Characterization of photosynthetic apparatus of pea chlorophyll mutants and their heterotic F_1 hybrids with standard genotype (cv. Torsdag)

O.B. VAISHLYA*, V.G. LADYGIN**, V.A. SOKOLOV*** and G.A. SEMENOVA**

*Department of Plant Physiology and Biotechnology, Tomsk State University, Lenin av. 36, Tomsk, 634050, Russia**
*Institute of Soil Science and Photosynthesis, Russian Academy of Sciences, Puschino, Moscow region, 142292, Russia***
*Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, Lavrentiev av. 10, Novosibirsk, 630090, Russia****

Abstract

In comparison to cv. Torsdag, in leaves of low-productive *Pisum sativum* L. chlorophyll mutants the decrease in chlorophyll content was caused by the decrease in cell number per unit volume. Qualitative changes in activities of photosystem (PS) 1 in mutant M2004, and quantitative changes of PS1 and PS2 in mutants M2004 and M2014 and in hybrids were also found. However, the activity of ribulose-1,5-bisphosphate carboxylase (RuBPC) in M2014, and those of RuBPC and glyceraldehyde phosphate dehydrogenase in M2004 and hybrids were higher than in cv. Torsdag. The hybrids inherited the normal structure of photosynthetic apparatus of standard genotype in parallel with the compensatory gene complex of M2004, which was expressed at many levels of organization. This may be the basis of hybrid vigour in this case.

Additional key words: absorption spectra of chloroplast pigments; carotenoids; glyceraldehyde phosphate dehydrogenase; palisade and spongy parenchyma; phosphoenolpyruvate carboxylase; photophosphorylation; photosystems 1 and 2; *Pisum sativum*; ribulose-1,5-bisphosphate carboxylase.

Introduction

The main purpose of this investigation was to contribute to clearing the physiological and biochemical nature of hybrid vigour of plants (cf. also Krebs *et al.* 1996). We

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*e-mail: vaishlya@fbr.tsu.tomsk.su
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tried to find structural and functional differences in mesostructure of chloroplasts and leaves of *chlorotica* mutants, initial standard genotype (cv. Torsdag), and their F_1 hybrids. Theoretically, the mutants used in this investigation should differ from cv. Torsdag only in a locus bearing the mutant gene. This was isolated and repeatedly backcrossed with the standard type to segregate subsequently in F_2. However, genetic analysis showed that heterosis was epistatic but not allelic which was used to formulate a new concept of heterosis (Sokolov 1989). The *chlorotica* mutation in M2004 is located in linkage group I.

Materials and methods

We used chlorophyll (Chl) mutants M2004 and M2014 produced by ethyleneimine treatment of *Pisum sativum* L. grain cv. Torsdag (T) and their F_1 (T×M2004 and M2004×T) hybrids. These hybrids show a stable heterosis effect in plant height and seed productivity: Torsdag (control) brings a 100 % seed yield, while M2004 20-30 %, M2014 2-3 %, T×M2004 120-140 %, and M2004×T 120-150 % of the control.

The experiments were done during 4 years according to the agrotechnical requirements for selection-genetical experiments on experimental plots of the Siberian Botanical Garden of the Tomsk State University, in the Institute of Cytology and Genetics of the Siberian Branch of Russian Academy of Sciences, and in the Institute of Soil Science and Photosynthesis of the Russian Academy of Sciences. Observations were made at seedling stage (4-5 leaves). For analyses, leaves that completed growth of the same age were used. The samples were always taken at 09:00 to 10:00 h in order to prevent circadian effects. The leaf material was frozen immediately in liquid N_2 or collected in plastic bags and rapidly transferred to the cold room for further processing.

Chl content was determined in 100 % acetone (Lichtenthaler and Wellburn 1983). Carotenoids were separated by thin-layer chromatography on *Silufol* plates, eluted, and their absorption was measured at 464 nm for β-carotene, 446 nm for lutein, 442 nm for violaxanthin, and 438 nm for neoxanthin (Ladygin *et al.* 1988). Absorption spectra of chloroplast pigments and their second derivatives were recorded at 296 K on a *Hitachi-356* spectrophotometer. Low-temperature fluorescence emission spectra were recorded at 77 K on a spectrofluorimeter made in our institute (Ladygin and Bil' 1981). Electrophoretic analysis of proteins and pigment-protein complexes in polyacrylamide gel was done as described in Michel *et al.* (1983). The number of PS1 and PS2 reaction centres was calculated from the values of EPR-signals of paramagnetic centres of PS1 (P_{700}^+) and plastosemiquinone PS2 at 77 K (Chetverikov 1983). The rate of primary photochemical reactions was recorded potentiometrically (Nishimura *et al.* 1962, Zabotin 1970).

