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Diurnal Changes of Phosphoenolpyruvate Carboxylase Activity in Leaves of Field-grown Maize Introduced into Tibetan Plateau*

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Abstract In the day course of photosynthesis of maize introduced into Tibetan Plateau, during its jointing stage, the phosphoenolpyruvate carboxylase (PEPC) activity in photosynthetic leaves undulated more gently and was always higher than the net photosynthetic rate (P_n) at every time point. By studying the variation of difference between P_n and PEPC activity throughout the day, the influence of environmental factors (e. g. light intensity and ambient temperature) and stomatal status on photosynthesis was analysed.

Key words Maize; Tibetan Plateau; Phosphoenolpyruvate carboxylase

引种到青藏高原大田的玉米叶片中磷酸烯醇式丙酮酸羧化酶活性的日变化

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摘 要 引种到青藏高原大田的玉米, 其拔节期的全天光合进程中, 叶片中磷酸烯醇式丙酮酸羧化酶 (PEPC) 活性总是大于相应时间点的净光合速率 (P_n), 且全天变化幅度较 P_n 缓和。通过研究 PEPC 活性和 P_n 之间差异的全天变化, 分析了环境因子 (如光强、气温) 和气孔状态对光合作用的影响。

关键词 玉米; 青藏高原; 磷酸烯醇式丙酮酸羧化酶
中图分类号: Q 945. 11 **文献标识码**: A

Definition of the limiting factors in the photosynthetic process is a prerequisite to genetically increasing the photosynthetic rate of crop species[1]. And it is also favorable to increase crop yield agronomically because photosynthetic rate is often the primary limitation for high yield under field conditions[2, 3]. So Gifford *et al.* proposed that improvement in the maximum rate of leaf photosynthesis may become essential to further increase in yield potential[4].

Under field environmental conditions, photosynthesis of leaves is always the result of complex diverse factors and reactions, such as CO_2 concentration (ambient and or intercellular), ambient temperature, light intensity, the activity of carboxylase and the capacity of electron transport etc. Furthermore, P_n (net photosynthetic rate) is indirectly associated with leaf water potential, leaf age and available soil nutrients as well[5]. So in order to improve crop management, it would be useful to understand the factors that limit photosynthesis in the field conditions.

It is well established that leaf Kranz anatomy and PEPC contribute to high photosynthetic ability

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in C_4 plants, including maize[6]. High activity of PEPC (phosphoenolpyruvate carboxylase) is an important necessary factor to maintain high photosynthetic rate in C_4 plants besides other ecological or/and physiological factors. By analyzing the differences between PEPC activity and photosynthetic rate over the day, it is feasible to study which factor(s) should be responsible for the diurnal changes of photosynthesis in field-grown maize which was introduced from low elevation plain to the Tibetan Plateau.

1 Materials and Methods

1.1 Plant material and growing conditions; Gas exchange measurements

These items were exactly same as what were reported in our former paper[7].

1.2 Assay of enzyme activity

1.2.1 Extraction of enzyme

The upper parts of three leaves on which gas exchange measurements were made at every time point were immediately excised and frozen in liquid N_2.

PEPC (EC4.1.1.31) was extracted by a modification of the method described by Hague and Sims[8]. Frozen leaf material (5g) was ground in 10mL ice-cold extraction solution [Tris-H_2SO_4 (0.1mol/L, pH8.0), containing 5% glycerol (v/v), 1% PVP (w/v), DTT (7mmol/L), EDTA (1mmol/L)] in a cold glass mortar. The homogenate was filtered through four layers of cheesecloth and centrifuged at 20000 × *g* for 15min, and the supernatant was measured for enzyme activity as soon as possible. All procedures were carried out at 4℃.

1.2.2 Enzyme activity assay

The activity of PEPC was assayed by measuring the decrease in A_{340nm} at 25℃ using a WFZ800-D3A digital spectrophotometer (Beijing, China). According to some researchers[9, 10, 11], the assay system contained (in μmol): Tris-HCl, pH 8.0 (100), PEP (sodium salt, 1.5), NADH (disodium salt, 0.17), $NaHCO_3$ (20), $MgCl_2$ (20), 20 units of malate dehydrogenase, an appropriate volume of enzyme extraction above and distilled water in a total volume of 3mL. The unit of PEPC activity was calculated as $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$, based on the estimate that 1g fresh weight of frozen leaf meterial was equivalent to $2.5 \times 10^{-3}\ m^2$ of leaf area.

2 Results and discussion

2.1 Diurnal trends of P_n and PEPC activity

As shown in Fig.1, P_n of maize leaves increased from 2.45 to 35.83 ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) over the period from 8:00 to 13:00; then fluctuated slightly from 13:00 to 19:00. After 19:00, P_n declined markedly. The maximum P_n (39.03 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) occurred at 15:00. These indicated obviously that the P_n vs time curve had a single peak[7]. While the activity of PEPC increased from 26.58 to 46.52 ($\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) from 8:00 to 13:00. The maximum activity of PEPC (46.95 $\mu mol\ CO_2 \cdot m^{-2} \cdot s^{-1}$) also occurred at 15:00. Then PEPC activity leveled off and fell gradually from 15:00 to 21:00. Apparently, the diurnal variation of PEPC activity exhibited a similar but gentle trend compared to that of leaf P_n.

