PHOTOSYNTHETICA 34 (3): 427-438, 1997

Comparison of chlorophyll fluorescence kinetics and photochemical activities of isolated chloroplasts in genetic analysis of *Lycopersicon esculentum* Mill. hybrids

H. SYNKOVÁ*, N. WILHELMOVÁ*, D. HOLÁ**, D. HAISEL*
and Z. ŠESTÁK*,+

Institute of Experimental Botany, Department of Physiology of Photosynthesis,
*Na Karlovce 1a, CZ-160 00 Praha 6, Czech Republic**
Department of Genetics and Microbiology, Faculty of Sciences, Charles University,
*Viničná 5, CZ-128 00 Praha 2, Czech Republic***

Abstract

In experiments with 5×5 diallel cross of tomato cv. Smržické contents of individual chlorophylls (Chl) and carotenoids (Car), Chl fluorescence kinetic parameters (F_v/F_m, q_P, q_N and Φ_{II}), activities of photosystem 2 and of the whole electron transport chain (Hill reaction) were compared during plant ontogeny with the aim to detect genetically determined differences among genotypes. The differences in Chl fluorescence kinetic parameters and Hill reaction activities were more affected by leaf development than by plant genotype, although the differences between reciprocal crosses were found in the former ones. Some additive genetic effects may participate in the genetic determination of q_N. Statistically significant non-additive genetic effects in inheritance of the total Chl content were found. Detailed pigment analysis revealed additivity together with differences between reciprocal crosses in individual Car and Chl. The highest general combining ability was found for the parental line AM.

Additional key words: carotenoids; electron transport chain; Hill reaction; hybrids; leaf development; PAM; photosystem 2; plant growth; tomato.

Received 10 January 1996, *accepted* 10 July 1997.

[+]Author for correspondence: fax: (+420-2) - 243 10 113; e-mail: sestak@site.cas.cz

Abbreviations: Car - carotenoids; Chl - chlorophyll; F_v/F_m - ratio of variable to maximal fluorescence in dark adapted state; F_v/F_0 - ratio of variable to minimum fluorescence; FeCy - ferricyanide; PD - 1,4-phenylene diamine; PS - photosystem; q_N - nonphotochemical quenching; q_P - photochemical quenching; Φ_{II} - actual quantum yield of photosystem 2 photochemistry.

Acknowledgements: The authors thank Mrs. M. Koubová and Mrs. L. Kolčabová for their skilful technical assistance. This research has been subvenced by grant No. A638106 from the Grant Agency of the Academy of Sciences of the Czech Republic.

Introduction

Chl fluorescence quenching analysis has been successfully used as a non-destructive method for screening and analysis of stress effects in various plant species (for reviews see Lichtenthaler and Rinderle 1988, Karukstis 1991, Schreiber and Bilger 1993). Its usefulness has been proved for evaluation of stress resistance or sensitivity of various crop plants, *e.g.*, chilling effects in tomato (Walker *et al.* 1990, Brüggemann and Dauborn 1993, Brüggemann and Linger 1994, Brüggemann *et al.* 1994a,b) or maize (Oberhuber *et al.* 1993), or selection of drought tolerant genotypes of wheat (Havaux *et al.* 1988). Chl fluorescence analysis is often used for prediction of CO_2 assimilation (cf. Krall and Edwards 1992, Edwards and Baker 1993). An attempt to establish the Chl fluorescence *in vivo* as a method for selection of more efficient genotypes has yielded inconsistent and contradictory results (Planchon *et al.* 1989, Krebs *et al.* 1996), leading sometimes to the conclusion that there are no apparent differences among photosynthetic efficiencies of inbreds and their hybrids. Also Jefferies (1992a,b) failed to discriminate effectively among different drought-treated genotypes of potato when he used values of Chl fluorescence transients. Nevertheless, Walker *et al.* (1990) recommend the F_O/F_P ratio for evaluating chilling tolerance of commercial cultivars of tomato and its hybrids with related wild species.

Photochemical activity of isolated chloroplasts has been used for the identification of inheritance mechanisms in various plant species. Several inbred and hybrid lines of maize differ in this trait, and positive heterosis in F1 generation has been detected (Ovchinnikova 1976, Kočová 1992). Statistically significant differences in photochemical activity have been found in soybean (Du *et al.* 1982) and in wheat (Zelenskiï *et al.* 1978), though Barta and Hodges (1970) have not observed any differences in three cultivars of wheat. Da Silva *et al.* (1995) have recommended the study of photosynthetic O_2 evolution by photoacoustic method for the evaluation of different plant genotypes.

In the present work, the Chl fluorescence and quenching analysis were compared with photochemical activities of isolated chloroplasts from five parental lines of tomato and their reciprocal hybrids with the goal to detect genetically determined differences in photosynthetic performance among tomato genotypes.