Leaf mesostructure was examined using cytase and maceration of tissue with 2.5 % acetic acid in 1 M HCl at 353 K (Mokronosov 1978). Number of chloroplasts per cell was determined in 120 cells for every variant. For electron microscopic measurements, leaves were fixed first in 1 % glutaraldehyde and then in 1 % osmic acid solution (Semenova 1985). CO_2 exchange was measured with the infrared gas

analyser *Infralyt-4*. The rate of O_2 production was determined using the Clark platinum electrode.

Chloroplasts were isolated from fresh leaves at 276 K by differential centrifugation (Rathnam and Edwards 1975). They were approximately 90 % intact and free from mitochondria as judged by phase contrast microscopy. The washed chloroplasts were lysed by resuspension in ice-cold 10 mM Tris-HCl (pH 8.0) with 1 mM $MgCl_2$, 0.1 mM EDTA for RuBPC and phosphoenolpyruvate carboxylase (PEPC) assays, and in ice-cold 0.05 M Tris-HCl (pH 7.8) with 5 mM sodium ascorbate, 3 mM cystein, 1 mM $MgCl_2$, and 5 mM dithiothreitol (DTT) for assays of other enzymes. *Triton X-100* (0.05 %, m/v) was added to resuspension medium to ensure complete lysis of the chloroplasts. Membranes were removed by centrifugation at 15 000×*g* for 15 min at 276 K in a *Beckman* rotor. The supernatant stromal fraction was decanted and kept on ice until use.

Enzyme activities were determined spectrophotometrically at 340 nm by *Specord UV-VIS*. All enzyme assays were done at least in triplicate. Before measurements, RuBPC was activated. Extracts were activated within 10 min at 303 K with 10 mM $NaHCO_3$, 10 mM $MgCl_2$, 0.1 mM EDTA, and 1 mM DTT in 0.1 M Tris-HCl (pH 8.1). Reactions were initiated by addition of the heat activated enzyme, assays were run for 15 min. RuBP was purchased from *Sigma*. The reaction medium contained for determination of activity of

RuBPC (4.1.1.39): Tris-HCl (pH 7.9) 0.1 M, $MgCl_2$ 30 mM, ATP 10 mM, glutathione 10 mM, NADH 4 mM, $NaHCO_3$ 50 mM, RuBP 6 mM

glyceraldehyde phosphate dehydrogenase (GAPD; 1.2.1.13): Tris-HCl (pH 7.8) 0.05 M, $MgSO_4$ 0.2 M, cysteine 0.1 M, ATP 31 mM, glutathione 26 mM, NADPH 3 mM, 3-PGA 10 mM

PEPC (4.1.1.31): Tris-HCl (pH 8.1) 0.05 M, $MgCl_2$ 50 mM, $NaHCO_3$ 100 mM, NaF 5 mM, NADH 6 mM, PEP 150 mM

malate dehydrogenase-NADPH (MDG-NADPH; 1.1.1.82): Tris-HCl (pH 8.2) 0.1 mM; DTT 20 mM, $MgCl_2$ 45 mM, oxaloacetic acid 0.5 mM, NADPH 0.3 mM

malic-enzyme-NADP (ME-NADP; 1.1.1.40): Tris-HCl (pH 8.0) 0.05 M, EDTA 0.1 mM, sodium malate 1.6 M, $MnCl_2$ 0.08 M, NADP 13 mM

Specific activity of enzymes was calculated using the extinction coefficient 6.22 and the water-soluble protein content (Bradford 1976). Reagents of the firms *Serva, Calbiochem, Sigma,* and *Reanal*, and mineral salts of the highest purity (Russia) were used. The Student *t*-test was used for statistical analysis.

