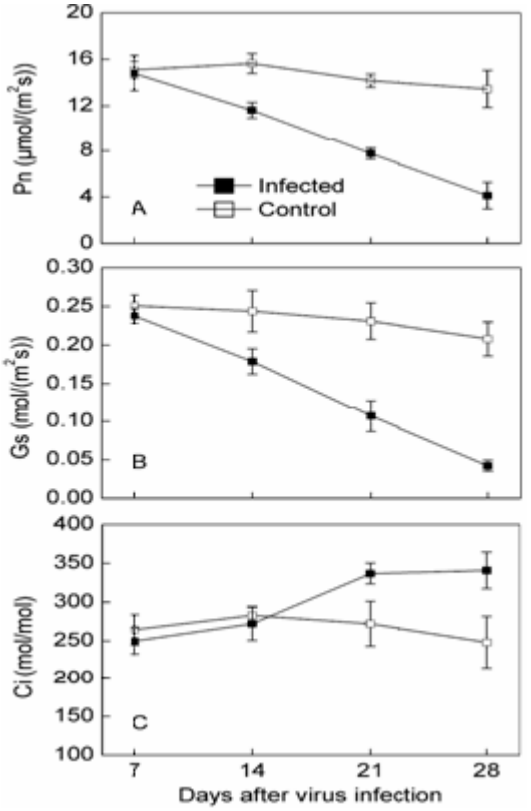

Fig. 1. Effects of BSMV infection on the net photosynthetic rate (*P*n) (A), stomatal conductance (*G*s) (B), and intercellular CO_2concentrations (Ci) (C) in the leaves of Centipede grass. Data are means±SE (n = 4)

Measurement of gas exchange and chlorophyll fluorescence

Leaf gas exchange and chlorophyll fluorescence were measured simultaneously according to Jin *et al.,* (Jin stem (LiCor-6400; LiCor Inc. USA) with an integrated fluorescence fluorometer (Li-6400–40) under ambient CO_2 concentrations and 21% O_2. The measurements were made on the second fully expanded leaf from the top of four randomly selected plants from each treatment, at a temperature of 25°C under a photosynthetic photon flux density (PPFD) of 1000 μmol/(m²s), relative humidity 60%. The maximum

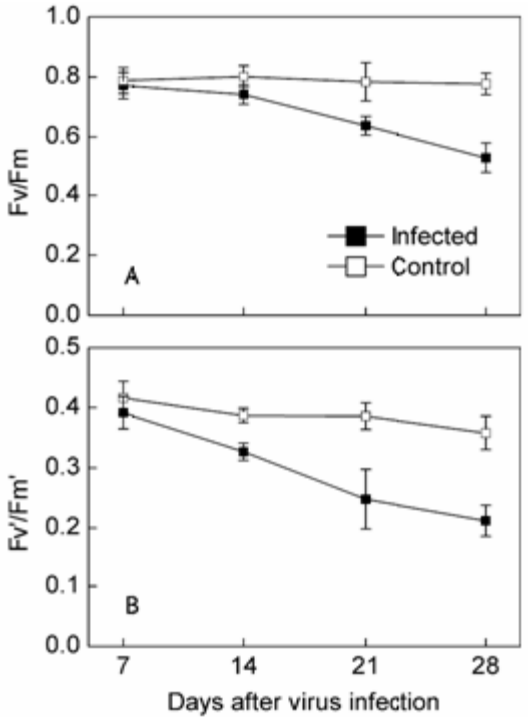

Fig. 2. Effects of BSMV infection on the maximum quantum yield of the primary photochemistry of PSII (Fv/Fm) (A), and efficiency of excitation energy capture by open PSII reaction centers (Fv'/Fm'2) (B) in the leaves of Centipede grass. Data are means±SE (n = 4)

quantum yield of the primary photochemistry of PSII (Fv/Fm) was calculated as (Fm-Fo)/Fm. The actual efficiency of total PSII centers [Φ_{PSII} = (Fm' -Fs)/Fm'], the efficiency of energy conversion of open PSII [Fv' /Fm' = (Fm' "Fo')/Fm'], photochemical quenching [qP = (Fm' "Fs)/(Fm' "Fo')] and the non-photochemical quenching [NPQ = Fm/Fm' -1] were calculated from measured parameters (Maxwell *et al.,* 2000).