Materials and methods

Plants: The complete diallel cross of five parental lines of tomato (*Lycopersicon esculentum* Mill. cv. Smržické, lines 17, 19, 20, 24, and AM) and their F1 hybrids was used for our analysis. Parental genotypes were homozygous with identical number of chromosomes ($2n = 24$), they had a similar habitus, and a similar leaf type. Seeds were sown in sterile sand and grown there till the secondary leaves started to appear. After approximately two weeks they were transferred to pots with soil. The plants were further twice repotted at the age of 30 and 70-80 d, respectively. The parental genotypes were grown in four replicates, and hybrid genotypes in two replicates containing 10 plants of each genotype.

All plants were grown in a greenhouse from September till January. Night temperature was maintained at a minimum of 15 °C, day temperature did not exceed 25 °C. The additional irradiance of approx. 200 μmol m^{-2} s^{-1} was used during winter. Plants 14, 30, 70-80, and 130 d-old were used for fluorescence kinetics measurements, and plants 70-80 and 130 d-old for electron transport measurements. Except for the 14 d-old seedlings, of which primary leaves were used, the first mature leaves from the top were used for measurement.

Chl fluorescence induction was measured after a 15 min dark period with the PAM Chlorophyll Fluorometer (*Walz*, Effeltrich, Germany) at room temperature and ambient CO_2 concentration on leaves attached to the plant. The measuring beam irradiance was 0.35 μmol m^{-2} s^{-1}, actinic irradiance 150 μmol m^{-2} s^{-1}, 700 ms saturated flashes of 2500 μmol m^{-2} s^{-1} were fired every 30 s. Data sampling, control, and some calculations were served by the *DA100* Data Acquisition System (*Walz*, Effeltrich, Germany). All Chl fluorescence parameters were calculated according to the nomenclature of van Kooten and Snel (1990). Quantum yield of photosystem 2 (PS2) photochemistry, Φ_{II}, was calculated as $(F_{m'} - F_s)/F_{m'}$ according to Genty *et al.* (1989).

Chl and carotenoid (Car) contents were determined in 80 % acetone extracts of leaf discs with a spectrophotometer *PU-8740* (*Philips Scientific*, Cambridge, U.K.) by the methods of Arnon (1949) and Lichtenthaler (1987), respectively. Leaf discs corresponding to 1 cm^2 were used also for determination of fresh and dry leaf matter. Detailed pigment composition was determined by the HPLC system (*Spectra-Physics*, San Jose, U.S.A.). The pigments were separated on a *Separon SGX C18* column (*Tessek*, Praha, Czech Republic) at a flow rate 0.17 mm^3 s^{-1} with a 10 mm^3 injection volume. Elution with acetonitrile/methanol/water (80:12:6) mixture for 6 min was followed by a linear gradient (0 - 100 %) of methanol within 8 min. The detection wavelength was 445 nm.

Photochemical activities of chloroplasts: Broken chloroplasts were isolated from the same leaves used for fluorescence measurement. The mid-veins of the leaves were stripped, the blades were sliced and blended in isolation medium [mM]: HEPES 50, sorbitol 350, $MnCl_2$ 1, $MgCl_2$ 1, EDTA 2, ascorbate 2, K_2HPO_4 0.5; with additions of 1 % polyvinylpyrrolidone and 0.5 % bovine serum albumine. After filtration and following centrifugation (2000×g, 5 min), the pellet was resuspended in the resuspending medium [mM]: Tricine 50 (pH 7.5), sorbitol 330, $MnCl_2$ 1, $MgCl_2$ 1, EDTA 2. Photochemical activities of the whole electron transport chain (Hill reaction) and PS2 were measured as oxygen evolution using a liquid phase Clark type oxygen electrode (*Hansatech*, King's Lynn, U.K.). The suspension of thylakoids was diluted in the assay medium [mM]: Tricine 40 (pH 7.5), NaCl 2, $MgCl_2$ 5, K_2HPO_4 2. Final Chl concentration was 50 g m^{-3}. Ferricyanide (FeCy) to a final concentration of 6.25 mM was added prior to the measurements, 1,4-phenylene diamine (PD) to a final concentration of 0.5 mM was added during the measurements. The measurements were done at 25 °C under an irradiance of 350 μmol m^{-2} s^{-1}.

Genetic analysis: The differences between genotypes and the effect of plant age were tested using the ANOVA method. The general (GCA) and specific (SCA) combining abilities of individual genotypes together with reciprocal effects of F1 hybrids were estimated according to Griffing (1956) with the aid of CBE programme (Wolf 1996). Analysis of a dialell cross and separation of individual components of genetic variation was made according to Hayman (1954), using the programme ADC (Krejčí, unpublished).

Results

Plant growth: The differences in growth among the parental lines were small and insignificant. The plants of lines 17 and 19 were usually higher, with a stronger stem and more numerous branches compared to the others till the age of 80 d, afterwards the differences almost disappeared. These two parental genotypes had a high fertility, and a sufficient amount of fruits and hybrid seeds was produced. The lowest fertility was observed in lines 20 and AM, whose hybrid fruit and seed production was insufficient. Hybrid plants did not show any apparent differences in growth in comparison with parental cultivars.

