DOI: 10.1007/s11099-015-0104-7 PHOTOSYNTHETICA 53 (2): 269-278, 2015

Photosynthetic activities, C_3 and C_4 indicative enzymes and the role of photoperiod in dormancy induction in 'Chunjie' peach

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Abstract

Our study examined the relationship between photosynthetic performance and activities of key photosynthetic enzymes to understand the photosynthetic variation and reasons for the variation during dormancy induction under different photoperiods in peach (*Prunus persica* L. cv. Chunjie). Furthermore, the study explained the changes in the key enzymes from the viewpoint of differential proteomics. The results showed that the leaf net photosynthetic rate (P_N) and stomatal conductance tended to decrease, while the intercellular CO_2 concentration rose, which indicated that the reduced P_N resulted from nonstomatal limitation. During the dormancy induction period, the activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phospho*enol*pyruvate carboxylase (PEPC) declined, which was the main reason for the reduced P_N. Two-dimensional electrophoresis maps and differential protein identification demonstrated that the decrease in activity of the photosynthetic enzymes was mainly due to enzymatic degradation. The enzyme degradation by a long-day treatment occurred later and to a lesser degree than that of the short-day treatment. In the long-day treatment, the carboxylation activity of Rubisco was higher than that of the control treatment, and the PEPC activity and the ratio of the PEPC/Rubisco activity were lower than the corresponding activities during the control treatment. These differences under long-day conditions were significant but did not occur in the short-day treatment, suggesting that the C_4 pathway might be more active under short-day conditions.

Additional key words: gas exchange; MALDI-TOF; nonstomatal limitation; Rubisco large subunit; two-dimensional electrophoresis.

Introduction

Dormancy of northern deciduous fruit trees is an important adaptive mechanism for plant survival in cold climates. It is essential that the dormant condition is established within the plant well in advance of the cold season. Dormancy is induced by several factors, such as low temperature, water deficit, short photoperiod, and a combination of these factors (Rinne *et al*. 2001, Kozlowski and Pallardy 2002, Heide and Prestrud 2005, Olsen 2006, Heide 2011). Photoperiod has been considered to play a major role (Fennel and Hoover 1991, Jian *et al*. 1997, Whitelam and Devlin 1997, Rohde *et al*. 2002, Cook *et al*. 2005, Rohde and Bhalerao 2007). However, the mechanism of dormancy induction is still unknown. Leaves are the main sites of both photoperiodic induction and photosynthesis (Knott 1934). P_N affects vegetative growth of the tree and the degree of conversion from the vegetative growth stage to dormancy. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) and phospho*enol*pyruvate carboxylase (PEPC) are key enzymes of the C_3 and C_4 photosynthetic pathways, respectively; their activities directly affect the P_N (Jiang *et al*. 1996, 2000). The ratio of the two enzyme activities indicates the relative predominance of the C_3 compared with the C_4 photosynthetic pathway. The photosynthetic efficiency of the C_4 pathway is higher than

Received 25 November 2013, *accepted* 2 May 2014.
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Abbreviations: C_i – intercellular CO_2 concentration; CK – control; g_s – stomatal conductance; IEF – isoelectric focusing; IPG – immobilized pH gradient strips; LD – long day; MALDI-TOF – matrix-assisted laser desorption/ionization time of flight; M_r – relative molecular mass; MS – mass spectrometry; PEP – phospho*enol*pyruvate; PEPC – phospho*enol*pyruvate carboxylase; PI – isoelectric point; PMF – peptide mass fingerprinting; P_N – net photosynthetic rate; SD – short day; 2-DE – two-dimensional electrophoresis.
Acknowledgements: We are grateful to Shandong Province Excellent Young Scientist Award Fund (No. BS2011NY011) and the National Natural Science Foundation of China (No. 31372050) for financial support of this study.
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that of the C_3 pathway and changes in environmental conditions affect the expression of the C_4 pathway in C_3 plants. In wheat flag leaves and ears, water stress enhances the enzyme activity of the C_4 pathway (Wei *et al*. 2003), and under heat stress, PEPC activity increases significantly, Rubisco decreases, and the ratio of PEPC/Rubisco increases (Xu *et al*. 2001). Rubisco activity of rice is significantly inhibited under conditions of photooxidation, while the PEPC activity increases (Jiao and Ji 1996). Some amphibious plants, such as water chestnuts, can differentiate into the C_4 mode under terrestrial conditions and into the C_3 mode under submersed conditions. This can be concluded because plants grown under terrestrial conditions show the photosynthetic enzyme activities typical of the NAD-malic enzyme-C_4 subtype, whereas those grown under aquatic conditions show decreased activities of the key C_4 enzymes and increased Rubisco activity (Ueno *et al*. 1988). Different stages of growth and development also affect the expression of the C_4 pathway enzymes in C_3 plants. The PEPC/Rubisco activity ratio in the stems of *Phyllostachys pubescens* increases during the rapid growth stage after shooting (Wang *et al*. 2012). For northern deciduous fruit trees, however, there is no research regarding a number of important questions. For example, how do photosynthesis and key enzymatic activities change during dormancy induction and in response to photoperiodic induction? Do the photosynthetic pathways change when the trees respond to photoperiodic induction, and if so, what changes occur?

Using the leaves of the special, protected 'Chunjie' peach cultivar as the test material, we examined the effects of different photoperiods on the induction of peach dormancy and analyzed the variations of the key photosynthetic enzyme activities and the photosynthetic pathways during the period of dormancy induction. Finally, differential protein analysis based on 2-dimensional electrophoresis (2-DE) separation and mass spectroscopic (MS) identification was used to explore reasons for the changes of P_N.

Materials and methods

Test materials and experimental design: This study was conducted with peach (*Prunus persica* L. cv. Chunjie) in greenhouse at the Science and Technology Innovation Park of Shandong Agricultural University, Tai'an (36.11°N, 117.08°E), in August–November 2011. The plant height and crown diameter were approximately 2.5–3 and 2–3 m, respectively, and the plants grew robustly with normal management. The average daylight duration was in the range of 10–14 h and the monthly temperature ranges were 21.1–28.5°C in August, 15.1–24.3°C in September, 9.7–20.6°C in October, and 4.4–12.8°C in November during the experimental period in Tai'an.

