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Photosynthetic activity of ripening tomato fruit

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Abstract

Gas exchanges, chlorophyll (Chl) *a* fluorescence and carboxylation activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and phosphoenolpyruvate carboxylase (PEPC) were determined in tomato (*Lycopersicon esculentum* Mill.) fruits picked at different developmental stages (immature, red-turning, mature, and over-ripe). The fruits did not show signs of CO_2 fixation. However, photochemical activity was detectable and an effective electron transport was observed, the values of Chl fluorescence parameters in green fruits being similar to those determined in the leaves. The RuBPCO activity, which was similar to those recorded in the leaves at the immature stage of the fruit, decreased as the fruit ripened. PEPC activity was always higher than RuBPCO activity.

Additional key words: chlorophyll fluorescence; fruit photosynthesis; gas exchanges; *Lycopersicon esculentum;* phosphoenolpyruvate carboxylase; ribulose-1,5-bisphosphate carboxylase/oxygenase; transpiration.

Introduction

Immature tomato fruits contain chlorophyll (Chl) and are able to fix CO_2 (Hetherington *et al.* 1998, Smillie *et al.* 1999); however, no net CO_2 assimilation is evident at any stage of development from fruit set to maturation. The developing fruits must therefore be largely non-autotrophic and import most of their carbon from the adjacent leaves where CO_2 assimilation occurs *via* ribulose 1,5-bisphosphate carboxylase (RuBPCO) and where sucrose is synthesised for translocation. With respect to the leaves, fruits fix very little ambient CO_2 *via* RuBPCO, the activity of which is much lower than that of the phosphoenolpyruvate carboxylase (PEPC) (Blanke and Lenz 1989).

Fruit photosynthesis was thought to resemble that of C4 and CAM species. Nevertheless, fruits do not have a well developed Kranz anatomy nor do they display important pH fluctuations during the day/night cycle, thus suggesting that fruit photosynthesis cannot be ascribed to the C4 or CAM mechanisms (for review see Blanke and Lenz 1989). The mechanisms of fruit photosynthesis therefore need further elucidation.

In order to contribute to understanding the machinery of CO_2 fixation and re-fixation in developing fruits of C3 species, gas exchanges, Chl *a* fluorescence, and carboxylation activities of RuBPCO and PEPC were determined in tomato (*Lycopersicon esculentum* Mill.) fruits picked at different developmental stages (immature, red-turning, mature, and over-ripe).

Materials and methods

Plants: Tomato plants (*Lycopersicon esculentum* Mill. var. Crimson) were grown in pots and supplied with complete nutrient solution (Hoagland's solution). The experiments were carried out twice in a greenhouse under natural conditions during late spring-early summer. Minimum and maximum air temperatures were 25 and 30-32 °C, respectively, with maximum irradiation around 900 μmol m^{-2} s^{-1} (PAR).

Gas exchange, Chl *a* fluorescence, pigment concentration, and carboxylation activities of RuBPCO and PEPC were measured on fruits picked at different developmental stages, *i.e.*, immature (I), red-turning (R),

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Abbreviations: Chl, chlorophyll; F_m, maximal fluorescence; F_v, variable fluorescence; F_0, ground fluorescence; IRGA, infrared gas analyser; PAR, photosynthetically active radiation; PS2, photosystem 2.

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mature (M), and over-ripe (O). The above parameters were also analysed on mature leaves.

Fruit gas exchange was measured in the laboratory using a temperature-controlled assimilation chamber connected to an IRGA in an open gas exchange system (*H. Walz*, Effeltrich, Germany) following the procedures reported by Nali *et al.* (1998) with the necessary modifications for fruit measurements. Measurements were made on the shoulder midway between the proximal end of the fruit and its equator.

Fruit Chl *a* fluorescence was measured using a pulse amplitude modulation fluorometer (*PAM-2000, H. Walz*, Effeltrich, Germany) as reported by Guidi *et al.* (1997). Fruit samples were kept in darkness for 40 min, following which the ground (F_0) and maximum fluorescence (F_m) were determined. The experimental material was then irradiated by 200 μmol m^{-2} s^{-1} PAR until in about 20 min a steady-state fluorescence (F) was reached. F_m' was determined after brief saturating irradiation (8 000 μmol m^{-2} s^{-1} for 0.8 s) and F_0' was detected after turning off the actinic radiation and far-red irradiating for 5 s.