Results

The light green colour of mutant leaves resulted from the decrease in contents of Chl *a,* Chl *b,* and carotenoids. The content of Chl (*a*+*b*) in M2004 decreased to 76 % and in M2014 to 55 % of the standard genotype (T), in hybrids it remained at the level of T (Table 1). Similar changes were observed in the contents of carotenoids. This might be caused by several factors: Chl content decrease in a single chloroplast, a decrease in cell number per unit of leaf surface, or both factors cooperating at the

Table 1. Chlorophyll (Chl) and carotenoid contents [mg kg^{-1}(f.m.)] in leaves, and relative amounts of individual carotenoids [% of total].

Pigment	Torsdag (T)	M2004	T×M2004	M2004×T	M2014
Chl *a*	1350±41	1030±38	1357±43	1365±52	750±23
Chl *b*	537±16	420±8	555±14	510±18	255±9
Chl (*a*+*b*)	1887±36	1450±19	1912±33	1875±28	1005±30
Carotenoids	590±25	441±13	592±19	598±21	312±10
β-carotene	[33]	[15]	[26]	[25]	[23]
Lutein	[37]	[43]	[39]	[42]	[43]
Violaxanthin	[19]	[31]	[23]	[24]	[21]
Neoxanthin	[11]	[11]	[12]	[9]	[13]

same time. Spectral properties of pigments are among the distinctive tests for Chl state in chloroplast thylakoids. At room temperature, absorption spectra of pigment extracts from chloroplasts of M2014, hybrids, and T did not differ (Fig. 1). In M2004 the second derivative of absorption spectrum showed an additional peak in absorption region of carotenoids at *ca.* 470 nm. Quantitative determination of carotenoids showed a two-fold decrease of β-carotene content in M2004 and an 1.7 times increase of violaxanthin compared to T (Table 1), and the same tendency was detected in M2014.

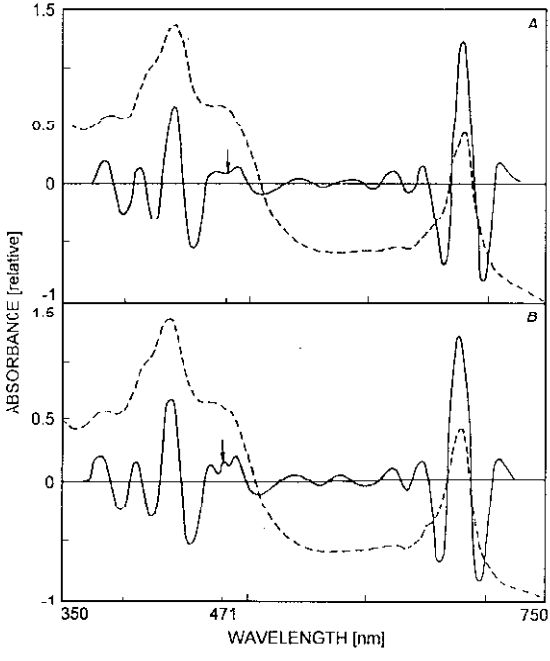

Fig. 1. Absorption spectra of pigments in 80 % acetone extracts from chloroplasts of pea leaves measured at room temperature (---) and their second derivatives (—). *A* - Torsdag; spectra of hybrids and M2014 were the same; *B* - M2004.

Table 2. Antenna sizes [number of chlorophyll *a* molecules] and numbers of reaction centres (RC) per leaf area [m^{-2}] and fresh mass [kg^{-1}] units for photosystems (PS) 1 and 2, and their ratios in pea chloroplasts.

	Antenna size		RC per area [$\times 10^{16}$]		RC per f.m. [$\times 10^{17}$]		RC PS2/PS1
	PS1	PS2	PS1	PS2	PS1	PS2	
Torsdag (T)	936±32	1114±56	10.53	8.85	11.00	9.24	0.84
M2004	1821±99	1301±81	4.21	5.89	3.78	5.29	1.40
T×M2004	918±43	975±48	10.42	8.75	10.89	9.15	0.84
M2004xT	864±39	929±17	11.65	10.84	11.88	11.05	0.93
M2014	1216±62	1871±96	4.66	3.03	3.80	2.47	0.65

There is a connection between β-carotene and reaction centres (RCs) of photosystems (Govindjee 1982). The decrease in β-carotene content in mutant chloroplasts was correlated with the decrease in number of RCs of PS1 and PS2. In M2004 chloroplasts the number of PS2 RCs was reduced 1.7 times and in M2014 3.7 times while the number of PS1 RCs decreased 3 times in both mutants (Table 2). According to this, antenna sizes of PS2 and PS1 were significantly increased in mutants. The hybrid plants were characterized by a tendency to increase the number of RCs of both photosystems, decrease the antenna size, and balance their ratio.