Three experimental treatments were used. In a long-day (LD) treatment, the day light duration was prolonged artificially with an average intensity of 350 μmol(photon) m^{-2} s^{-1} to provide a 16 h day/8 h night photoperiod under ambient temperature conditions. In a short-day (SD) treatment, the day light duration was shortened by shading to provide the 8 h day/16 h night photoperiod under ambient temperature conditions. Control conditions (CK) were ensured under the natural light intensity, light duration, and temperature at the place. Each treatment consisted of ten trees, and sampling was repeated three times.

Determination of dormancy status: Ten one-year-old shoots (the same orientation and height from the beginning to the end, three replications) were collected randomly at 7 or 10 d intervals. The clean-water method was used to determine the dormancy status (Jian *et al*. 1997, Wang *et al*. 2008). The leaves and 5 cm first buds were removed. The shoots were held in an incubator under the following conditions: day/night temperatures of 25/21°C, light intensity of 40 μmol(photon) m^{-2} s^{-1}, 14 h day/10 h night photoperiod, and a relative humidity of 80–90%. When the time required for the first budding of 60% or more of the young shoots exceeded 10 d, dormancy was considered to have been induced. When the buds did not burst within six weeks, it was regarded as natural dormancy.

Measurements of photosynthesis and the activities of key photosynthetic enzymes: Photosynthetic parameters of gas exchange were measured using the *TPS-2 Photosynthesis System* (*PP Systems*, UK). Mature leaves in the middle of the new shoots were selected to measure P_N, g_s, and C_i were recorded simultaneously.

The Rubisco (EC 4.1.1.39) and PEPC (EC 4.1.1.13) enzyme activities were measured according to the method described in Li and Li (1989), with slight modifications. The enzymes were extracted from 0.5 g leaf tissue that was ground to a fine homogenate in 3 ml of 100 mM, precooled Tris-HCl buffer (including 5% glycerol, 1% PVP, 1 mM EDTA, and 10 mM β-mercaptoethanol, pH = 8.2) with precooled mortar and pestle. The homogenate was centrifuged for 20 min at 15,000 ×*g* at 4°C. The supernatant, the crude enzyme extract, was stored at 4°C before use.

The carboxylation activity of Rubisco was measured spectrophotometrically using methods described by Li and Li (1989), with slight modification. The reaction solution contained 1 M Tris-HCl buffer (pH 8.0), 0.1 M $MgCl_2$, 1 mM EDTA, 50 mM ATP, 50 mM dithiothreitol (DTT), 2 mM NADH (3 ml of each above reagent), 0.1 ml of 200 mM $NaHCO_3$, 0.8 ml of ddH_2O, and 0.1 ml of 9 mM RuBP. The reaction solution was preheated for 10 min in a 30°C water bath before the addition of 0.1 ml of a mixture of 3-phosphoglycerate kinase and 3-phosphoglyceraldehyde dehydrogenase (2.49×10^{-23} g/2.49×10^{-23} g), and the initial optical absorption value (E0) was measured at 340 nm, the measuring time was 1 min. A 0.1 ml aliquot

of Rubisco crude enzyme extract was then added to start the reaction, and the optical absorption during the reaction (E1) was immediately measured at 340 nm every 30 s, the measuring time was 1 min. The enzyme activity was calculated from the difference between E1 and E0 and expressed in μmol mg^{-1} min^{-1}.

The PEPC activity was measured according to Shi *et al.* (1979) using slightly modified methods. The reaction solution contained 0.1 ml of 100 mM Tris-HCl buffer (pH = 9.2), 0.1 ml 10 M $MgCl_2$, 0.1 ml 10 mM $NaHCO_3$, 0.2 ml of 40 mM PEP (phospho*enol*pyruvate), 0.3 ml of 1 mg ml^{-1} NADH (pH = 8.9), and 0.3 ml of malate dehydrogenase (approximately 1.74×10^{-23} g). The reaction solution was preheated for 10 min at 28°C water bath before the reaction was started by adding 0.1 ml of PEPC enzyme extracting solution. PEPC activity of the solution was determined in μmol mg^{-1} min^{-1} using the changes in optical absorption measured at 340 nm, the measuring time was 1 min, the same as in case of Rubisco activity.

Total protein extraction and determination: The total soluble proteins of the peach leaves were extracted by the trichloroacetic acid/acetone precipitation method. The 2-DE experiments were conducted using an 800 μg sample volume and immobilized pH gradient (IPG) gel strips (24 cm) covering a pH range of 4–7.

Protein content in the supernatant was determined by the modified Bradford method (Ramagli and Rodriguez 1985), using bovine serum albumin (*Sigma*) as a standard.

Two-dimensional gel electrophoresis: The first dimension was isoelectric focusing (IEF), which was carried out on an *Ettan IPGphor Manifold* (*GE Healthcare*, UK). Hydration buffer [8 M urea, 4% (w/v) 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS), 0.5% (v/v) immobilized pH gradient (IPG) buffer, 0.28% (w/v) DTT, 0.002% (v/v) bromophenol blue] and sample solution mixed and centrifuged at 40,000 × *g* for 10 min at room temperature, the supernatant was added into IEF unit with the IPG strip (*GE Healthcare*, UK, pH = 4–7, 24 cm). The settings were as following: 30 V for 12 h, 500 V for 1 h; 1,000 V for 1 h, and 8,000 V for 9 h at 20°C, 50 μA per strip.

After the IEF, the IPG strips were equilibrated for 15 min in 15 ml equilibration buffer [6 M urea, 75 mM Tris-HCl (pH = 8.8), 30% (v/v) glycerol, 2% (w/v) sodium dodecyl sulfate (SDS)] supplemented with 1% (w/v) DTT. A second equilibration step of 15 min with the same equilibration buffer and the same volume, containing now 2.5% (w/v) iodoacetamide was carried out afterwards. The IPG strips were then sealed with 0.5% agarose in SDS running buffer at the top of slab gels (280 × 210 × 1 mm) polymerized from 12.5% (w/v) acrylamide and 0.1% N, N′-methylenebisacrylamide. The gels were poured between low fluorescent and bind-silane treated glass plates. The SDS-PAGE step was performed at 15°C in *Ettan Dalt II* tank (*GE Healthcare*, UK) at 5 W per gel for 45 min, 17 W/gel for 8 h, until the bromophenol blue dye front was about 1 cm from the bottom of the gel. The gels were fixed 45 min in 10% acetic acid and 40% ethyl alcohol after the second dimension on SDS-PAGE, then visualized with Coomassie Brilliant Blue (CBB) staining with 0.1% (w/v) CBB G-250, 10% (w/v) ammonium sulfate containing 7 Crystal water, 2% (v/v) phosphoric acid, and 25% methanol for about 20 h. Then the gels were bleached with distilled water.