Pigment contents: Mean values from analyses at the four sampling dates showed the highest Chl content calculated per leaf area in parental line 24, and per leaf dry matter in parental line 17; the lowest Chl content was in lines 19 and 20 (Tables 1 and 2). Hybrids showed higher Chl content than the parent lines, but the differences were not statistically significant. The parental lines 24 and AM had the highest ratio

Table 1. Chlorophyll (Chl) fluorescence characteristics and total Chl contents and Chl/Car (carotenoids) ratio calculated per units of leaf area [mg m^{-2}] or per units of dry leaf matter [g kg^{-1}] in parental lines (17, 19, 20, 24, AM) and in all hybrids derived from each parental line. Values are means of all measurements throughout plant ontogeny.

	17		19		20		24		AM	
	parent	hybrid	parent	hybrid	parent	hybrid	parent	hybrid	parent	hybrid
Chl *a/b*	2.88	3.01	2.93	3.04	2.85	2.89	3.03	2.97	3.02	2.96
Chl [mg m^{-2}]	181.9	216.2	167.1	212.7	184.3	225.9	206.3	217.5	185.0	208.4
[g kg^{-1}(d.m.)]	26.8	33.0	24.2	31.9	22.2	32.2	25.6	32.0	24.8	31.5
Chl/Car	7.74	7.33	7.51	7.33	7.45	7.28	7.88	7.36	7.35	7.34
F_v/F_m	0.82	0.83	0.83	0.84	0.82	0.83	0.82	0.83	0.82	0.83
F_v/F_0	4.85	4.94	4.95	5.06	5.00	4.92	4.82	4.89	4.99	4.99
q_P	0.84	0.87	0.82	0.86	0.83	0.87	0.81	0.86	0.8	0.86
q_N	0.27	0.26	0.29	0.25	0.30	0.24	0.26	0.25	0.27	0.26
Φ_{II}	0.73	0.73	0.74	0.74	0.74	0.73	0.73	0.73	0.73	0.73

of Chl *a/b*, nevertheless, the differences between parental lines and hybrids were of a varying size. No significant changes were observed in hybrid lines in the ratio of Chl/Car.

Table 2. Values of statistical significance (*p*) from analysis of variance of pigment amounts and chlorophyll (Chl) fluorescence characteristics. Analysed are effects of tomato genotype ($N = 24$), plant age ($N = 3$), reciprocal crossing ($N = 1$), differences between parental and hybrid lines ($N = 1$). Chl/fm = Chl per unit of fresh leaf matter, Chl/area= Chl per unit of leaf area, Chl/dm = Chl per unit of leaf dry matter.

	genotype	plant age	reciprocal crossing	parents × hybrids
Chl *a/b*	0.207	0.000	0.096	0.299
Chl/area	0.496	0.000	0.578	0.719
Chl/fm	0.999	0.000	0.917	0.940
Chl/dm	0.917	0.000	0.821	0.887
Chl/Car	0.991	0.000	0.816	0.574
F_v/F_0	0.474	0.000	0.598	0.397
q_P	0.966	0.000	0.805	0.540
q_N	0.919	0.000	0.787	0.507
Φ_{II}	1.000	0.000	0.677	0.428

Generally, the pigment contents calculated per units of fresh and dry matter were independent of genotype, hybridization, and reciprocal crossing (Table 2), but they were significantly dependent on plant age (Fig. 1*A*). The highest pigment contents

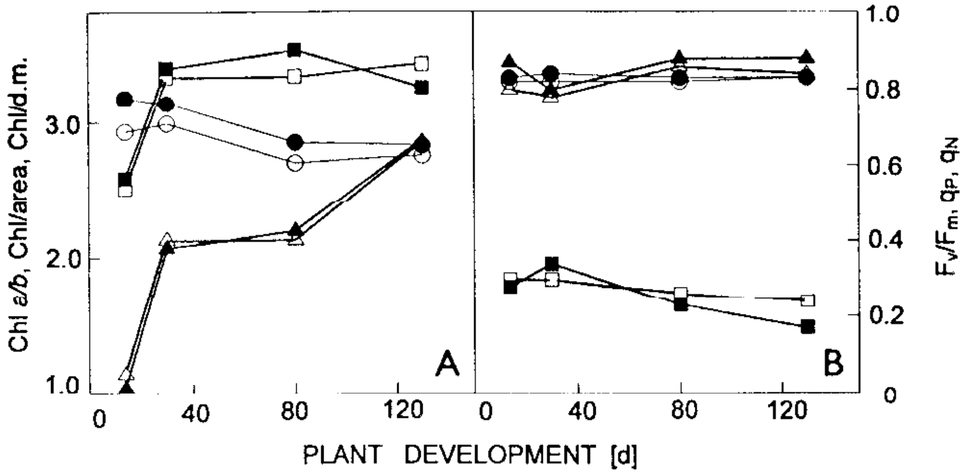

Fig. 1. Average values of the ratio of chlorophyll (Chl) *a/b* (○, ●), Chl (*a*+*b*) calculated per units of leaf area (□, ■, ×10^{-2} mg m^{-2}) and leaf dry matter (Δ, ▲, ×10^{-1} g kg^{-1}) (*A*), and average values of Chl fluorescence characteristics (*B* - ○, ● = F_v/F_m; □, ■ = q_N; Δ, ▲ = q_P) in five parental lines (*open symbols*) or twenty hybrid lines (*closed symbols*) during plant ontogeny.

were found in mature fruit producing plants (130-d-old). No significant differences between parents and hybrids were found during plant ontogeny.