Antioxidant enzyme activity determination

Leaf tissue (1.0 g) was ground in liquid nitrogen in 8.0 ml 50 mM K-phosphate buffer (pH 7.8) containing 0.2 mM EDTA, 4% (w/v) PVP-40. The homogenate was centrifuged at 15,000 × *g* for 2 min at 4°C. The enzyme activity of each sample was measured four times at 25°C. Activities of superoxide dismutase (SOD), ascorbate peroxidase (APX) and catalase (CAT) were assayed according to the methods of Verma and Mishra (Verma*et al.,* 2005).

Determination of malondialdehyde (MDA) content

The MDA content was determined by thiobarbituric acid (TBA) reaction according to the method of Jin et al (Jin *et al.,* 2011). About 0.8 g of each sample was homogenized in 5 mL of 0.5% (v/v) trichloroacetic acid (TCA). After centrifuging at 12000g for 10 min, 1 ml of extract was taken and 4 ml 0.6% TBA in 20% TCA was added. The mixture was heated in a boiling water bath for 15 min and then cooled quickly in an ice bath. The resulting mixture was centrifuged at 12,000 *g*for 10 min, and

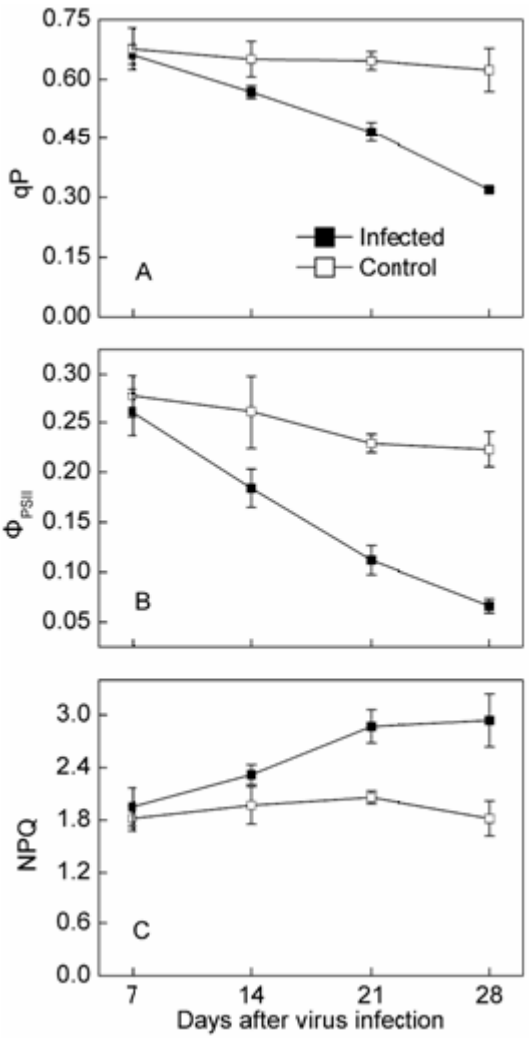

Fig. 3. Effects of BSMV infection on the quantum yield of PSII electron transport (Φ_{PSII}) (A), photochemical quenching (qP) (B) and the non-photochemical quenching (NPQ) (C) in the leaves of Centipede grass. Data are means±SE (n = 4).

the specific absorbance of products and nonspecific background-absorbance were read at 532 and 600 nm.

RESULTS

The photosynthetic rate (Pn), stomatal conductance (Gs) and intercellular CO2 concentration (Ci) remained stable in healthy centipede grass leaves (Fig. 1). After 21 days of treatment, there was a slightly decline in Pn and Gs. The BSMV infection did not appear to significantly influence leaf photosynthetic rates on the first measurement date (days 7, Fig. 1); however, at after approximately 14 days infection, BSMV significantly reduced the leaf photosynthetic performance of centipede grass. At 21 and 28 days infection, Pn were decreased by 45.2% and 70.3%, respectively. Virus infection can not only reduce the Pn, and also can reduce the Gs in centipede grass leaves. At 21 and 28 days infection, Gs were decreased by 53.7% and 79.8%,