To specify these changes we examined pigment-protein complexes of chloroplasts. Gel electrophoresis showed many Chl-containing bands in all variants: LH_0, CP1, LH_1, LH_2, CP2, LH_3, and FP. Electron microscopic study showed that the ultrastructure of hybrid plastids was analogous to that of T. M2014 chloroplasts had the same ultrastructureas T, but the plastids had a stretched form (Fig. 2). In M2004 we detected also the light-distinct orientation of grana as a consequence of agranal thylakoid destruction, but the chloroplast ultrastructure did not differ substantially from that of T. Thus we supposed a specific change in PS1 structure of mutants which was confirmed by low-temperature fluorescence emission spectra of Chl: the spectra of hybrids and T were similar (Fig. 3). The height in M2004 of the long-wavelength emission peak, which belongs to the complex of Chl *a* with proteins of PS1 (Govindjee 1982), was increased in comparison with T, and this peak was shifted by 7 nm into the short-wavelength region. The fluorescence spectrum of M2014 differed from that of T mainly by a two-fold increase in heights of the 686 and 697 nm bands which belong to the complex of Chl *a* with PS2 proteins. This might be caused by the sharp decrease in Chl content as well as by changes in interaction of the light-harvesting complex with RCs as a consequence of the increase in antenna size. Analysis of the second derivative of low-temperature fluorescence emission spectra revealed the existence of the same Chl spectral forms in all studied variants, at 648, 661, 670, 676, 678, 684, 690, 696, and 708 nm (Ladygin and Bil' 1981).

The differences in pigment system affected the rates of primary photosynthetic reactions. Thus, the rate of electron transport along the cyclic pathway (CPP) in isolated chloroplasts of the M2004 and M2014 was decreased three times compared

