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BRIEF COMMUNICATION

Effect of heat stress during grain filling on phospho*enol*pyruvate carboxylase and ribulose-1,5-bisphosphate carboxylase/oxygenase activities of various green organs in winter wheat

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Abstract

In two winter wheat (*Triticum aestivum* L.) cultivars differing in their response to high temperature, JD8 (tolerant) and J411 (sensitive) we studied the effect of heat stress on the activities of phospho*enol*pyruvate carboxylase (PEPC) and ribulose-1,5-bisphosphate carboxylase (RuBPC) in green organs during grain-filling. There were significantly higher PEPC activities and lower RuBPC activities in each of the non-leaf organs (awn, glume, lemma, peduncle, and sheath) than in the flag leaf blade. Under heat stress for 12 d, the activity of RuBPC quickly declined and the activity of PEPC first increased and later declined in all organs, resulting in a great increase of the PEPC/RuBPC ratios in the organs, particularly in non-leaf organs which had a higher PEPC/RuBPC than the flag leaf blade in all times. The PEPC activity and PEPC/RuBPC ratio in every organ of JD8 were higher than those in the same organ of J411. Thus the differences in PEPC activities and PEPC/RuBPC may be associated with the differences in photosynthetic heat tolerance among the organs of the same plant or between the two cultivars.

Additonal key words: awn; flag leaf; glume; lemma; peduncle; sheath; *Triticum aestivum.*

In most Northern regions of China, the day temperatures during grain filling of wheat are often over 30 °C, significantly higher than the optimum leaf photosynthesis temperature (*ca*. 23–25 °C), which has become a key restraint for further increase in grain yield (Zou 1988). Effect of heat stress on wheat photosynthesis has often been studied in leaf blades (Anil *et al.* 1986, Al-Khatib and Paulsen 1990, Scott *et al.* 1990, Mohanty 2003), but only rarely in other green organs (Blum 1986). The contribution of ear photosynthesis to grain mass varies between 10–44 % (Evans *et al.* 1975) and photosynthesis of peduncle and sheath is very important to grain filling (Wang *et al.* 2001). In the previous paper (Xu *et al.* 2001) we reported that the heat tolerance of the non-leaf green organs was stronger than that of flag leaf blade. During heat stress, the declining extent of net photosynthetic rate (P_N) in ear was smaller than that in flag leaf blade, and P_N values of peduncle and sheath were relatively stable, too. Thus there is a large difference in photosynthetic adaptability to high temperature between non-leaf organs and leaf blades, but the mechanism of this difference is little known. We presumed that the difference in photosynthetic adaptability to high temperature might relate to the difference in C_4 photosynthesis enzyme activity among organs (Xu *et al.* 2001). For examining this assumption, we investigated the activities of PEPC and RuBPC in various green organs of wheat under normal and high temperature environments during grain filling.

Two winter wheat (*Triticum aestivum* L.) cultivars differing in their response to high temperature, JD8 (tolerant) and J411 (sensitive), were planted in the experimental field of China Agriculture University in Beijing, China. At anthesis the plants of similar growth status were marked in plots of each cultivar. In order to simulate high temperature stress, transparent plastic sheds were set in the treatment plots of the two cvs. on the 12th day after anthesis (cf. Xu *et al.* 2001). The duration of high temperature treatment was 12 d. Temperature inside and outside the sheds was observed 7 times every day. The highest and lowest temperatures in treatment plots between 09:00 and 18:00 were 34±2.2 and 30±1.0 °C, respectively. There was a 3–5 °C difference in mean day temperature

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and no difference in the lowest night temperature (17±1.0 °C) between treated and untreated (control) plots. Irradiance was similar for treatment and control. At 3 d before heat treatment all plots were irrigated to ensure plants do not suffer drought stress. The sampling time was 0, 3, 8, and 12 d after start of high temperature treatment, *i.e*. 12, 15, 20, and 24 d after anthesis, respectively. The green organs, *i.e*. flag leaf blade, sheath, peduncle, glume, lemma, and awn, were sampled and investigated for PEPC and RuBPC activities.