Table 3. Pigment contents (N - neoxanthin, V - violaxanthin, A - antheraxanthin, Z - zeaxanthin, L - lutein, Chl *a* - chlorophyll *a*, Chl *b* - chlorophyll *b*, β-car - β-carotene) [mg m^{-2}] in just mature leaves of 80-d-old plants of tomato lines 17, 19, 20, 24 and AM and their hybrids. Means of 3 samples, each was 2-3 times analysed by HPLC.

	Chl *a*	Chl *b*	Chl *a/b*	β-car	L	V	Z	N	A
17×17	281.4 ± 8.9	91.3 ± 2.4	3.08	13.5 ± 0.3	22.1 ± 0.8	9.8 ± 0.3	0.42 ± 0.02	5.8 ± 0.2	0.20 ± 0.01
17×19	296.6 ± 9.5	95.1 ± 1.8	3.12	12.9 ± 0.4	23.1 ± 0.5	10.0 ± 0.2	0.46 ± 0.02	6.0 ± 0.3	0.18 ± 0.01
17×20	284.8 ± 8.2	93.4 ± 2.7	3.05	13.4 ± 0.2	22.4 ± 0.9	9.6 ± 0.3	0.40 ± 0.02	6.0 ± 0.2	0.21 ± 0.01
17×24	286.4 ± 8.6	94.1 ± 1.9	3.04	13.5 ± 0.4	23.2 ± 0.6	10.3 ± 0.4	0.45 ± 0.02	6.0 ± 0.2	0.22 ± 0.01
17×AM	303.4 ± 9.1	95.7 ± 2.2	3.17	13.5 ± 0.3	22.6 ± 0.6	10.5 ± 0.2	0.46 ± 0.02	6.4 ± 0.3	0.21 ± 0.01
19×19	279.3 ± 8.4	92.7 ± 1.4	3.01	13.9 ± 0.4	22.9 ± 0.5	10.8 ± 0.3	0.39 ± 0.01	6.2 ± 0.2	0.22 ± 0.01
19×17	283.9 ± 7.2	94.1 ± 2.5	3.02	13.5 ± 0.2	23.7 ± 1.0	10.3 ± 0.3	0.40 ± 0.02	5.8 ± 0.1	0.19 ± 0.01
19×20	273.9 ± 8.7	90.4 ± 1.2	3.03	14.3 ± 0.3	22.2 ± 0.4	10.8 ± 0.4	0.46 ± 0.02	5.6 ± 0.2	0.22 ± 0.01
19×24	279.0 ± 7.9	91.4 ± 2.1	3.05	13.2 ± 0.3	23.5 ± 0.8	10.6 ± 0.3	0.40 ± 0.02	5.8 ± 0.2	0.24 ± 0.01
19×AM	288.2 ± 8.1	98.2 ± 1.5	2.93	12.8 ± 0.2	22.4 ± 0.5	10.8 ± 0.3	0.42 ± 0.02	6.0 ± 0.2	0.23 ± 0.01
20×20	279.6 ± 9.2	91.2 ± 2.2	3.07	13.8 ± 0.3	22.5 ± 0.9	9.6 ± 0.3	0.42 ± 0.02	6.0 ± 0.1	0.23 ± 0.01
20×17	275.7 ± 10.3	92.1 ± 1.7	2.99	13.3 ± 0.4	22.0 ± 0.7	10.4 ± 0.2	0.41 ± 0.01	6.3 ± 0.2	0.22 ± 0.01
20×19	271.5 ± 8.5	90.9 ± 2.4	2.99	12.5 ± 0.4	22.2 ± 0.5	10.2 ± 0.4	0.43 ± 0.02	6.2 ± 0.3	0.19 ± 0.01
20×24	278.1 ± 8.4	93.4 ± 0.9	2.98	13.2 ± 0.3	23.4 ± 0.4	9.8 ± 0.3	0.41 ± 0.01	6.5 ± 0.2	0.21 ± 0.01
20×AM	292.5 ± 9.5	99.1 ± 1.9	2.95	13.0 ± 0.5	22.7 ± 0.5	10.4 ± 0.3	0.42 ± 0.02	6.0 ± 0.2	0.18 ± 0.01
24×24	271.5 ± 8.3	91.4 ± 1.1	2.97	12.9 ± 0.6	22.0 ± 0.5	10.5 ± 0.3	0.44 ± 0.02	6.1 ± 0.1	0.23 ± 0.01
24×17	268.3 ± 8.7	89.4 ± 1.6	3.00	13.4 ± 0.4	22.0 ± 1.1	10.6 ± 0.4	0.45 ± 0.01	6.0 ± 0.2	0.20 ± 0.01
24×19	274.8 ± 10.8	91.6 ± 1.8	3.00	13.2 ± 0.3	22.6 ± 0.8	10.4 ± 0.3	0.45 ± 0.02	5.9 ± 0.1	0.23 ± 0.01
24×20	269.9 ± 9.1	90.8 ± 2.3	2.97	12.5 ± 0.5	22.3 ± 0.7	10.0 ± 0.3	0.41 ± 0.02	6.1 ± 0.2	0.22 ± 0.01
24×AM	285.4 ± 6.7	98.6 ± 2.8	2.89	13.2 ± 0.4	22.3 ± 0.7	10.3 ± 0.4	0.41 ± 0.02	6.2 ± 0.2	0.22 ± 0.01
AM×AM	324.0 ± 12.2	113.5 ± 1.7	2.85	14.1 ± 0.5	23.7 ± 0.6	11.0 ± 0.3	0.44 ± 0.02	7.1 ± 0.3	0.21 ± 0.01
AM×17	325.9 ± 9.4	111.3 ± 2.4	2.93	13.4 ± 0.5	22.9 ± 0.4	10.5 ± 0.3	0.47 ± 0.02	6.7 ± 0.2	0.21 ± 0.01
AM×19	322.0 ± 10.6	110.0 ± 1.8	2.93	13.7 ± 0.3	22.5 ± 0.5	10.5 ± 0.2	0.45 ± 0.01	6.9 ± 0.3	0.20 ± 0.01
AM×20	327.1 ± 11.7	111.8 ± 2.2	2.93	13.0 ± 0.6	23.0 ± 0.5	10.9 ± 0.2	0.42 ± 0.02	6.7 ± 0.2	0.19 ± 0.01
AM×24	320.8 ± 1.9	110.5 ± 1.4	2.90	13.8 ± 0.5	23.2 ± 0.8	11.0 ± 0.4	0.49 ± 0.02	6.9 ± 0.3	0.20 ± 0.01

