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Reduced activity of glycine decarboxylase: effects on photosynthesis and respiration in potato leaves

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Introduction

Potato, the typical C3 plant loses almost 15% of the assimilated CO_2 by decarboxylation of glycine in the glycolate cycle. Glycine decarboxylase (GDC, EC 2.1.2.10) is an enzyme catalyzing the conversion of two molecules of glycine to serine with concomitant evolution of one molecule of CO_2 as the source of photorespiration. With the aim to reduce photorespiration and increase photosynthetic productivity the transformants of potato with suppressed expression of GDC have been constructed (Bauwe, 1998). In this study components of photosynthetic and respiratory CO_2 exchange in photosynthesizing leaves of potato control plants and of its transformants were determined.

Materials and methods

Tubers of potato control plants (*Solanum tuberosum* L., var. Desireé) and transformants P1 and P15 with 50-75% decreased activity of GDC were obtained from Dr. Hermann Bauwe. Plants were grown in soil under combined illumination of high pressure sodium discharge lamp LU400/HO/T/40NG (LUCALOX, Hungary) and of high pressure mercury-vapour fluorescent lamp LRF 250W E40 (POLAMP, Poland) at the following conditions: irradiance 250-300 µmol m^{-2} s^{-1}, 12h/12h light/dark, day/night temperature 22/16°C. Eight week old control plants and ten week old plants of transformants were used in experiments which were performed on fully expanded leaves of upper levels under normal conditions: CO_2 – 370 µL L^{-1}, O_2 – 210 mL L^{-1}, PPFD – 750 µmol m^{-2} s^{-1}, T - 25ºC. Using a radiogasometric method (Pärnik, Keerberg, 1995) the following characteristics of photosynthetic and respiratory CO_2 exchange were determined: rates of net and true photosynthesis, rate of photorespiration, rate of respiration in the light, rate of respiration in the dark, rates of carboxylation and oxygenation of ribulose-1,5-bisphosphate (RuBP) and specificity factor of Rubisco. Amino acids were measured by means of the analyzer AAA T339 (MIKROTECHNA, Praha).

Results

The rate of true photosynthesis was highest in leaves of control plants (10.8 µmoles CO_2 m^{-2} s^{-1}). In transformant P15 it was about 2 times lower, P1 showed an intermediate value. The same relationship was found for the total rate of intracellular decarboxylation (photorespiration+respiration) in the light: 1.8, 1.0

and 0.8 μmoles $CO_2\ m^{-2}\ s^{-1}$ in leaves of control plants, P1 and P15, respectively. Potential photosynthesis P_P, measured under nonphotorespiratory conditions, was decreased in P1 1.6 and in P15 5 times (Table 1).

Table 1. Rates of true photosynthesis $\mathbf{P_T}$ and respiratory CO_2 fluxes in leaves of potato control plants and transformants **P1** and **P15** under normal conditions. Potential photosynthesis $\mathbf{P_P}$ was measured under 600 μL L^{-1} CO_2 and 15 mL L^{-1} O_2.

			Photorespiration	Respiration	
	P_T	P_P		In the light	In the dark
			μmol $CO_2.m^{-2}.s^{-1}$		
Control (Desiree)	10.7 ± 0.5	26.4 ± 1.7	1.56 ± 0.18	0.21 ± 0.09	1.37 ± 0.12
P1	7.1 ± 0.4	16.4 ± 0.8	0.69 ± 0.21	0.33 ± 0.12	0.90 ± 0.05
P15	4.7 ± 0.2	5.3 ± 1.2	0.29 ± 0.14	0.54 ± 0.11	0.88 ± 0.04

No differences were detected in the relative rate of total intracellular decarboxylation of control plants and transformants. Lower photorespiration in transformants was compensated by higher respiration of plants. (Table 2).

Table 2. Components of respiration in leaves of potato control plants and transformants **P1** and **P15** under normal conditions in the light.

	Control plant	P1	P15
	% of true photosynthesis		
Photorespiratory decarboxylation:			
t o t a l	**14.6 ±1.2**	**9.7 ±2.5**	**6.2 ±2.5**
primary photosynthates	12.4 ±1.0	8.2 ±2.4	5.2 ±2.5
stored photosynthates	2.2 ±0.7	1.5 ±0.6	1.0 ± 0.5
Respiratory decarboxylation:			
t o t a l	**2.0 ±0.7**	**4.6 ±1.4**	**11.4 ±2.2**
primary photosynthates	1.7 ±0.6	3.9 ±1.2	9.7 ±2.1
stored photosynthates	0.3 ± 0.1	0.7 ±0.6	1.7 ± 0.3

In all measurements the main substrates of decarboxylation were primary photosynthates, about 14% the true photosynthesis. The contribution of stored photosynthates was in medium 2.5% (Table 2).

The specificity of Rubisco was determined in attached leaves and calculated from the ratio of rates of carboxylation and oxygenation of RuBP (P_C /P_O) according to the formula:

$$S = P_C [O_2]_W / P_O [CO_2]_W,$$

where $[O_2]_W$ and $[CO_2]_W$ are oxygen and CO_2 concentrations in liquid phase at mesophyll cell walls.