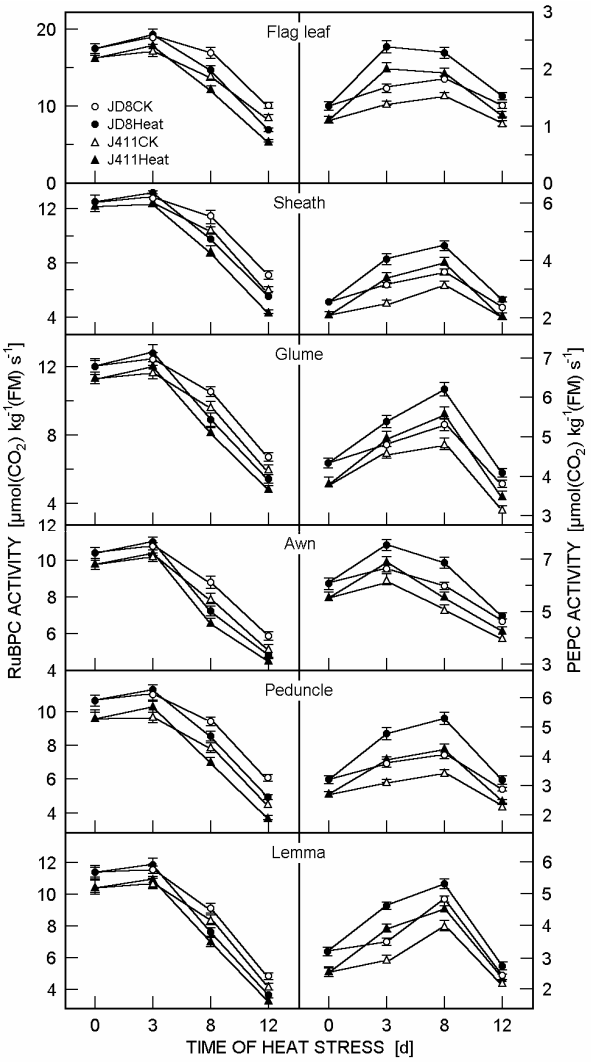

Fig. 1. RuBPC (*left*) and PEPC (*right*) activities in flag leaf blade, sheath, glume, awn, peduncle, and lemma of wheat plants under normal (CK) and heat stress (HS) environments (n = 3).

The extraction of enzyme was carried out according to Sayre *et al*. (1979) with slight modifications. Green tissue (0.2 g) was ground with a mortar and pestle (2 °C) containing a small amount of sand and 3.0 cm^3 of grinding media consisting of 0.1 M Tris-HCl (pH 7.8), 100 mM $MgCl_2$, 1 mM EDTA, 20 mM mercaptoethanol, 100 kg m^{-3} glycerin, and 10 kg m^{-3} polyvinylpyrrolidone. Following a centrifugation at 15 000×g for 10 min at 4 °C, the supernatant was used for enzyme assay.

RuBPC activity was assayed by the method of Camp *et al*. (1982). Enzyme extract was added to a reaction mixture that contained 50 mM Tricine-NaOH (pH 7.9), 10 mM KCl, 1 mM EDTA, 2 mM dithiothreitol (DTT), 0.2 mM NADH, 5 mM ATP, 15 mM $MgCl_2$, 10 mM $NaHCO_3$, 5 mM phosphocreatin, 2 units per cm^3 creatine phosphokinase, 4 units per cm^3 each of NAD-dependent glyceraldehyde-3-P dehydrogenase and 3-P-glycerate kinase in a final volume of 1 cm^3. It was incubated at 25 °C for 5 min. Reactions were initiated by addition of 0.5 mM RuBP.