In 80 d-old plants a more detailed pigment analysis was done by the HPLC method (Table 3). At this phase of plant development (start of flowering), the highest and statistically significant Chl *a* and *b* contents were found in the parental line AM and in its hybrids. The Chl *a/b* ratio was the lowest in this line and its hybrids. The differences in contents of individual carotenoids found among parental lines and between reciprocal hybrids were small and usually statistically insignificant (Table 3). The highest number of significant differences was again connected with the line AM.

Chl *a* fluorescence characteristics were affected more by plant age than by genotype. The differences between parental genotypes and hybrids were mostly statistically insignificant (Table 2). The highest quantum yields of PS2 (Φ_{II}) were observed in 70-80 d-old plants, the lowest ones were found in primary leaves of the 14 d-old plants. No significant differences in F_v/F_m were found between parental lines and their hybrids (Tables 1 and 2).

Photochemical (q_P) and non-photochemical (q_N) quenching showed some dependence on plant age (Fig. 1*B*) and less or no dependence on plant genotype. The highest q_P values were found in the parental line 17 and its hybrids, the lowest ones in the inbred lines AM and 24 (Table 1).

The highest q_N was found in the line 20, the lowest one in the line 24. The differences between parents and their hybrids were mostly statistically insignificant, although a slightly lower q_N was observed in hybrids than in the parent plants. Some decrease of q_N was observed in all plants with increasing age (Fig. 1*B*).

Photochemical activities: In all parental lines with the exception of line 24 (values not shown), higher activities of the Hill reaction (acceptor FeCy) and PS2 (acceptor PD) were found in chloroplasts from leaves of 130-d-old plants than in chloroplasts from leaves of the 80-d-old plants. Both the Hill reaction and PS2 activities of chloroplasts isolated from parental lines were higher than those of their F1 hybrids, with the exception of line 24 (Table 4). The highest photochemical activities were found in line 17 and the lowest ones in line 24.

Table 4. Photochemical activities [μmol(O_2) kg^{-1}(Chl) s^{-1}] of isolated chloroplasts from different parental lines of tomato and their F1 hybrids. The activities were measured with phenylenediamine (PS2) or ferricyanide (Hill) as electron acceptors. Values are means of all measurements throughout plant ontogeny.

	17		19		20		24		AM	
	parent	hybrid	parent	hybrid	parent	hybrid	parent	hybrid	parent	hybrid
PS2	6.64	3.01	4.54	2.97	3.24	2.45	2.71	3.17	4.02	2.58
Hill	3.30	1.56	1.82	1.47	1.94	1.32	1.48	1.80	2.35	1.32

Genetic analysis: Analysis of variance of diallel crosses showed statistically significant non-additive genetic effects participating in the inheritance of Chl (*a*+*b*) content calculated per leaf area unit (Table 5). However, when expressed per fresh matter or dry matter unit, these effects were not statistically significant. The presence of the additive genetic effects was found only for the Chl *a/b* ratio. The differences between genotypes in Chl/Car ratio were not statistically significant. However, detailed analysis of individual photosynthetic pigment contents made in 80 d-old plants enabled us to greatly diminish the non-genetic component of variability and examine thus the nature of the genetic effects more thoroughly (middle part of Table 5). The additivity together with the differences between reciprocal crosses were the main causes of genetic variation in the content of all xanthophylls but lutein, as well as Chl *a* and Chl *b*. Non-additive genetic effects were observed for the contents of neoxanthin, antheraxanthin, zeaxanthin, and β-carotene. There were no genetically determined differences between genotypes in lutein content (Table 5). The highest general combining ability was found for the parental line AM; the hybrid 17×19 together with its reciprocal crosses showed a good specific combining ability for these traits.