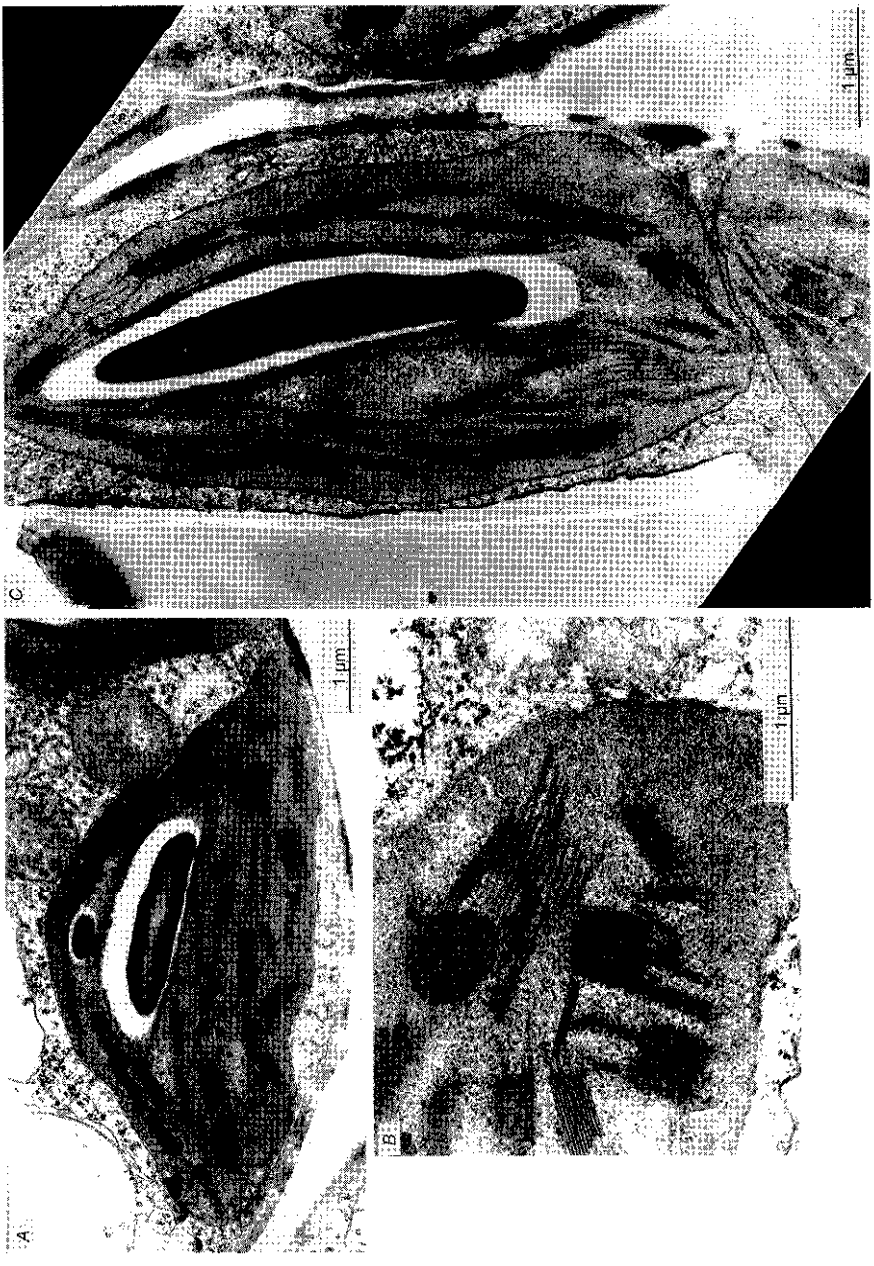

Fig. 2. Chloroplast ultrastructure of pea leaves. *A* – Torsdag; *B* – M2004; *C* – hybrid M2004×T.

to T (Table 3). However, the deficit in photosynthetic ATP was partly compensated by an increase in noncyclic photophosphorylation (NCPP) rate. The Hill reaction rate was also slightly increased in mutants in comparison with T. High productive hybrids were characterized by maximal rates of CPP and Hill reaction and an intermediate rate of NCPP.

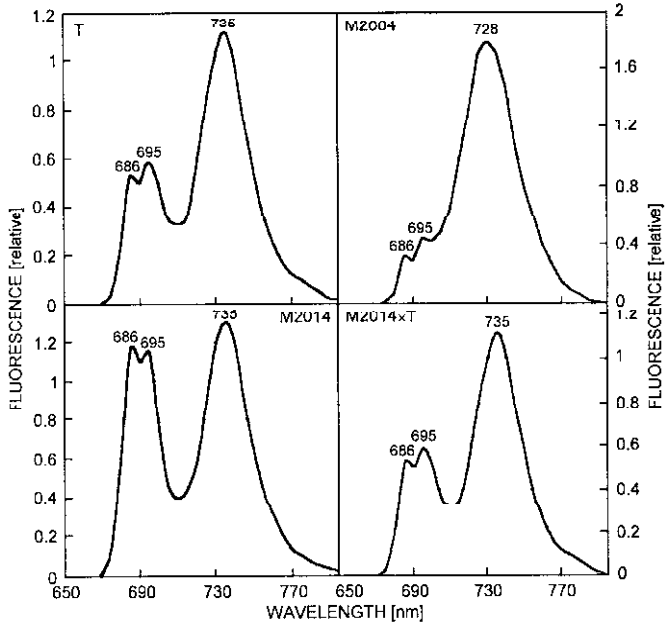

Fig. 3. Low temperature fluorescence emission spectra (77 K) of pea leaves of cv. Torsdag (T), mutants M2004 and M2014, and one hybrid.

The changes in fine structure of the photosynthetic apparatus (Table 5) were in accordance with CO_2 uptake and O_2 production (Table 4): these activities decreased in mutants and increased in hybrids in comparison with T. Tendency of changes in the rate of O_2 production was similar to that of Hill reaction. This confirmed calculations of assimilation number and photosynthesis of single chloroplast which were decreased in mutants and increased in hybrids.