Image capture, analysis and identification with MALDI-TOF/TOF MS: The images of the gels were obtained using the image scanner *Umax Powerlook 2100* (*GE Healthcare*, UK) at 300 dpi and a 16-bit grayscale pixel depth and then analyzed with *ImageMaster™ 2D Platinum Software Version 5.0*. Changes in the abundance of protein spots exceeding 2-fold were considered evidence for the differential expression of the proteins. After digesting with trypsin (mass spectrometry grade), the peptide spots were sequenced using MALDI-TOF/TOF-MS (*BGI*, China) and identified by searching the SWISS-PROT and NCBInr databases.

Statistical analysis: The data were analyzed by *SPSS version 19.0*, graphs were finished with *SigmaPlot 10.0*.

Results

Dormancy process definition and photoperiodic role: The time course of bud burst and new shoot growth during all three treatments is shown in Fig. 1. For the CK treatment, the number of days to the first burst was prolonged as the day length shortened and the temperature fell. The average length of new shoots was 59.9 cm on September 15 and showed no further increase. The buds collected at this time burst at 12 d, indicating the beginning of dormancy induction. The buds stopped bursting after November 10, which signified that the buds were transferred into the natural dormancy period. For the LD treatment, the number of days to the first burst was also prolonged as the temperature fell, and the buds entered into dormancy induction on September 22, which was 7 d later than those in the CK treatment and sprouted no later than November 17. Maintaining the unchanged photoperiod, new shoots eventually stopped in the SD treatment by September 8, at which time sprouting required 13.2 d,

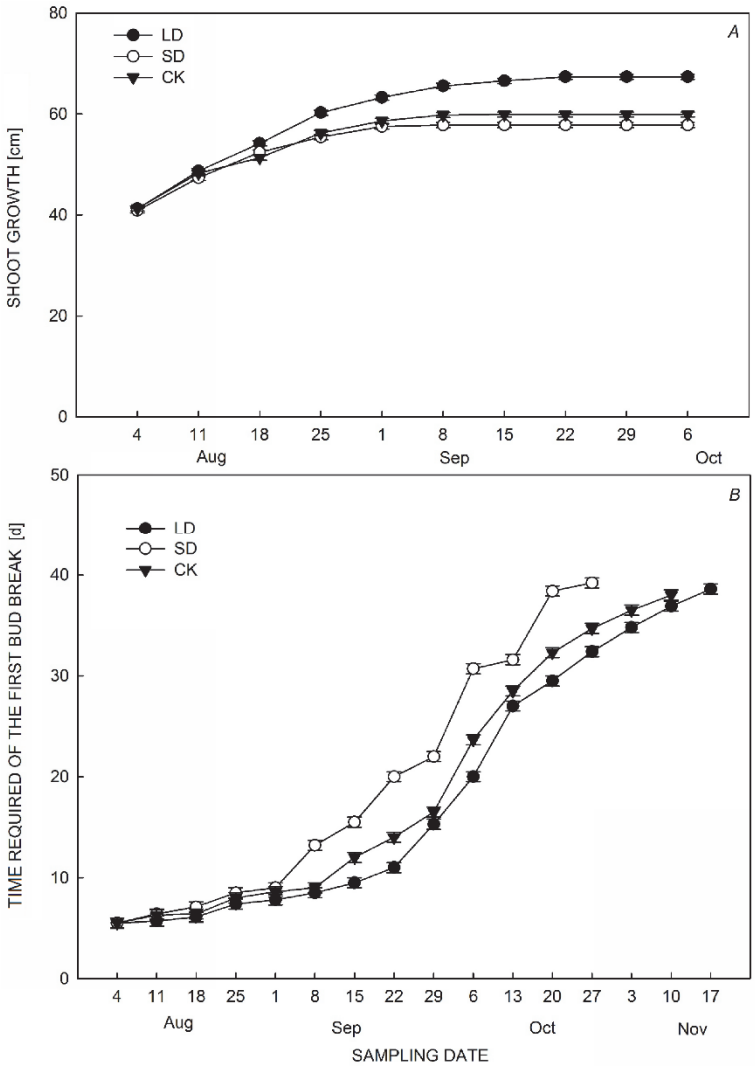

Fig. 1. Effects of photoperiod treatments on shoot growth (*A*) and days required of the first bud break (*B*) in peach (*Prunus persica* L. cv. Chunjie). Means ± SD (n = 10). CK – control treatment, LD – long-day treatment, SD – short-day treatment.

indicating that the buds entered the dormancy induction period. The buds stopped bursting on November 3, which was 14 d earlier than those in the CK treatment. Clearly, the SD exposure significantly affected the dormancy induction and also shortened the dormancy induction period; however, the LD photoperiod postponed it.

Photosynthetic parameters: The variation in the P_N of peach leaves under the different photoperiods is shown in Fig. 2*A*. The P_N of all three treatments was the highest before the dormancy induction period and then began to decrease during dormancy induction. The change was similar in leaves from the LD and CK treatments, but the peak was 10 d later in the LD than that in the CK treatment. At 11.25 μmol(CO_2) m^{-2} s^{-1} in the LD treatment, the peak in the LD treatment was 1.26 times higher that of the peak in the CK treatment; the difference was highly significant. The minimum value at 2.65 μmol(CO_2) m^{-2} s^{-1} in the LD treatment did not differ significantly from that of the CK treatment, and the natural dormancy period was entered at this level. The P_N maximum time was 10 d earlier for the SD than that for the CK treatment. The P_N remained in the range of 3.40–2.83 μmol(CO_2) m^{-2} s^{-1} when the natural dormancy period was entered and continued to decrease to 1.85 μmol(CO_2) m^{-2} s^{-1} by the end of the treatment period. This final value was 18.7% lower than that of the CK treatment, and the difference was highly significant.