Comparing the trends of PEPC activity and P_n (Fig.1), it was interestingly found that fluctuation of PEPC activity was clearly less than that of P_n, probably meaning that P_n should be associated

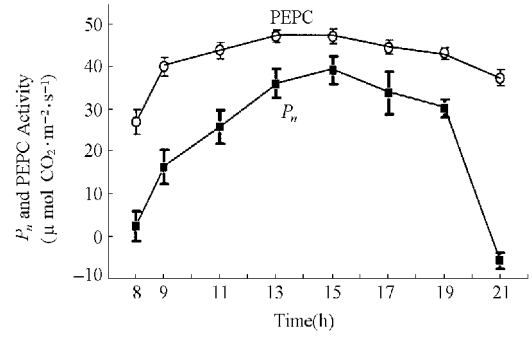

Fig.1 Diurnal changes of P_n and PEPC activity. Every point stands for the mean of 15 (for P_n) or 3 (for PEPC activity) values from three leaves. Vertical bars indicate the standard errors (P_n, net photosynthetic rate; PEPC, phosphoenolpyruvate carboxylase.)

with and more sensitive to more endogenous and exogenous factors than PEPC. Furthermore, PEPC activity always exceeded P_n throughout the day, as was similarly reported in ribulose 1, 5-bisphosphate carboxylase study[12]. It is known that the substrate for PEPC is HCO_3^-, rather than CO_2 because PEPC does not contain biotin[13]. And P_n was measured at air level of CO_2, while PEPC activity was measured at saturating HCO_3^- whose concentration was greater than 1000 mol/L. Thus measured PEPC activity in fact indicated the potential capacity which photosynthetic leaves could reach under certain optimal conditions theoretically. From this viewpoint, we could propose that P_n of introduced maize leaves still have some space to be increased.

2.2 Differences between PEPC activity and P_n

We could reasonably suppose that the difference between P_n and PEPC activity would be constant if the influence of each factor associated with photosynthesis would not vary throughout the day. In fact, the difference between P_n and PEPC activity exhibited some variations over the day as shown in Fig. 2.

As shown in Fig. 2, nearly constant differences form 8: 00 to 9: 00 mean that the increase of PEPC activity should be chiefly responsible for that of P_n, while other factors be secondary. It has been reported that photosynthetic PEPC activity was subject to reversible light activation by protein phosphorylation in maize leaves[11, 14, 15]. So it should be reasonably concluded that increasing PAR (Fig. 3) was the ultimate impetus of increasing P_n in this period.

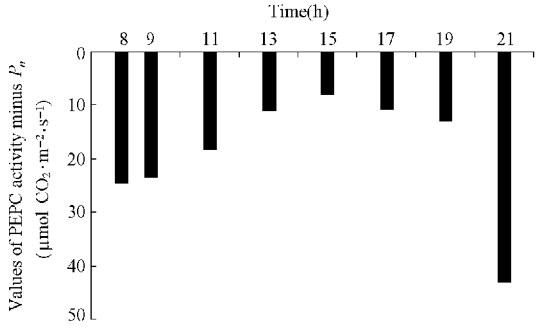

Fig. 2 The difference values between the means of P_n and of PEPC activity throughout the day. (P_n, net photosynthetic rate; PEPC, phosphoenolpyruvate carboxylase.)

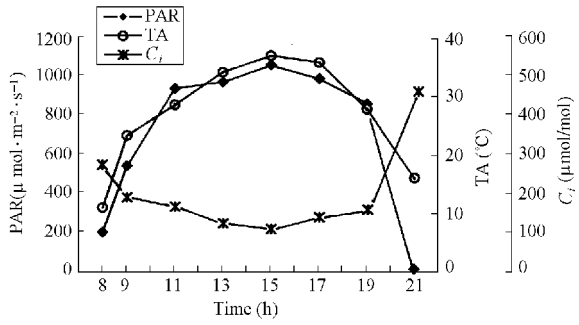

Fig. 3 Diurnal variations of photosynthetically active radiance (PAR), ambient temperature (TA) and intercellular CO_2 concentration (Ci). (These data were cited from the paper of Yang *et al* [7])

Form 9: 00 to 15: 00, due to that declining C_i was disadvantageous to P_n (Fig. 3), the increasing of P_n (Fig. 1) and gradual decrease of differences (Fig. 2) suggested that factors other than PEPC activity should play more important parts to increase P_n and the most possible ones were PAR and ambient temperature. Especially at 15: 00 when C_i was at its minimum, the P_n exhibited its maximum. This means that the other factors except C_i must be in an optimal situation for leaf photosynthesis. Therefore, we suggested that supplying CO_2 fertilizer of certain content to maize community should be efficient to promote P_n further.

As shown in Fig. 1, 2 and 3, P_n declined gently and the differences gradually became larger despite the high level of PEPC activity and increasing C_i from 15: 00 to 19: 00. Besides decreasing of PAR and ambient temperature, the accumulation of photosynthetic product (e. g. soluble carbohydrate) would also contribute to this trend[16], though there existed different views[17, 18].

After 19: 00, according to the criterion suggested by Farquhar and Sharkey[19], the stomatal factor was not the reason for P_n decreasing because of increasing of C_i. Considering the high PEPC activity, the factor which should be responsible for decreasing of P_n was the sharp decreasing PAR (Fig. 3), because enzyme reactions of CO_2 assimilation require assimilatory power (ATP and

NADPH) from electron transport, which in turn depends on absorbed irradiance[5].

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