Table 3. Photochemical activities of pea chloroplasts. FeCy - ferricyanide.

Activity of	Torsdag (T)	M2004	T×M2004	M2004×T	M2014
Cyclic photophosphorylation [mmol(ATP) kg^{-1}(Chl) s^{-1}]	99.7±5.0	31.1±0.3	120.6±5.8	125.0±3.3	34.7±2.5
Noncyclic photophosphorylation [mmol(ATP) kg^{-1}(Chl) s^{-1}]	91.4±2.2	161.1±3.9	114.4±4.4	107.8±3.1	190.6±5.3
Hill reaction activity [mmol(FeCy) kg^{-1}(Chl) s^{-1}]	158.6±4.7	176.1±5.0	208.3±7.8	232.2±6.1	174.4±4.7
Ratio P/2e	0.58	0.91	0.55	0.47	1.10

Table 4. Photosynthetic activity and mesostructure parameters of pea leaves.

Characteristic	Torsdag (T)	M2004	T×M2004	M2004×T	M2014
Potential photosynthesis [$\mu g(CO_2)\ m^{-2}\ s^{-1}$]	236±3	167±6	306±8	322±8	128±3
Rate of O_2 production [$nmol(O_2)\ m^{-2}\ s^{-1}$]	112±7	62±3	165±11	155±12	87±5
Photosynthesis of single chloroplast [$\times 10^6$(molecule CO_2) $chloroplast^{-1} \cdot s^{-1}$]	16.6	10.7	26.7	25.9	12.7
Assimilation number [$\mu g(CO_2)\ kg^{-1}(Chl)\ s^{-1}$]	408	339	556	556	336

The differences between pea mutants and T could equally well be explained by changes in cell number. The comparative study of leaf mesostructure revealed the basic cause of decrease in Chl content in the mutants. The number of Chl containing cells per leaf area unit in these plants was lower than in the normal cells in palisade and spongy tissues (Table 5). The number of chloroplasts in M2004 palisade cells was increased in comparison with T, but in M2014 there were no similar changes. The hybrid T×M2004 had the same number of chloroplasts in both types of tissues as T and the hybrid M2004×T had 3 chloroplasts more than the control spongy cells. In accordance with this, the saturation of leaf with photosynthetizing organelles was maximal in hybrids and minimal in the low-productive M2014. This decrease was not so significant in palisade cells and was at the control level in spongy cells in M2004.

Table 5. Mesostructure parameters of pea leaves in palisade (P) and spongy (S) tissues. Numbers of cells per m^2 of leaf area in thousands, numbers of chloroplasts in cell in units and per m^2 in millions.

Characteristic		Torsdag (T)	M2004	T×M2004	M2004×T	M2014
Cells per m^2 [$\times 10^3$]	P	53.0±2.0	34.0±1.0	55.0±2.0	54.0±3.0	36.0±2.0
	S	81.0±4.0	58.0±3.0	98.0±4.0	97.0±5.0	58.0±2.0
Chloroplasts per cell	P	18.0±0.5	25.0±1.7	19.0±0.6	20.0±2.9	17.0±0.4
	S	10.0±0.2	15.0±0.8	10.0±0.2	13.0±0.9	10.0±0.2
Chloroplasts per m^2	P	9.5±0.3	8.5±0.6	10.5±0.5	10.8±0.4	6.1±0.1
	S	8.1±0.2	8.7±0.4	9.8±0.3	12.6±0.6	5.8±0.1

All studied forms differed in the parameter CVC (chloroplasts volume coefficient), *i.e.*, the volume of a cell that is "served" by one chloroplast (Mokronosov 1978). Thus, the maximum CVC value among the examined variants supposed an increased cell volume provided with ATP, NADPH, and metabolites at the expense of activity of one chloroplast. There was a two-fold decrease in this

parameter in M2004 and a 1.5-fold increase in M2014 compared to T. These changes in CVC value were caused by the increase in number of chloroplasts per cell in M2004, but by the increase of cell volume of both types in tissue in M2014.