Fig. 2*B* shows the changes in stomatal conductance (g_s) at the different stages of the photoperiodic induction. The g_s values in all three treatments decreased after the dormancy induction period. The g_s value in the LD treatment was not significantly different from that in the CK treatment at the early stage of the dormancy induction period but was higher and lower than that of the CK treatment at the intermediate and late stages, respectively. In the SD treatment, the g_s value declined rapidly and was significantly lower than that in the CK treatment once the peach tree entered dormancy induction, but there was no difference between these treatments after October 11, which indicated that the resistance of the different stages was induced by different photoperiodic conditions. Long days improved late resistance, whereas short days

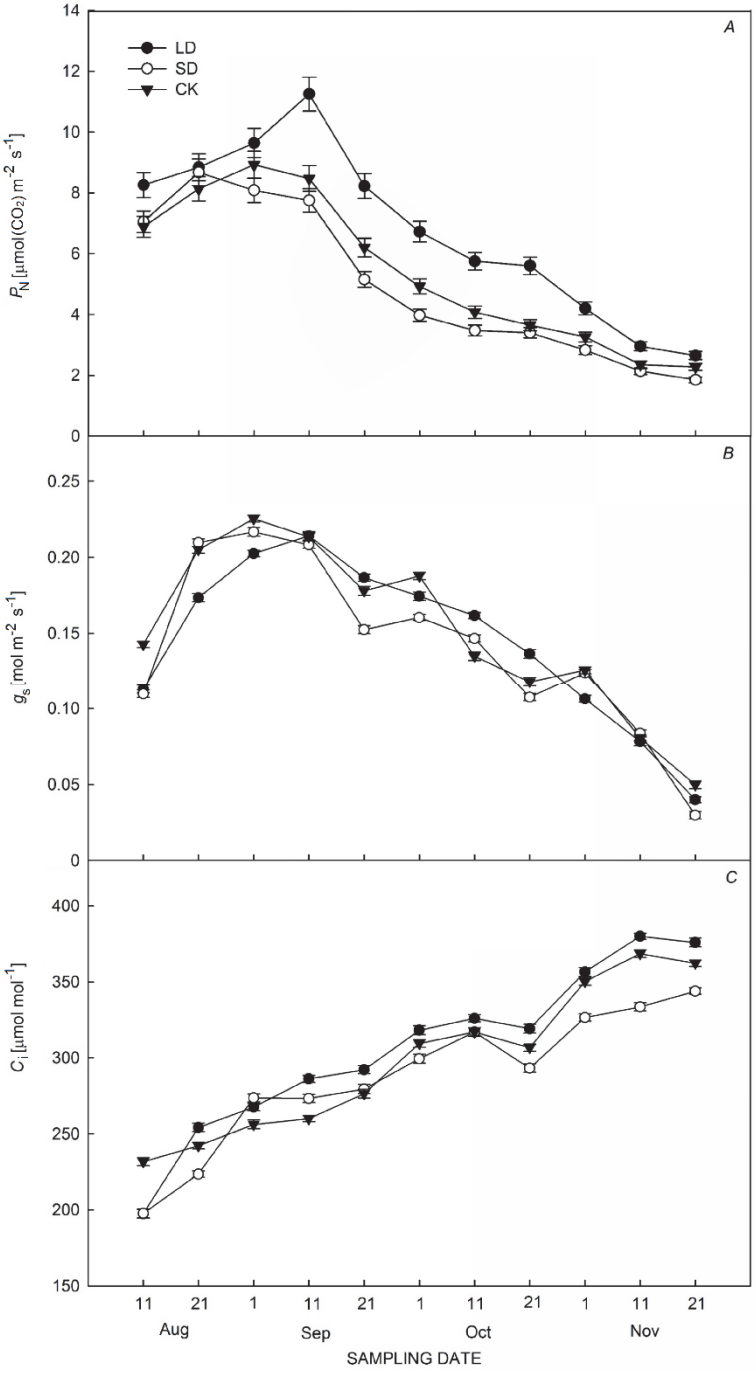

Fig. 2. Photosynthetic parameter changes in peach (*Prunus persica* L. cv. Chunjie) leaves during the photoperiodic dormancy induction. Photosynthetic parameters refer to net photosynthetic rate (P_N) (*A*), stomatal conductance (g_s) (*B*), and intercellular CO_2 concentration (C_i) (*C*). Means ± SD (n = 5). CK – control treatment, LD – long-day treatment, SD – short-day treatment.

facilitated early resistance because the trees could respond more rapidly.

The intercellular CO_2 concentration (C_i) data are shown in Fig. 2*C*. The C_i values of all treatments showed a varying upward trend during the dormancy induction period. The C_i trends were in the opposite direction than that of the g_s and P_N. The C_i trends in the LD and CK treatments were the same; although the numerical value was higher for the LD than that of the CK treatment, the difference was not significant during the entire period of dormancy induction. After entering the dormancy induction period, the g_s in the SD treatment also increased. There was no significant difference between the SD and CK treatments before November 21; however, a significant difference developed after that date.

Key photosynthetic enzyme activities: Rubisco is a key enzyme of the C_3 photosynthetic pathway. The carboxylation activity of Rubisco showed a downward trend overall during dormancy induction (Fig. 3*A*). The peak in the carboxylation activity of the leaves appeared 7 d later in the LD than in the CK treatment; the activity was