Table 5. Levels of statistical significance (*p*) from analysis of variance of photosynthetic pigment contents, chlorophyll (Chl) fluorescence characteristics, and photochemical activity of chloroplasts made in 5×5 diallel crosses of tomato.

Characteristic	Additive genetic effects	Non-additive genetic effects	Effects of rec. crossing	Non-genetic variation
Chl (dry mass)	0.489	0.184	0.091	0.000
Chl (fresh mass)	0.177	0.684	0.437	0.000
Chl (leaf area)	0.083	0.021	0.590	0.000
Chl *a/b*	0.001	0.280	0.406	0.000
Chl/Car	0.109	0.658	0.164	0.000
Neoxanthin	0.000	0.030	0.000	0.056
Violaxanthin	0.000	0.498	0.208	0.189
Antheraxanthin	0.000	0.000	0.000	0.000
Zeaxanthin	0.001	0.001	0.000	0.711
Lutein	0.374	0.112	0.280	0.953
β-carotene	0.124	0.018	0.001	0.921
Chl *a*	0.000	0.282	0.000	0.155
Chl *b*	0.000	0.101	0.000	0.524
F_v/F_m	0.263	0.268	0.039	0.006
q_P	0.263	0.376	0.000	0.000
q_N	0.016	0.151	0.000	0.000
PS2	0.357	0.307	0.997	0.000
Hill reaction	0.450	0.303	0.959	0.003

The differences between reciprocal crosses were probably the main cause of the observed genetic variation in Chl fluorescence characteristics (which was very small). Some additive genetic effects probably participated in the genetic determination of q_N. A genetic variation was not found for the photochemical activity of PS2 and the Hill reaction with FeCy (Table 4). Parental line 19 displayed the highest general combining ability for most of the fluorescence characteristics examined.

Discussion

Our experiments were designed to clarify whether the Chl fluorescence quenching analysis can be used as a tool for evaluating photosynthetic capacity of inbred lines of *L. esculentum* and their hybrids. Simultaneously, the electron transport activities of chloroplasts isolated from these plants and photosynthetic pigment contents were measured. All the measured parameters were highly dependent on plant age and less or not at all on the plant genotype. Therefore it is difficult to make a genetic analysis of photosynthesis in tomato using only the Chl fluorescence kinetics.

The Chl fluorescence parameters such as F_v/F_m or Φ_{II} currently used for detection of stress effects (cf. Lichtenthaler and Rinderle 1988) were useless for genetic analysis of photosynthesis in tomato plants grown under "optimal" conditions. Especially Φ_{II} almost did not change during plant development and among plant genotypes.

Both quenching coefficients (q_P and q_N) showed under these conditions a high sensitivity to plant age or actual plant state. Generally, a statistically significant decrease of q_N and increase of q_P and F_v/F_m in hybrid lines in the 130 d-old, *i.e.* mature and fruit producing, plants (see Fig. 1*B*) showed that hybrids might utilize radiant energy more efficiently than the parental lines. Unfortunately, the differences in these parameters among parental cultivars were small, so no striking performance could be expected in their hybrids.

Our results are in agreement with the fact that leaf age controls the content of pigments (cf. Šesták 1985). All literature on Chl fluorescence and quenching characteristics shows that leaf age induced differences are of a similar size as those induced by genetic origin or stress (Šesták and Šiffel 1997). This fully holds for our experiments with tomato. John *et al.* (1995) found in an original tomato cultivar similar significant changes in Chl content, net photosynthetic rate, and the F_v/F_m ratio with leaf age, but in the transgenic 1-amino-cyclopropane-1-carboxylic acid oxidase antisense plants there were no statistically significant differences in F_v/F_m between leaves of different position. The F_v/F_m ratios of leaves of three *L. peruvianum* lines originating at various altitudes (20, 1650, and 2400 m a.s.l.) reacted differently to moderate chilling and high irradiance (Brüggemann *et al.* 1994a). However, Gruenzweig *et al.* (1993) did not find differences in this value between 12- and 18-d-old third leaves of tomato cv. Rehovot, nor an effect of soil solarization. In experiments with F_2 plants derived from a selfed F_1 plant of *L. esculentum* cv. Solentos×*L. peruvianum* LA 2157 cross, Brüggemann *et al.* (1994b) found a fairly high heritability (H^2 = 0.83-0.92) for quenching coefficients q_P and q_I (photoinhibition dependent quenching). In other experiments with the tomato cv. Abunda the coefficients q_P and q_N depended both on leaf age and photoinhibitory chilling, while q_N was practically unaffected by aging and chilling (Brüggemann and Dauborn 1993). No correlation between Chl fluorescence parameters on the one hand and net photosynthetic rate and growth parameters on the other hand was found in eight tomato genotypes grown at suboptimal temperature (Janssen *et al.* 1995). Nevertheless, differences between genotypes could be distinguished using Chl fluorescence parameters such as low temperature discontinuity in F_P *versus* temperature curve, and temperature dependence of F_P and maximal fluorescence rise.