PEPC activity was measured spectrophotometrically at 340 nm using a *Varian 100 UV* spectrophotometer and by coupling the PEP reaction to the oxidation of NADH with malate dehydrogenase (MDH) according to Blanke and Ebert (1992). The enzyme extract was added to a solution, which in 1 cm^3 of final volume contained 50 mM Tris-HCl (pH 7.8), 10 mM $MgCl_2$, 0.25 mM EDTA, 5.0 mM $NaHCO_3$, 2.0 mM DTT, 4 unit MDH, 0.1 mM NADH, and 2.0 mM PEP. The reaction was started by the addition of tissue extract.

The assay of RuBPC activity of various organs in the two cultivars showed (Fig. 1) that the enzyme activities of control plants were maintained relatively high during 12–15 d after anthesis and then declined. The enzyme activities of various organs were high to low in the order of flag leaf blade, sheath, glum, lemma, awn, and peduncle, and the enzyme activity in flag leaf blade was significantly higher than that in the non-leaf organs. The trend in variation of RuBPC activity in each organ was not affected by high temperature treatment during the grain filling, but the declining rates of enzyme activities in the different organs were significantly higher in high temperature treatment plants than in control plants. RuBPC activities in all organs were higher in JD8 than in J411, while under high temperature the declining RuBPC activities in the organs were relatively lower in JD8 than in J411.

PEPC activity was found in all green organs of the two cvs. (Fig. 1). In the control during grain filling stage, PEPC activities in various organs were high to low in the order awn, glum, lemma, peduncle, sheath, and flag leaf blade, and the value of PEPC activity in flag leaf blade was significantly lower than the value in each non-leaf organ. Under high temperature treatment, PEPC activity of every organ in the treated plants obviously increased to an optimum between days 3 and 8, and was significantly higher than that in control plants. Twelve days after high temperature treatment, PEPC activities of the treated plants swiftly declined, but there were no significant differences between treated and untreated plants. Under both normal and high temperature, JD8 showed relatively higher PEPC activities in all organs than J411.

The ratio of PEPC/RuBPC activities may reflect a relation of activities of C_4 pathway enzyme to C_3 pathway enzyme in CO_2 fixation. This ratio (Table 1) of each

organ tended to increase with grain growth. In the green organs, PEPC/RuBPC was high to low in the order awn, glume, lemma, peduncle, sheath, and flag leaf blade, and the ratio in each non-leaf organ was significantly higher than that in flag leaf blade. It may indicate that the relative role of C_4 photosynthetic function is more important in non-leaf organs, especially in ears, than in the flag leaf blade. Compared with control, high temperature treatment significantly increased the PEPC/RuBPC ratio of each organ in the two cvs. and with increasing heat treatment duration the organ PEPC/RuBPC trended to increase more. PEPC/RuBPC ratios in all non-leaf organs were higher in JD8 than in J411. Nevertheless, in wheat cultivars also the contents of small and large subunits of RuBPCO differ (Muranaka *et al*. 2002) which may also be reflected in the final enzyme activity.

During the grain filling of wheat, heat stress reduces P_N and leads to the decrease of grain mass (Scott *et al.* 1990, Xu *et al.* 2001). However, there are remarkable differences in heat stress sensitivity among green organs of wheat. Blum (1986) showed that awns maintained a higher optimum temperature for photosynthesis (32 °C) than flag leaves (25 °C). Xu *et al*. (2001) reported that under heat stress the decrease of photosynthetic activity in the flag leaf blade was significant, while in the ear and other non-leaf organs the activity was fairly constant. Apparently, the thermotolerance of non-leaf organs was stronger than that of the flag leaf blade. The difference in photosynthetic thermotolerance among organs may be explained by the difference in biochemistry of photosynthesis (Blum 1986).

Table 1. PEPC/RuBPC ratios of various green organs in tolerant (JD8) and sensitive (J411) wheat cultivars under heat stress. Values in parentheses are PEPC/RuBPC ratios of the various green organs under normal (CK) environment.