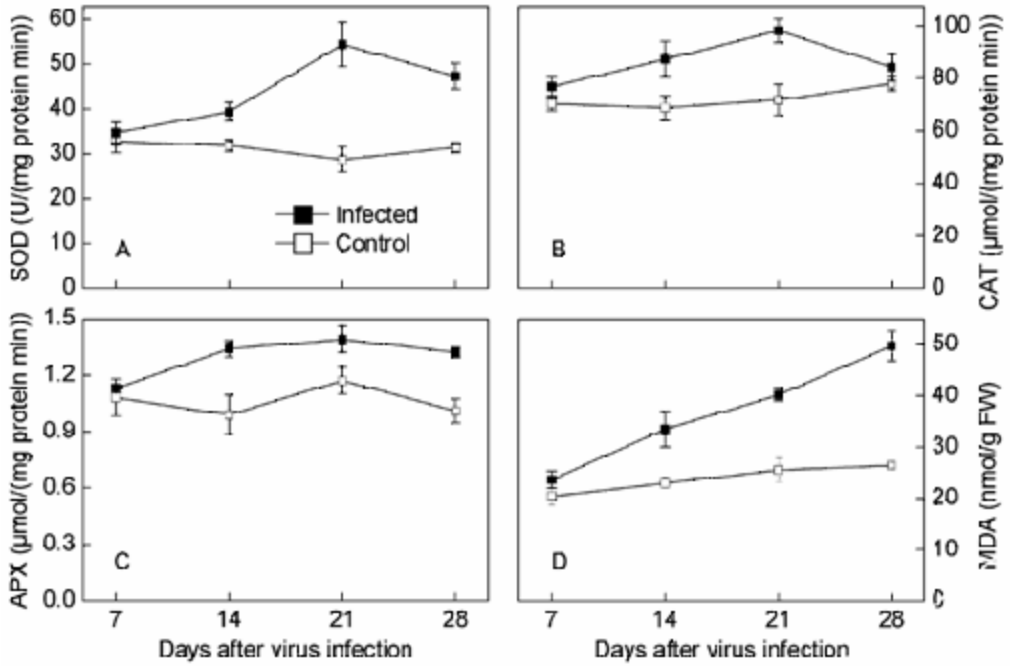

Fig. 4. Effects of BSMV infection on the maximum quantum yield of the primary photochemistry of PSII (Fv/Fm) (A), and efficiency of excitation energy capture by open PSII reaction centers (Fv2 /Fm2) (B) in the leaves of Centipede grass. Data are means±SE (n = 4)

respectively. However, Ci did not change significantly on the first two measurement date (days 7 and 14), and then Ci was increased gradually with increasing of infection time (Fig.1).

Fv/Fm, Fv'/Fm', qP and Φ_{PSII} maintain a high level in the healthy control leaves (Figure 2, 3). In this study, Fv/Fm was not affected until 14 days of infection. With the progress of infection, Fv/Fm and Fv'/Fm' were largely decreased (Figure 2). At 21 and 28 days infection, qP was decreased by 27.8% and 48.9%, respectively (Fig. 3). Φ_{PSII} was decreased significantly after BSMV infection in Centipede grass, and the extent of decline was significantly greater than the Fv/Fm and Fv'/Fm' (Figure 2, 3). At 21 and 28 days infection, Φ_{PSII} was decreased by 51.1% and 70.4%, respectively. NPQ remained unchanged in healthy control leaves during the measurement period, while NPQ was gradually increased in the infected leaves. At 21 and 28 days infection, NPQ was increased by 39.3% and 61.5%, respectively (Fig. 3).

In the present study, SOD, APX and CAT activities and MDA content remains unchanged in the healthy control leaves (Figure 4). However,

there were remarkable increases in SOD, CAT and APX activities until 21 days of infection. At 28 days infection, SOD, APX and CAT activities began to decline. However, MDA content was increased after BSMV infection. At 21 and 28 days infection, MDA content was increased by 57.3% and 88.2%, respectively (Fig. 4).

DISCUSSION

Both stomatal and non-stomatal limiting factors affect photosynthesis. When Gs and Ci is changed in the same way, we can determine that the Pn changes are caused by stomatal limitation (Jin *et al.,* 2009). In the present study, the Pn and Gs were significantly reduced in the infected plants. Meanwhile, the Ci was increased in the BSMV infected plants compared with controls. These results indicate that the reductions of Pn of the infected plants were not due to stomatal factors but a low capacity of CO_2-fixation cycle (Holzberg *et al.,* 2002). This result is consistent with previous studies in broad bean (Li *et al.,* 2006) and stem mustard (Guo *et al.,* 2005).

Virus infection would lead to the destruction of the chloroplast structure, the damnification of the oxygen evolving complex and the light-harvesting pigment protein complex in PSII, thereby lead to the reduction in photosynthetic CO_2 assimilation rate (Funayama *et al.,* 1997, Li *et al.,* 2006). Fv/Fm is a parameter widely used to indicate the maximum quantum efficiency of PSII. This parameter is widely considered to be a sensitive indication of plant photosynthetic performance with healthy samples, and is closely related to the degree of the photosynthesis photoinhibition (Maxwell *et al.,* 2000). It did not obvious change in Fv/Fm under the moderate BSMV infection (0-14 days), which indicated that moderate BSMV infection did not result in photosynthesis photoinhibition, and that there was no obvious damage in the PSII complex (Wilhelmová *et al.,* 2005). With the increase of BSMV infection time, the Fv/Fm is significantly reduced. Fv'/Fm' represents the effective quantum yield, it indicate the primary light energy capture efficiency of open PS II reaction center (Maxwell *et al.,* 2000). The low qP values indicated more reduced Q_A in PSII and less reduced plastoquinone pool (Kramer *et al.,* 2004).