Experiments with maize genotypes (Krebs *et al.* 1996) were more promising for the use of Chl fluorescence analysis in detecting genetic differences between parental lines and their hybrids than the experiments with tomato. Nevertheless, also in the maize experiments the effect of plant age and growing season was often more expressed than the effect of genotypes.

Photochemical activities of isolated chloroplasts have been successfully used for genetic analysis (Ovchinnikova 1976, Kočová 1992). In our experiments with broken chloroplasts the differences between parental and hybrid lines in this trait were usually statistically insignificant. The observed dependence of Hill activities on plant age coincided with the results of Chl fluorescence measurements, *i.e.*, an increase in electron transport activities in 130 d-old plants in comparison with the younger ones. However, the chloroplast isolation itself is a strong intervention into the plant cell with possible consequences on photochemical activities, and it is therefore difficult to compare the results of both procedures. Thus, in leaves of transgenic *Pssu-ipt* tobacco almost no changes in Chl fluorescence parameters have been observed, although a higher than 30 and 70 % inhibition of Hill activity with FeCy and methylviologen, respectively, was found in isolated chloroplasts compared to control wild type tobacco (Synková *et al.* 1997 and unpublished).

Heterosis in pigment content has been found in maize and tobacco (Mencáková 1967, Titok *et al.* 1994, Krebs *et al.* 1996). Although the pigment contents in tomato are mostly affected by ontogeny, both the non-additive effect in total Chl content calculated per leaf area and additive effect in Chl *a/b* were proved.

References

Arnon, D.I.: Copper enzymes in isolated chloroplasts. Polyphenoloxidase in *Beta vulgaris*. - Plant Physiol. **24**: 1-15, 1949.

Barta, A.L., Hodges, H.F.: Characterization of photosynthesis in cold hardening winter wheat. - Crop Sci. **10**: 535-538, 1970.

Brüggemann, W., Dauborn, B.: Long-term chilling of young tomato plants under low light. III. Leaf development as reflected by photosynthesis parameters. - Plant Cell Physiol. **34**: 1251-1257, 1993.

Brüggemann, W., Klaucke, S., Maas-Kantel, K.: Long-term chilling of young tomato plants under low light. V. Kinetic and molecular properties of two key enzymes of the Calvin cycle in *Lycopersicon esculentum* Mill. and *L. peruvianum* Mill. - Planta **194**: 160-168, 1994a.

Brüggemann, W., Linger, P.: Long-term chilling of young tomato plants under low light. IV. Differential responses of chlorophyll fluorescence quenching coefficients in *Lycopersicon* species of different chilling sensitivity. - Plant Cell Physiol. **35**: 585-591, 1994.

Brüggemann, W., Linger, P., van Heusden, S.: Quantitative photosynthesis-related traits and restriction fragment length polymorphisms for mapping chilling tolerance genes in tomato. - In: Dörffling, K., Brettschneider, B., Tantau, H., Pitham, K. (ed.): Crop Adaptation to Cool Climates. Pp. 415-422. European Commission, Brussels - Luxembourg 1994b.

da Silva, W.J., Prioli, L.M., Magalhães, A.C.N., Pereira, A.C., Vargas, H., Mansanares, A.M., Cella, N., Miranda, L.C.M., Alvarado-Gil, J.: Photosynthetic O_2 evolution in maize inbreds and their hybrids can be differentiated by open photoacoustic cell technique. - Plant Sci. **104**: 177 - 181, 1995.

Du, W.C., Wang, Y.M., Tan, K.H.: Differences in photosynthetic activity between soybean varieties (strains) and relationship with yield. - Acta agron. sin. **8**: 131-135, 1982.

Edwards, G.E., Baker, N.R.: Can CO_2 assimilation in maize leaves be predicted accurately from chlorophyll fluorescence analysis? - Photosynth. Res. **37**: 89-102, 1993.

Genty, B., Briantais, J.-M., Baker, N.R.: The relationship between the quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. - Biochim. biophys. Acta **990**: 87-92, 1989.

Griffing, B.: Concept of general and specific combining ability in relation to diallel crossing systems. - Aust. J. biol. Sci. **9**: 463-493, 1956.

Gruenzweig, J.M., Rabinowitch, H.D., Katan, J.: Physiological and developmental aspects of increased plant growth in solarised soils. - Ann. appl. Biol. **122**: 579-591, 1993.

Havaux, M., Ernez, M., Lannoye, R.: Correlation between heat tolerance and drought tolerance in cereals demonstrated by rapid chlorophyll fluorescence tests. - J. Plant Physiol. **133**: 555-560, 1988.

Hayman, B.I.: The analysis of variance of diallel crosses. - Biometrics **10**: 235-244, 1954.