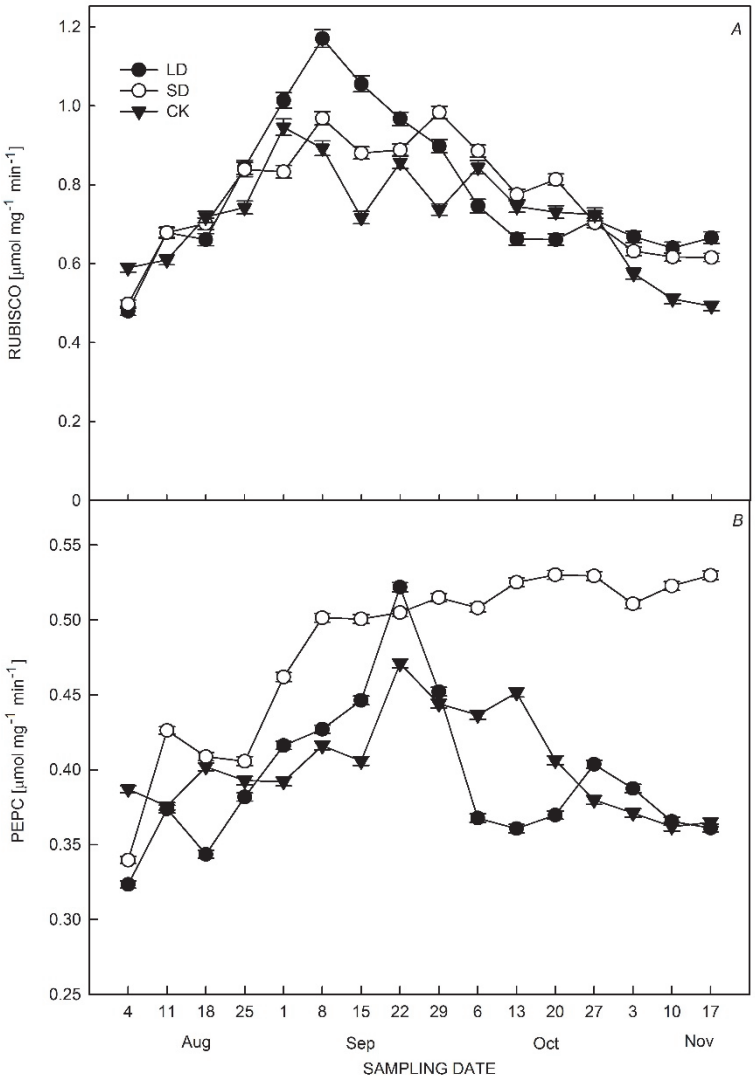

Fig. 3. Effects of photoperiod on activities of ribulose-1,5-bisphosphate carboxylase (Rubisco) (*A*) and phospho*enol*pyruvate carboxylase (PEPC) (*B*) in peach (*Prunus persica* L. cv. Chunjie) leaves during dormancy induction. Means ± SD (n = 5). CK – control treatment, LD – long-day treatment, SD – short-day treatment.

1.167 μmol mg^{-1} min^{-1} on September 8, and then declined. The carboxylation activity values were higher in the LD than in the CK treatment, and the differences were significant or highly significant, except the values from October 6 to 27. In the SD treatment, the carboxylation activity peak occurred on September 8. The carboxylation activity declined throughout the dormancy induction period and was significantly or highly significantly higher in the SD than that in the CK treatment, lower than that in the LD treatment.

PEPC is a key enzyme of the C_4 photosynthetic pathway. The first PEPC activity peak (0.524 μmol mg^{-1} min^{-1}) observed in the LD treatment appeared on September 22 (Fig. 3*B*). The activity decreased during the dormancy induction period and was significantly lower in the LD than that in the CK treatment of the mid-dormancy stage. The PEPC activity of SD increased during the treatment period, which was also higher in the SD than in the CK treatment significantly.

Photosynthetic pathways: The ratio of PEPC and Rubisco reflects to some extent the relative active degree of both photosynthetic pathways in leaves. Fig. 4 shows temporal changes in the ratio of the PEPC and Rubisco activities among all treatments. The courses of all treatments were substantially similar. The ratio of PEPC/Rubisco was significantly lower in the LD than that in the CK treatment at corresponding dates; however, the ratio was higher in the SD than that in the CK treatment, and the difference was highly significant, especially in the middle and later periods of the dormancy induction period (after October 6).

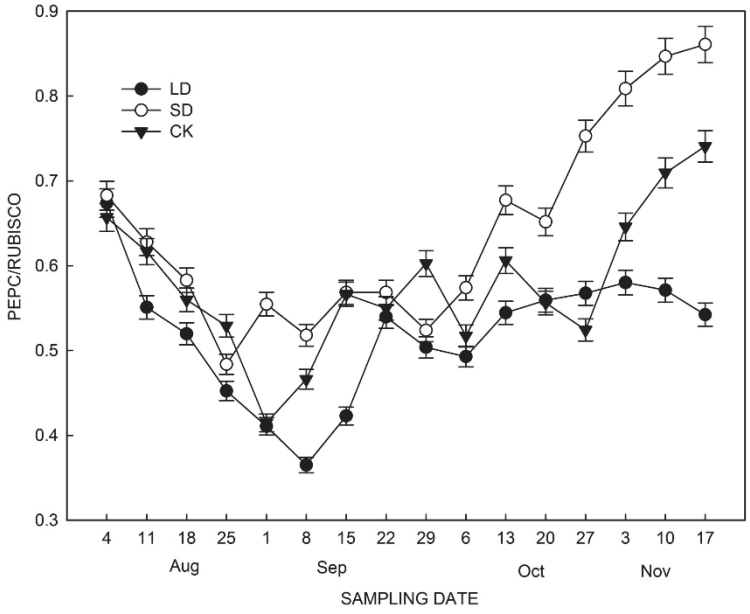

Fig. 4. Effects of photoperiod treatments on phospho-*enol*pyruvate carboxylase/ribulose-1,5-bisphosphate carboxylase ratio (PEPC/Rubisco ratio) in peach (*Prunus persica* L. cv. Chunjie) leaves during dormancy induction. Means ± SD (n = 5). CK – control treatment, LD – long-day treatment, SD – short-day treatment.

Differential proteomic analysis: To further explore the reasons for the decline in the photosynthetic enzyme activity, we analyzed the total leaf proteins using 2-DE technology. More than 500 active points were filtered from each gel, including 30 differentially expressed proteins identified by MALDI-TOF/TOF-MS, of which 12 were identified as the Rubisco large subunit (Fig. 5, Table 1). The apparent molecular mass of these large subunits were all less than the theoretical molecular mass (57.3 KDa), but their PMF (peptide mass fingerprinting) maps were different from one another. We speculated that the Rubisco large subunits filtered from the gel were different degraded fragments of the Rubisco protein. The matching ratio of the identified proteins ranged from 15 to 50%, and the Mowse scores were higher than the threshold value, indicating reliable identification results. As can be seen from Fig. 5 and Table 1, there were 12 degradation fragments of the large Rubisco subunits and their expression was upregulated during photoperiodic induction in both LD and SD treatments. Thus, we confirmed that the expression of the main Rubisco protein in the leaves was downregulated and the extent of the downregulation was lesser in the LD than that in the SD treatment with the deepening of dormancy induction. There were more degradation fragments in the SD than in the LD treatment, explaining the greater inhibition of carbon assimilation in the SD treatment. The comprehensive 2-DE maps also demonstrated that the degradation of Rubisco frequently occurred in the intermediate stage of the dormancy induction period and occurred later and to a lesser extent in the LD than in the SD treatment.