Values of S (control - 104.0 ±5.3; P1 - 143.9 ± 9.9; P15 - 120 ± 6.2) are significantly higher in transformants than in control plants. No differences in the Rubisco specificity were found in the measurements *in vitro* (H.Bauwe, personal communication). To explain this disagreement it must be taken into account that the measurements *in vivo* are based on the determination of the rate of RuBP oxygenation from the stoichiometry of the glycolate cycle assuming that 2 oxygenation reactions are needed for the production of 1 molecule of CO_2 in the reaction of glycine decarboxylation. Apparently this assumption is not valid for the transformants. It means that in transformants a portion of glycine is not decarboxylated by GDC but transported out of the glycolate cycle resulting in a

change of the stoichiometry between RuBP oxygenation and photorespiration. This is consistent with the higher content of the nonprotein glycine in transformants as compared to control plants. During illumination of plants from 9.00 to 16.00 (7 hours) the content of glycine in leaves of transformants enhanced 3 times up to 6 mmol m^{-2} (Fig.1). At this time concentration of glycine in control leaves was about 100 times lower - 0.06 mmol m^{-2} (Fig.2).

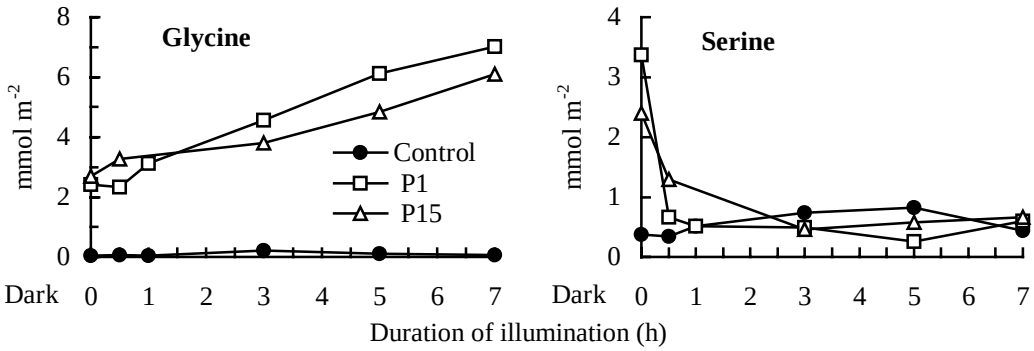

Fig.1. Content of nonprotein glycine and serine in leaves of potato plants and transformants P1 and P15 as function of duration of illumination.

During the night a bulk of glycine was converted to serine which was quickly metabolized via the glycolate cycle after switching on the light in the next morning (Fig.1). Steady state level of serine was almost the same for control plants and transformants P1 and P15 (Fig.1,2).
To study the influence of the high level of glycine concentration on plant growth control plants and transformant P1 were illuminated continuously during 10 days. Plants survived this period but growth of transformants P1 was strongly suppressed. Amino acids content was measured after 10 days of continuous illumination. Glycine concentration in P1 leaves was somewhat higher than after 7 hours illumination. Serine level was almost the same. In control plants the content of glycine and serine did not changed during 10 days continuous illumination (Fig.2).

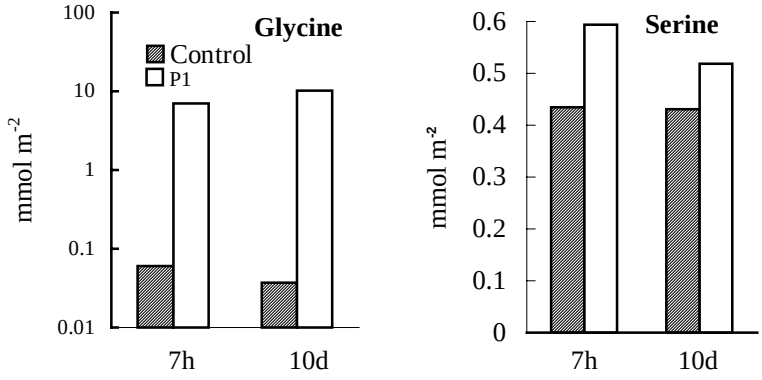

Fig.2. Content of nonprotein glycine and serine in leaves of potato control plants and transformant P1 after short-term (7h) and long-term (10 days) continuous illumination.

Discussion

In barley leaves with reduced activity of GDC (63-47% of wild type) photosynthesis was suppressed only in photorespiratory conditions where incorporation of glycerate-3-phosphate into RPPC (reductive pentose phosphate cycle) for RuBP regeneration was reduced (Wingler et.al., 1997). In potato transformants with reduced activity of GDC (50-25% of control) photosynthesis was suppressed also in non-photorespiratory conditions (Table 1). This suppression may be caused by lower content of Rubisco or by reduced rate of RuBP regeneration.

A compensatory relationship between photorespiration and respiration in the light was found – decrease of the first results in an increase of the other (Table 2). It means that transformants don't have any advantage with respect to respiratory losses during photosynthesis in the light. Respiration in potato control plants and transformants P1 and P15 was suppressed by light. In transformants with lower photorespiration the extent of light suppression was smaller than in control plants. This is consistent with our previous findings that respiration was not inhibited in nonphotorespiratory conditions at high CO_2 (Pärnik et. al., 1998).

Enhanced values of specificity of Rubisco of transformants and high glycine content in leaves suggest that in transformants a portion of glycine is not decarboxylated by GDC but transported out of the glycolate cycle. It was shown that the superfluous glycine was not transported out of leaves to tubers. After 4 h exposure of a leaf to $^{14}CO_2$ about 84% of radioactivity was found in the exposed leaf, 4% in other leaves, 12 % in stem and roots and only traces of radioactivity in tubers.

Apparently a portion of accumulated glycine in P1 and P15 was consumed in respiratory processes. It may explain the fact that transformants with impaired ability to decarboxylate glycine by GDC survived the 10 day continuous illumination.

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