The actual efficiency of photosystem 2 (PS2) was measured as (F_m' – F)/F_m' (Genty *et al.* 1989). The photochemical quenching coefficient (q_P) was determined as (F_m' – F)/(F_m' – F_0') and the non-photochemical quenching coefficient (q_{NP}) as (F_m – F_m')/(F_m – F_0') (Schreiber *et al.* 1998). The intrinsic efficiency of PS2 in dark-adapted material was measured as F_v/F_m, where the maximal variable fluorescence F_v was determined as $F_m - F_0$.

Carboxylation activities: Fruit used for gas exchange and Chl fluorescence was divided into two portions: one portion was used for Chl content analysis according to Moran (1982), another portion was frozen and crushed to a fine powder at the temperature of liquid N_2 and maintained at –80 °C. The powder was extracted in a mortar or in a glass homogeniser containing ice-cold extraction medium containing 0.25 M Tris-HCl (pH 7.8), 0.05 M $MgCl_2$, 0.0025 M EDTA, and 37.5 mg dithiothreitol. The amounts of buffer utilised were dependent on the quantity and the mass of the fruit sample. The ratio between sample fresh mass and amount of buffer was about 1 : 1. The homogenate was then filtered through 4 layers of cheesecloth and centrifuged for 10 min at 10 000×*g*. An aliquot of the resulting supernatant was used for protein determinations with the dye-binding method (*Biorad*, Richmond, USA) using BSA as the standard.

Initial RuBPCO and PEPC activities were assayed according to Usuda (1985) and Holaday *et al.* (1992), with minor modifications. The activities were expressed on the basis of protein content.

Statistical analysis: All experiments were repeated three times. For comparison of the means, analysis of variance (ANOVA) followed by the least significant difference (LSD) test was used.

Results and discussion

Transpiration rate regularly increased during fruit development (Table 1), but the trend of CO_2 evolution as measured either in light or dark was unclear (Table 1). CO_2 evolution in the dark was significantly higher than in light with the exception of the over-ripe stage. These results agree with reports of Willmer and Johnston (1976) and Bravdo *et al.* (1977). So, tomato fruit did not show net CO_2 fixation and Blanke and Lenz (1989) proposed to identify net fruit photosynthesis as the difference between the respiration rates in the light and in the dark. However, this estimation is grossly incorrect. In tomato fruit during the different development phases a difference between the CO_2 evolution in the dark and in the light (see Table 1) is evident. CO_2 evolution in the dark and in the light was higher at the immature stage and this may indicate the presence of an intense metabolic activity in the fruit which also determines a re-fixation of CO_2 *via* PEPC.

Tomato fruit contained Chl which was retained up to a very advanced stage of ripening, as reported also by other authors (Clijsters 1969, Pantastico 1975, Phan 1975, Jones 1981). Chl content declined during fruit maturation (Table 1) but never dropped to nil. Also the Chl *a/b* ratio decreased with fruit development, ranging from 2.4 to 0.5 from green to over-ripe fruits. The more pronounced decrease in Chl *a* than in Chl *b* is essentially due to chlorophyllase activity (Rhodes and Wooltorton 1967).

The presence of Chl in fruit tissue indicates that the fruits are able to receive energy for photosynthesis. This was confirmed also by Chl fluorescence measurements. Ground (F_0) and maximal (F_m) fluorescence, as well as the F_v/F_m ratio, decreased significantly during fruit development, reaching low values at the over-ripe stage (Table 1). F_v/F_m, which indicates the intrinsic efficiency of PS2 photochemistry in absence of PAR, showed in green tomato fruits a mean value of 0.83, which is close to the average of the values found in leaves of a wide range of C3 species (Björkman and Demmig 1987). A fluorescence signal was detected also in mature and over-ripe fruits, thus indicating that the electron transport system was effective in tomato fruits even at late stages of maturation.

Photochemical (q_P) and non-photochemical (q_{NP}) quenching coefficients as well as the actual quantum yield of PS2 (Φ_{PS2}) were determined only in green and ripe fruits (Table 1). Photochemical quenching was higher than the non-photochemical one in both stages, while actual quantum yield of PS2 under steady state photosynthesis was significantly higher in green fruits. Actual photosynthetic efficiency is determined by the relative distribution of absorbed photon energy to photochemical and non-photochemical pathways (Schreiber *et al.* 1998). The allocation of energy to these pathways is indicated by the quenching coefficients. The fruits were able to maintain the PS2 acceptor in oxidised form as indicated by the high values of q_P. As far as electron transport is concerned, fruits during their maturation are able to carry out the regular photochemical process, so it is unlikely that photosynthesis is limited by electron transport.