Discussion

Opinions still vary concerning the functions of the photoperiod and temperature in dormancy induction. Jian *et al*. (2004) considered the photoperiod to be the only factor inducing dormancy, with a little effect of low temperatures in the late summer. However, certain experiments indicate that low temperature is the main factor inducing the dormancy of apple trees (Heide and Prestrud 2005). Moreover, the plant dormancy could be apparently induced in some warm areas only by short days and low temperatures (Stewart *et al*. 1990, Heide and Prestrud 2005). In the present study (Fig. 1), the dormancy induction period of the Chunjie peach occurred in the late summer and early autumn. The buds in all treatments entered the dormancy induction and natural dormancy, with the only difference in the length of time required to reach the two dormancy stages, which indicated that the day length was only a cofactor in the induction process and acted mainly before dormancy induction, while the gradual decrease in the natural temperature (from August to November) was the major inducing factor.

There may be several reasons for this dormancy induction pattern. First, the photoperiod treatments began in August, when the temperature followed a gradual downward trend (but did not reach the low-temperature range before October). The buds were able to enter the dormancy induction period under the LD photoperiod, which may be relevant to the signal of decreasing temperature. Temperature signals contribute to the timing of photoperiodic growth cessation and bud set (Rohde *et al*. 2011). The trees in the LD treatment might enter the dormancy induction period later than those exposed to natural conditions because of high expression levels of certain proteins conducive to leaf growth, such as ATP synthase (Guo *et al*. 2009, González *et al*. 2012). The

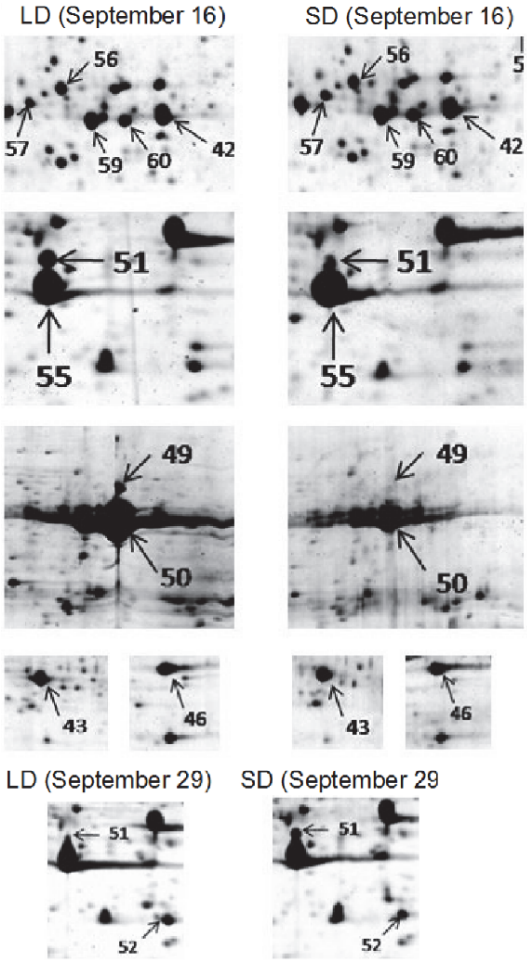

Fig. 5. Close-up views of representative two-dimensional electrophoresis (2-DE) gels of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) large subunits in long-day (LD) and short-day (SD) treatment peach (*Prunus persica* L. cv. Chunjie) leaves.

buds in the SD treatment entered the dormancy induction period because of the short-day signal. In the northern region, the shortening of the day length predicts falling temperatures or even low-temperature stress in the near future, thus the trees respond more rapidly by advancing the dormancy process. Secondly, the short-day and low-temperature induction signals produced different temporal response patterns. Short days induced the dormancy characteristic first and the cold resistance later, whereas low temperatures induced the cold resistance first and the dormancy characteristic later, which produced the difference in dormancy mechanisms between the long- and short-day treatments (Wang *et al*. 2008). Finally, the species variation in sensitivity to photoperiod and temperature is due to the variation among species in their long-term living environments. Different species or areas might generate different results (Heide and Prestrud 2005, Heide 2008, 2011), which may at least partially explain the controversy regarding dormancy induction by photoperiod and temperature.

As the main organ of photosynthesis in higher plants, leaves bear the important tasks of manufacturing assimilates and providing energy. Rubisco functions as a carboxylase in the C_3 photosynthetic carbon reaction and as an indispensable dioxygenase in photorespiration. The enzyme is composed of 8 large subunits (56 KDa) and 8 small subunits (14 KDa). Research shows that an adverse environment can affect plant photosynthesis and damage photosystem function (Sun *et al*. 2008, 2009; Zhang *et al*. 2009, 2010; Sun and Li 2010), so does the dormancy. Generally, two types of factors affect photosynthesis: one is stomatal limitation and the other is nonstomatal limitation. When g_s, C_i, and P_N are all reduced or all increased, it can be considered that P_N change was primarily due to stomatal limitation. Declines in P_N that are accompanied by decreases in the g_s and increases in the

Table 1. Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) large subunits and ribulose-1,5-bisphosphate carboxylase (RuBPC) of peach (*Prunus persica* L. cv. Chunjie) leaves identified by peptide mass fingerprinting (PMF). Protein spot numbers are listed with the same as those given in Fig. 5. Changes in accumulation regard the long-day (LD) treatment as benchmark value. The arrow direction shows changes in accumulation of protein spots in the short-day (SD) treatment.

Spot	Accession number	Protein name	Organism	pI/Mr	Coverage rate	Accumulation change
42	gi|9909908	RuBPC	*Pachynema junceum*	5.33/19.19	41.95%	↑
43	gi|9909908	RuBPC	*Pachynema junceum*	5.28/19.19	23.56%	↑
46	gi|194400588	Rubisco large subunit	*Codonopsis kawakamii*	7.03/49.01	22.37%	↓
49	gi|533062	Rubisco large subunit	*Rhodotypos scandens*	6.57/52.06	29.03%	↓
50	gi|313183830	Rubisco large subunit	*Prunus persica*	6.57/53.08	41.89%	↓
51	gi|156454194	Rubisco large subunit	*Omphacomeria acerba*	6.35/51.36	36.90%	↓↑
52	gi|313183830	Rubisco large subunit	*Prunus persica*	6.57/53.08	32.21%	↓
55	gi|38147280	Rubisco large subunit	*Floerkea proserpinacoides*	6.24/52.08	20.17%	↓
56	gi|37194725	Rubisco large subunit	*Chrysophyllum oliviforme*	6.57/52.01	18.28%	↓
57	gi|38147280	Rubisco large subunit	*Floerkea proserpinacoides*	6.24/52.08	20.17%	↓
59	gi|9910021	Rubisco large subunit	*Turpinia occidentalis*	6.65/24.97	22.22%	↑
60	gi|9909908	RuBPC	*Pachynema junceum*	5.28/19.19	23.56%	↑