Φ_{PSII} reflect actual photochemical efficiency under the partial shutdown of thePSIIreaction center, which isclosely related to electron transport rate (Maxwell *et al.,*2000; Jin *et al.,* 2010). $\ddot{O}_{PSII}$ was lower in the infected plants than in control plants, which indicated that linear electron transport rate was reduced. In contrast to our results, (Guo *et al.,* 2005) reported turnip mosaic virus (TuMV) infection did not reduce the electron transport rate in leaves of stem mustard. The quantum yield of PSII electron transport was affected by Fv'/Fm' and qP (Jin *et al.,* 2010, 2011). After BSMV infection, qP andFv'/Fm' were largely decreased in centipede grass. These results suggested that both the number of open PSII reaction centers and the efficiency of light energy transformation by PSII reaction centers were decreased in the BSMV infected plants, which decreased the capacity of linear electron transport and resulted in a decline in CO_2 assimilation.

BSMV infection led to an excess of excitation energy, since the photosynthetic rate was decreased. The excess energy will cause damage to the photosynthetic apparatus if this energy was not dissipated safely (Jin *et al.,* 2009, 2010). NPQ has been suggested to play a crucial role in the response of plants to virus infection (Li *et al.,* 2006). In this study, a notable rise in NPQ can greatly help to dissipate the excessive excitation energy and efficiently prevent the photosynthetic structure from being affected by BSMV infection. However, The NPQ is dependent on pH gradient across the thylakoid membrane (ΔpH), which associated with electron transport (Shikanai *et al.,* 2002; Štroch *et al.,* 2004). Therefore, it was worthy to further study what process generates the ΔpH necessary to support this requisite NPQ increase, because the linear electron transport rate was decreased in the infected plants.

Virus infection could increase SOD activity in plants, thus relieving the injury from reactive oxygen species (ROS) (Liu *et al.,* 2009). MDA is a product of lipid peroxidation. In this study, the MDA content was gradually increased, which indicated that ROS were accumulated, thereby resulting in membrane lipid peroxidation. SOD CAT and APX can keep membrane system from active oxygen and other peroxide free radicals hurt (Jin *et al.,* 2011). SOD, APX and CAT activities were increased before 21 days infection, which

suggested that SOD, CAT, and APX could partially remove these ROS under such conditions. After 28 days infection, SOD, APX and CAT activities was gradually decreased, and this decreases were accompanied by a significantly elevated MDA content (Fig. 4), which indicates that the ROS was not be effectively removed. At the same time, the magnitude of increase of NPQ was largely decreased, so that the electrons could more easily leak to O_2 to generate ROS.