Table 1. CO_2 evolution, transpiration rate, total chlorophyll (Chl) content, Chl *a/b* ratio, Chl fluorescence parameters, photochemical and non-photochemical quenching coefficients q_P and q_{NP}, actual quantum yield of photosystem 2 (PS2) - Φ_{PS2}, and carboxylation activities of ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBPCO) and phosphoenolpyruvate carboxylase (PEPC) of tomato fruits as measured at different developmental stages (I, immature; R, red-turning; M, mature; O, over-ripe). Means of 4 replicates. For each row, means followed by the same letters are not significant different for $p<0.05$.

	Fruit development stage			
	I	R	M	O
CO_2 evolution [μmol m^{-2} s^{-1}] in light	2.53a	1.55b	1.96b	2.28a
CO_2 evolution [μmol m^{-2} s^{-1}] in dark	3.50a	1.99b	3.22a	1.98b
Transpiration rate [mmol(H_2O) m^{-2} s^{-1}]	0.19c	0.67bc	1.21b	1.80a
Chl [mg kg^{-1}(FM)]	24.40a	7.03b	5.63b	7.52b
Chl *a/b*	2.44a	0.91b	0.47c	0.43c
F_0	43a	29ab	18bc	4c
F_m	265a	93b	25c	5d
F_v/F_m	0.832a	0.704b	0.337c	0.200c
q_P	0.858	0.879	-	-
q_{NP}	0.195	0.107	-	-
Φ_{PS2}	0.673	0.622	-	-
RuBPCO [mol(CO_2) kg^{-1}(protein) s^{-1}]	0.015a	0.011ab	0.009b	0.005c
PEPC [mol(CO_2) kg^{-1}(protein) s^{-1}]	0.032a	0.023b	0.010c	0.010c
Leaves:				
RuBPCO [mol(CO_2) kg^{-1}(protein) s^{-1}]	0.0152			
PEPC [mol(CO_2) kg^{-1}(protein) s^{-1}]	0.0015			

Carboxylation activity (expressed on the basis of protein content) of both RuBPCO and PEPC declined with fruit maturation (Table 1). RuBPCO activity in green fruits was similar to that recorded in leaf tissues, whereas PEPC activity was about 2-fold than the RuBPCO activity, in accordance with the results reported by Laval-Martin *et al.* (1977) and Bravdo *et al.* (1977). Compared to RuBPCO, PEPC activity was much higher in green and red-turning fruits. However, this kind of carboxylation is not aimed at producing assimilates for fruit growth but to re-fix respiratory CO_2 and to accumulate malate which acts as osmoticum in maintaining positive cell turgor, the driving force for cell enlargement and fruit growth.

In conclusion, we showed that during different developmental stages the tomato fruits did not show CO_2 assimilation; however, fruit tissues had a consistent photochemical activity. The results confirmed in fruit tissues the presence and functionality of major photosynthetic structures: Chl content and fluorescence analyses showed that the photochemical efficiency of PS2 in fruit is similar to that of leaves. RuBPCO activity was found in all different stages even if almost lower than that recorded in the leaves. The activity of PEPC in tomato fruit was higher than the RuBPCO activity during the first phases of fruit development. Thus, fruit tissue can photosynthesise and fix CO_2, even if the higher fruit metabolic and respiratory activities "hide" this assimilatory activity. It is suggested, in accordance with other authors (Bravdo *et al.* 1977, Blanke and Lenz 1989, Proietti *et al.* 1999), that fruit is partially able to provide the carbon skeleton and energy necessary during its growth.

On the basis of these results the question why the tomato fruits did not show net assimilation of CO_2 at any stage of development remains unanswered. This feature is common in sink organs showing high PEPC activity and very low photosynthetic efficiency such as in maize ears (Soldatini *et al.* 1982). In this particular case the dark CO_2 fixation in the metabolism of tissues exhibiting low photosynthesis did not aim to produce photosynthates but to produce C_4 acids, in particular malate, the first stable product of CO_2 fixation *via* PEPC. Malate is a key substrate for respiration during fruit ripening (Toldman-Andersen and Hansen 1997).

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