C_i can be attributed to nonstomatal limitation (Gao *et al*. 1993). Xu (1997) also notes that a reduction in the C_i is an indispensable condition of photosynthesis affected by stomatal limitation; however, an increase in the C_i is the most reliable criterion for nonstomatal limitation of photosynthesis. In this study, the P_N and the g_s both decreased, but the C_i increased (Fig. 2) during the dormancy induction, which demonstrated that the P_N declined due to nonstomatal limitation. This study also showed that the activities of PEPC and Rubisco were reduced (Fig. 3), indicating that the degradation of carboxylase was the main reason for nonstomatal limitation. Many degraded fragments of large Rubisco subunits were found through the identification and analysis of differentially expressed proteins (Fig. 5, Table 1), indicating that the reduced Rubisco activity occurred because of the degradation of the enzyme protein. Bi *et al*. (2011) obtained similar results with cucumber. However, the PEPC protein was not identified. There are several possible reasons for the apparent absence of PEPC. Firstly, the molecular mass of the main PEPC protein spot is approximately 110 KDa, which categorizes PEPC among the high molecular mass proteins on the 2-DE gel map (the 2-DE experiment reveals protein molecular mass in the range of 11.4 to 116.0 KDa). Additionally, the PEPC location could be on the upper edge of the map, the testing method may be ineffective, or most likely, there is a low level of PEPC expression in peach leaves.

The photosynthetic carbon metabolic pathway is not static but is influenced by environmental conditions, and the C_4 pathway has been found to exist in certain C_3 plants, *e.g*. soybean, rice, and citrus (Li *et al*. 2001, Li and Jiao 2005, Hu 2007, Man *et al*. 2009). One pathway may shift to another or change regarding the expression intensity of the different photosynthetic enzymes in different growth periods and environments (Niu *et al*. 2004). In this study, the ratio of PEPC/Rubisco in leaves was significantly or highly significantly lower in the LD than that in the CK treatment and was higher in the SD than that in the CK treatment (Fig. 4). Compared to LD conditions, we speculated that the C_4 photosynthetic pathway under SD conditions might be more active. This adjustment in the photosynthetic carbon assimilation pathway under different environmental conditions can mean that the tree is better adapted to specific ecological environments. There are many physiological and biochemical changes in the response of trees to photoperiodic induction throughout the growing season and into dormancy. Adjustments in photosynthetic performance constitute only a small proportion of these changes, and research will continue on the other changes and their mechanisms.

Conclusion: Short days promoted dormancy induction of peach buds, while long days delayed this function. The P_N declined during dormancy induction due to nonstomatal limitation, which was mainly caused by the decreased activity and degradation of the photosynthetic enzymes. The C_4 photosynthetic pathway under short-day conditions was more active than under long-day conditions during the dormancy induction.

References

Bi H.G., Wang M.L., Jiang Z.S. *et al*.: [Impacts of suboptimal temperature and low light intensity on the activities and gene expression of photosynthetic enzymes in cucumber seedling leaves.] – Chin. J. Appl. Ecol. **22**: 2894-2900, 2011. [In Chinese]

Cook N.C., Bellen A., Cronjé P.J.R. *et al*.: Freezing temperature treatment induces bud dormancy in 'Granny Smith' apple shoots. – Sci. Hortic.-Amsterdam **106**: 170-176, 2005.

Fennell A., Hoover E.: Photoperiod influences growth, bud dormancy, and cold acclimation in *Vitis labruscana* and *V. riparia*. – J. Am. Soc. Hortic. Sci. **116**: 270-273, 1991.

Gao H.Y., Zou Q., Cheng B.S.: [Study on the stomatal and nonstomatal limitation during the diurnal course of photosynthesis in soybean leaves.] – Acta Bot. Boreal. **13**: 96-102, 1993. [In Chinese]

González L.M.G., Kayal W.E., Ju C.J.T. *et al*.: Integrated transcriptomic and proteomic profiling of white spruce stems during the transition from active growth to dormancy. – Plant Cell Environ. **35**: 682-701, 2012.

Guo C.F., Sun Y., Lai C.C., Zhang M.Q.: [Proteomic analysis of tea leaf under polyethylene glycol stress.] – J. Tea Sci. **29**: 79-88, 2009. [In Chinese]

Heide O.M., Prestrud A.K.: Low temperature, but not photoperiod, controls growth cessation and dormancy induction and release in apple and pear. – Tree Physiol. **25**: 109-114, 2005.

Heide O.M.: Interaction of photoperiod and temperature in the control of growth and dormancy of *Prunus* species. – Sci. Hortic.-Amsterdam **115**: 309-314, 2008.

Heide O.M.: Temperature rather than photoperiod controls growth cessation and dormancy in *Sorbus* species. – J. Exp. Bot. **8**: 1-8, 2011.

Hu L.M.: [Study on the photosynthesis and C4 pathway photosynthetic characteristics in citrus.] – Ph D Thesis, Huazhong Agric. Univ., 2007. [In Chinese]

Jian L.C., Li P.H., Sun L.H., Chen T.H.H.: Alteration in ultrastructure and subcellular localization of Ca^{2+} in poplar apical bud cells during the induction of dormancy. – J. Exp. Bot. **48**: 1195-1207, 1997.

Jian L.C., Lu C.F., Deng J.M. *et al*.: [Inducing factor and regulating role of intracellular Ca^{2+} level for woody plant bud dormancy.] – Chin. J. Appl. Environ. Biol. **10**: 1-6, 2004. [In Chinese]

Jiang D.A., Lu Q., Weng X.Y. *et al*.: [Regulation of Rubisco carboxylation activity and photosynthetic rate by Rubisco activase during leaf senescence in rice.] – J. Zhejiang Univ.-Sc A **26**: 119-124, 2000. [In Chinese]

Jiang D.A., Weng X.Y., Lu Q. *et al*.: [Effect of potassium on diurnal variations in P_N, Hill reaction and SOD activities in rice plant.] – Acta Phytophys. Sin. **22**: 87-93,1996. [In Chinese]

Jiao D.M., Ji B.H.: [Changes of the photosynthetic electron transport and photosynthetic enzyme activities of two rice varieties under photooxidation condition.] – Acta Agron. Sin. **2**: 43-49, 1996. [In Chinese]

Knott J.E.: Effect of a localized photoperiod on spinach. – P. Am.