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REFERENCES

1. Fu DY, Hong J, Chen JS, Wu JX., Accumulation of coat protein of *Turnip mosaic virus* in host chloroplast and its effect on PSII activity. *J. Plant Physiol. Mol. Biol.* 2004; **30**: 34-40.
2. Funayama S, Hikosaka K, Yahara T., Effects of virus infection and growth irradiance on fitness components and photosynthetic properties of *Eupatorium makinoi* (Compositae). *Am. J. Bot.* 1997; **84**: 823–829.
3. Guo D-P, Guo Y-P, Zhao J-P, Liu H, Peng Y, Wang Q-M, Chen J-S, Rao G-Z., Photosynthetic rate and chlorophyll fluorescence in leaves of stem mustard (*Brassica juncea* var. *tsatsai*) after turnip mosaic virus infection. *Plant Sci.* 2005; **168**: 57–63.
4. Guo XQ, Wun FJ, Zhu HC., Effect of PVY infection on photosynthesis of tobacco. *J. Zhejiang Univ*. (Agr. Life Sci.) 2000; **26**(1): 75-78.
5. Holzberg S, Brosio P, Gross C, Pogue GP., *Barley stripe mosaic virus*-induced gene silencing in a monocot plant. *Plant J*. 2002; **30**: 315-327.
6. Jin SH, Li XQ, Jia XL., Genotypic differences in the responses of gas exchange, chlorophyll fluorescence, and antioxidant enzymes to aluminum stress in *Festucaarundinacea*. *Russ. J. Plant Physiol*. 2011; **58**: 560-566.
7. Jin S, Li X, Hu J, Wang J., Cyclic electron flow around Photosystem I is required for adaptation to high temperature in a subtropical forest tree, *Ficusconcinna*. J. Zhejiang Univ.-Sci. B 2009; **10**: 784-790.
8. Jin SH, Li XQ, Zheng BS, Wang JG., Response of photosynthesis and antioxidant systems to high-temperature stress in *Euonymus japonicus* seedlings, *For. Sci*. 2010; **56**: 172-180.
9. Kramer DM, Johnson G, Kiirats O, Edwards GE., New fluorescence parameters for the determination of Q_A redox state and excitation energy fluxes. *Photosynth. Res.*2004; **79**: 209-218.
10. Li X, Jin S., Effects of Drought Stress on Gas Exchange and Chlorophyll Fluorescence Characteristics in *Eremochloa ophiuroides*. *Chin Agri. Sci. Bull*., 2010; **26**: 170-174.
11. Li Y-H, Hong J, Xie L, Yang Y, Zhou X-P, Jiang D-A., Effects of *broad bean wilt virus 2* isolate infection on photosynthetic activities and chloroplast ultrastructure in broad bean leaves. *J. Plant Physiol. Mol. Biol*. 2006; **32**: 490-496.
12. Liu L, Hou X, Wang Li, Chen X., Changes of four protective enzyme activities and relationships to resistance in non-heading Chinese cabbage after infection of Turnip mosaic virus. *J. Nanjing Agri. Univ.* 2009; **32**: 14-18.
13. Maxwell K, Johnson GN., Chlorophyll Fluorescence – A Practical Guide, *J. Exp. Bot.* 2000; **51**: 659–668.
14. Naidu RA, Krishnan M, Ramanujam P, Ggnanam A, Nayudu MV., Studies on peanut green mosaic infected peanut (*Archishypogaea*L.) leaves. I. Photosynthesis and photochemical reactions. *Physiol. Plant Pathol*. 1984; **25**: 181-190.
15. Peng X-X, Qu SM., Advance in research of barlye stripe mosaic virus. *Virol. Sin*. 1989; **4**: 329-335.
16. Peng YH, Lei JL, Huang LF, Yu JQ., Effects of *Potato virus Y* infection on chloroplast ultrastructure, photosynthesis and chlorophyll fluorescence quenching in potato leaves. *ActaPhytopathol. Sin*. 2004; **15**(3): 189-190
17. Reinero A, Beachy RN., Association of TMV coat protein with chloroplast membranes in virus-infected leaves. *Plant Mol. Biol*. 1986; **6**: 291-301.
18. Reinero A, Beachy RN., Reduced photosystem II activity and accumulation of viral coat protein in chloroplast of leaves infected with tobacco mosaic virus. *Plant Physiol.* 1989; **89**: 111-116.
19. Shikanai T, Munekage Y, Kimura K., Regulation of proton-to-electron stoichiometry in photosynthetic electron transport: physiological function in photoprotection. *J. Plant Res.* 2002; **115**(1): 3-10.
20. Štroch M, Špunda V, Kurasová I., Non-radiative dissipation of absorbed excitation energy within

photosynthetic apparatus of higher plants. *Photosynthetica* 2004; **42**(3):323-337.

21. Sun X, Qiu B., Sequence analysis of barley stripe mosaic virus China strain (BSMV-CH) RNA1. *Sci. Agri. Sin.* 2008; **41**(7):1969-1974
22. Sun X, Zhao W, Qing L, Yang S., Comparing and analyzing the tripe gene block sequence of Barley stripe mosaic virus China strain. *Acta. Phytopathol. Sin.* 2010; **40**(4): 433-437.
23. Verma S, Mishra SN., Putrescine Alleviation of Growth in Salt Stressed *Brassica junceaby* Inducing Antioxidative Defense System, *J. Plant Physiol*. 2005; **162**: 669–677.
24. Wang CM, Shi DJ, Zhu SF, Tian B, Wei NS., Effects of *Cucumber mosaicvirus*infection on photosynthetic activities of tobacco leaves and chloroplasts. *Acta Bot. Sin.* 2000; **42**(4): 388-392.
25. Wilhelmová N, Procházková D, Šindelárová M, Šindelár L., Photosynthesis in leaves of *Nicotianatabacum*L. infected with tobacco mosaic virus. *Photosynthetica* 2005; **43**(4): 597-602.