Cv.	Organ	Duration of heat stress			
		0 d	3 d	8 d	12 d
J411	Awn	0.53	0.63 (0.57)	0.79 (0.60)	0.88 (0.71)
	Glume	0.34	0.41 (0.40)	0.68 (0.50)	0.73 (0.53)
	Peduncle	0.28	0.38 (0.32)	0.59 (0.44)	0.67 (0.50)
	Lemma	0.24	0.36 (0.27)	0.65 (0.47)	0.66 (0.49)
	Sheath	0.17	0.27 (0.19)	0.44 (0.30)	0.46 (0.31)
	Flag leaf blade	0.07	0.12 (0.09)	0.17 (0.12)	0.23 (0.13)
JD8	Awn	0.55	0.65 (0.58)	0.89 (0.63)	0.93 (0.73)
	Glume	0.36	0.42 (0.38)	0.70 (0.50)	0.76 (0.57)
	Peduncle	0.29	0.43 (0.34)	0.63 (0.46)	0.68 (0.47)
	Lemma	0.27	0.39 (0.29)	0.70 (0.52)	0.69 (0.47)
	Sheath	0.20	0.31 (0.24)	0.46 (0.31)	0.47 (0.32)
	Flag leaf blade	0.08	0.14 (0.09)	0.17 (0.12)	0.24 (0.14)

PEPC and other C_4 photosynthetic enzyme systems are active in ears of wheat, and the photosynthetic metabolism of ears has at least some characters of C_4 pathway or C_3-C_4 intermediate (Singal *et al.* 1986, Bort *et al.* 1996, Li and Hao 1999). Our present results indicated again presence of PEPC in all green organs of wheat. Furthermore, this activity was significantly higher in each non-leaf organ (ear, sheath, peduncle) than in the flag leaf blade, and so was the PEPC/RuBPC ratio. With grain filling, the PEPC/RuBPC ratios in all organs tended to increase. The activity was relatively higher in non-leaf organs than in leaf blades, and higher in the later phase than in the earlier phase during grain filling. As the thermotolerance of PEPC is stronger than that of RuBPC, and the photosynthetic optimum temperature of C_4 pathway is obviously higher than that of C_3 pathway (Pearcy and Ehleringer 1984), the difference in the PEPC activity may result in the difference in the photosynthetic thermotolerance among the green organs. The higher PEPC activities in non-leaf organs may account for their stronger photosynthetic thermotolerance relative to leaf blades. During the late stage of grain filling, due to swift senescence of leaf blades and rapid decrease in RuBPC activity, the carbon assimilation functions and thermotolerance of non-leaf organs may maintain a fairly stable photosynthetic productivity in the whole wheat plant.

Under high temperature stress RuBPC activity in every organ swiftly decreased, while the organ PEPC activity of the treated plants obviously increased and was significantly higher than in the control plants. A short period of heat stress may induce the increase of PEPC activity in the organs. The different responses of PEPC and RuBPC to high temperature resulted in the increase of PEPC/RuBPC ratio in the each organ of treated plants, which may be related to the adaptability of plants to heat stress. Jiao and Ji (1996) also showed in rice that the leaf PEPC activity followed an induced increase under photooxidation condition. Probably, the induced increase of PEPC activity in the C_3 plants may partially compensate for the negative effects to photosynthesis caused by the

rapid decrease of RuBPC activity under stress. In wheat, this compensation function could be an important mechanism of photosynthetic thermotolerance in the green non-leaf organs, which had higher PEPC activity and PEPC/RuBPC ratio than the leaf blades.

The present results also showed that both PEPC activity and PEPC/RuBPC of each organ were higher in the heat tolerant cultivar JD8 than in the heat sensitive J411, therefore the high PEPC activity in the whole plants, especially in non-leaf organs, may be an important biochemical characteristic of the cvs. of high photosynthetic efficiency and heat tolerance.

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