Janssen, L.H.J., Van Oeveren, J.C., Van Hasselt, P.R., Kuiper, P.J.C.: Genotypic variation in chlorophyll fluorescence parameters, photosynthesis and growth of tomato grown at low temperature and low irradiance. - Photosynthetica **31**: 301-314, 1995.

Jefferies, R.A.: Effects of drought on chlorophyll fluorescence in potato (*Solanum tuberosum* L.). I. Plant water status and the kinetics of chlorophyll fluorescence. - Potato Res. **35**: 25-34, 1992a.

Jefferies, R.A.: Effects of drought on chlorophyll fluorescence in potato (*Solanum tuberosum* L.). II. Relations between plant growth and measurements of fluorescence. - Potato Res. **35**: 35-40, 1992b.

John, I., Drake, R., Farrell, A., Cooper, W., Lee, P., Horton, P., Grierson, D.: Delayed leaf senescence in ethylene-deficient ACC-oxidase antisense tomato plants: molecular and physiological analysis. - Plant J. **7**: 483-490, 1995.

Karukstis, K.K.: Chlorophyll fluorescence as a physiological probe of the photosynthetic apparatus. - In: Scheer, H. (ed.): Chlorophylls. Pp. 769-795. CRC Press, Boca Raton - Ann Arbor - Boston - London 1991.

Kočová, M.: [Photochemical activity of isolated chloroplasts of maize (*Zea mays* L.) with respect to heterosis expression and complementation.] - Genetika Šlechtění (Praha) **28**: 37-51, 1992. [In Czech.]

Krall, J.P., Edwards, G.E.: Relationship between photosystem II activity and CO_2 fixation in leaves. - Physiol. Plant. **86**: 180-187, 1992.

Krebs, D., Synková, H., Avratovščuková, N., Kočová, M., Šesták, Z.: Chlorophyll fluorescence measurements for genetic analysis of maize cultivars. - Photosynthetica **32**: 595-608, 1996.

Lichtenthaler, H.K.: Chlorophylls and carotenoids - pigments of photosynthetic biomembranes. - In: Colowick, S.P., Kaplan, N.O. (ed.): Methods in Enzymology. Vol. 148. Pp. 350-382. Academic Press, San Diego - New York - Berkeley - Boston - London - Sydney - Tokyo - Toronto 1987.

Lichtenthaler, H.K., Rinderle, U.: The role of chlorophyll fluorescence in the detection of stress conditions in plants. - CRC crit. Rev. anal. Chem. **19**: S29-S85, 1988.

Mencáková, A.: Genetic analysis of chlorophyll content in maize and tobacco. - Photosynthetica **1**: 77-88, 1967.

Oberhuber, W., Dai, Z.-Y., Edwards, G.E.: Light dependence of quantum yields of Photosystem II and CO_2 fixation in C_3 and C_4 plants. - Photosynth. Res. **35**: 265-274, 1993.

Ovchinnikova, M.F.: [The complementation of maize chloroplasts.] Sel'skokhoz. Biol. **11**: 675-679, 1976. [In Russ.]

Planchon, C., Sarrafi, A., Ecochard, R.: Chlorophyll fluorescence transient as a genetic marker of productivity in barley. - Euphytica **42**: 269-273, 1989.

Schreiber, U., Bilger, W.: Progress in chlorophyll fluorescence research: major developments during the past years in retrospect. - Progr. Bot. **54**: 151-173, 1993.

Šesták, Z.: Chlorophylls and carotenoids during leaf ontogeny. - In: Šesták, Z. (ed.): Photosynthesis During Leaf Development. Pp. 76-106. Academia, Praha; Dr W. Junk Publ., Dordrecht - Boston - Lancaster 1985.

Šesták, Z., Šiffel, P.: Leaf-age related differences in chlorophyll fluorescence. - Photosynthetica **33**: 347-369, 1997.

Synková, H., Van Loven, K., Valcke, R.: Increased content of endogenous cytokinins does not delay degradation of photosynthetic apparatus in tobacco. Photosynthetica **33**: 595 608, 1997.

Titok, V.V., Lemesh, V.A., Rusinova, O.V., Podlisskikh, V.L.: Leaf area, chlorophyll content and biomass of tomato plants and their heterotic hybrid under *in vitro* culture. - Photosynthetica **30**: 255-260, 1994.

van Kooten, O., Snel, J.F.H.: The use of chlorophyll fluorescence nomenclature in plant stress physiology. - Photosynth. Res. **25**: 147-150, 1990.

Walker, M.A., Smith, D.M., Pauls, K.P., McKersie, B.D.: A chlorophyll fluorescence screening test to evaluate chilling tolerance in tomato. - HortScience **25**: 334-338, 1990.

Wolf, J.: User's Manual for the Software Package CBE, Version 4.0 (A Universal Program for Estimating Crossbreeding Effects). - Research Institute of Animal Production, Prague-Uhříněves 1996.

Zelenskiĭ, M.I., Mogileva, G., Shitova, I., Fattakhova, F.: Hill reaction of chloroplasts from some species, varieties and cultivars of wheat. - Photosynthetica **12**: 428-435, 1978.