Soc. Hortic. Sci. **31**: 152-154, 1934.
Kozlowski T.T., Pallardy S.G.: Acclimation and adaptive responses of woody plants to environmental stresses. – Bot. Rev. **68**: 270-334, 2002.
Li C.F., Li L.R.: [Comparison between the spectrophotometric method and ^{14}C-labelled method of measuring RuBPCase activity.] – Plant Physiol. Comm. **1**: 49-50, 1989. [In Chinese]
Li W.H., Lu Q.T., Hao N.B. *et al*.: [C_4 pathway enzymes in soybean leaves.] – Acta Bot. Sin. **43**: 805-808, 2001. [In Chinese]
Li X., Jiao D.M.: [Transgenic rice overexpressing C_4 photosynthetic genes and their application in breeding.] – Mol. Plant Breed. **3**: 550-556, 2005. [In Chinese]
Man W.Q., Du W.G., Hao N.B.: [Study on soybean breeding for high photosynthetic efficiency.] – Soybean Sci. **28**: 382-387, 2009. [In Chinese]
Niu S.L., Jiang G.M., Li Y.G.: [Environmental regulations of C_3 and C_4 plants.] – Acta Ecol. Sin. **24**: 308-314, 2004. [In Chinese]
Olsen, J.E.: Mechanisms of dormancy regulation. – Acta Hortic. **727**: 157-165, 2006.
Ramagli L.S., Rodriguez L.V.: Quantitation of microgram amounts of protein in two-dimensional polyacrylamide gel electrophoresis sample buffer. – Electrophoresis **6**: 559-563, 1985.
Rinne P.L.H., Kaikuranta P.M., van der Schoot C.: The shoot apical meristem restores its symplasmic organization during chilling-induced release from dormancy. – Plant J. **26**: 249-264, 2001.
Rohde A., Bastien C., Boerjan W., Thomas S.: Temperature signals contribute to the timing of photoperiodic growth cessation and bud set in poplar. – Tree Physiol. **31**: 472-482, 2011.
Rohde A., Bhalerao R.P.: Plant dormancy in the perennial context. – Trends Plant Sci. **12**: 217-223, 2007.
Rohde A., Prinsen E., De Rycke R. *et al*.: PtABI3 impinges on the growth and differentiation of embryonic leaves during bud set in poplar. – Plant Cell **14**: 1885-1901, 2002.
Shi J.N., Wu M.X., Cha J.J.: [Studies on plant phospho*enol*-pyruvate carboxylase – I. Separation and properties of PEP carboxylase isoenzymes.] – Acta Phytophysiol. Sin. **5**: 225-235, 1979. [In Chinese]
Stewart C.R., Martin B.A., Reding L., Cerwick S.: Respiration and alternative oxidase in corn seeding tissues during germination at different temperatures. – Plant Physiol. **92**: 755-760, 1990.
Sun L., Li S.C.: [Effects of UV-B stress on photosynthetic activity and antioxidase of *Chlorella vulgaris Antarctic* strain.] – J. Jiangxi Norm. Univ. **34**: 536-540, 2010. [In Chinese]
Sun S., Zhang L.T., Wang J.X. *et al*.: [Effects of low temperature and weak light on the functions of photosystem in *Prunus armeniaca* L. leaves in solar greenhouse.] – Chin. J. Appl. Ecol. **19**: 512-516, 2008. [In Chinese]
Sun Y.Y., Sun Y.J., Wu H.Z., Ma J.: [Effects of water stress on activities of nitrogen assimilation enzymes and photosynthetic characteristics of rice seedlings.] – J. Plant Nutr. Fert. **15**: 1016-1022, 2009. [In Chinese]
Ueno O., Samejima M., Muto S., Miyachi S.: Photosynthetic characteristics of an amphibious plant, *Eleocharis vivipara*: Expression of C_4 and C_3 modes in contrasting environments. – P. Natl. Acad. Sci. USA **85**: 6733-6737, 1988.
Wang H.B., Wang X.D., Cheng C.G. *et al*.: [Natural inducing factors of peach bud dormancy and roles of Ca^{2+} in the dormancy induction.] – Chin. J. Appl. Ecol. **19**: 2333-2338, 2008. [In Chinese]
Wang X.X., Liu L., Zhang J. *et al*.: [Changes of photosynthetic pigment and photosynthetic enzyme activity in stems of *Phyllostachys pubescens* during rapid growth stage after shooting.] – Chin. J. Plant Ecol. **36**: 456-462, 2012. [In Chinese]
Wei A.L., Wang Z.M., Zhai Z.X., Gong Y.S.: [Effect of soil drought on C_4 photosynthesis enzyme activities of flag leaf and ear in wheat.] – Sci. Agric. Sin. **36**: 508-512, 2003. [In Chinese]
Whitelam G.C., Devlin P.F.: Roles of different phytochromes in *Arabidopsis photomorphogenesis*. – Plant Cell Environ. **20**: 752-758, 1997.
Xu D.Q.: [Some problems in stomatal limitation analysis of photosynthesis.] – Plant Physiol. Comm. **33**: 241-244, 1997. [In Chinese]
Xu X.L., Wang Z.M., Zhang J.P.: [Effect of heat stress on photosynthetic characteristics of different green organs of winter wheat during grain-filling stage.] – Acta Bot. Sin. **43**: 571-577, 2001. [In Chinese]
Zhang H.X., Wu N.B., Hu L.T., Hong H.: [The effects of different treatments with UV-B radiation stress on seedling growth and character of chlorophyll fluorescence of *Vicia faba* L.] – J. Southwest China Nor. Univ. **35**: 105-110, 2010. [In Chinese]
Zhang Z.S., Zhang L.T., Gao H.Y. *et al*.: [Research of the photoinhibition of PSI and PSII in leaves of cucumber under chilling stress combined with different light intensities.] – Acta Agric. Sin. **42**: 4288-4293, 2009. [In Chinese]