The degree of chloroplast saturation of leaf area unit was well in line with the raising of plateau of the dependence of photosynthesis on irradiance in the hybrids and with the lowering of it in the mutants (Fig. 4). The curves of both hybrids were

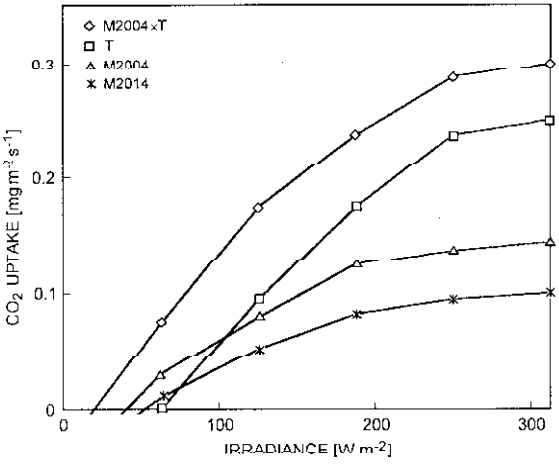

Fig. 4. Dependence of net photosynthetic rate on irradiance for pea leaves of cv. Torsdag (T), mutants M2004 and M2014, and one hybrid.

similar. CO_2 uptake in both the hybrids and mutants began at lower irradiance than that of T. This may be connected with the activation of photosynthetic enzymes (Woodrow and Berry 1988, Stitt and Quick 1989). In M2004, the activities of all studied photosynthetic enzymes were increased in comparison with T (Table 6). RuBPC and GAPD are the key enzymes of Calvin cycle, PEPC, NADPH-MDG, and NADP-ME are the enzymes of alternative photosynthetic ways. The CO_2 flow by alternative pathways in C_3 plants is only 1-3 % of the total CO_2 fixation. However, it is important because the activities of PEPC and NADPH MDG are increased under

Table 6. Activities of enzymes of photosynthetic metabolism [unit mg^{-1}(protein)] in pea chloroplasts.

Enzyme	Torsdag (T)	M2004	T×M2004	M2004×T	M2014
Ribulose-1,5-bisphosphate carboxylase	83±4	150±9	95±5	148±8	117±6
Glyceraldehyde phosphate dehydrogenase	312±11	949±28	492±21	546±33	147±8
Phosphoenolpyruvate carboxylase	5.7±0.2	8.5±0.3	6.4±0.2	8.1±0.3	3.7±0.4
NADPH-malate dehydrogenase	29±1.4	83±2.5	45±4.0	71±3.1	62±3.0
NADP-malic enzyme	3.6±0.1	4.8±0.2	4.2±0.1	4.6±0.2	6.0±0.3

stress and when the ATP/NADPH ratio in chloroplasts is changed (Edwards and Walker 1986).

Although the activity of RuBPC was higher in M2014 than in T, the activity of GADP that determines the use of atmospheric CO_2 in Calvin cycle was two times decreased. Simultaneously, there was a two-fold activation of alternative pathway enzymes PEPC, NADPH-MDG, and NADP-ME. The hybrid M2004×T had the same activity of RuBPC as M2004, but the reciprocal hybrid T×M2004 had RuBPC activity similar to that of T. Similar results were found for the activities of NADPH-MDG and PEPC. The activities of GAPD and ME were intermediate between the parent forms.

Discussion

Our experiments showed that the decrease in Chl content in leaves of mutants was caused by the decrease in number of cells per unit of leaf area. In spite of that, the main reason of poor green colouration of mutants was more complex. There were essential qualitative changes in the structure of PS1 in M2004: the destruction of agranal thylakoids where the functionally active PS1 is localized, and shift of the major band of PS1 fluorescence (735 nm) into the short-wavelength region by 7 nm. An almost two-fold increase in this fluorescence band corresponded to the increase of PS1 antenna size. The increase of the 686 and 695 nm bands of fluorescence emission that belong to PS2 caused changes in M2014. The numbers of RCs of PS1 and PS2 decreased in both mutants in comparison with T, but in M2004 mainly PS2 was changed. These differences are not only the result of the cell number decrease. The proteins of RCs are encoded by chloroplast genes, but around 20 mutations affecting RCs are found in the nuclear genome. In spite of Mendelian inheritance of the mutation type *chlorotica* (*chi*) found in M2004 (Shumnyĭ *et al.* 1982), both chloroplast and nuclear genomes may participate in the previous phenomenon. Probably it is one of the reasons of the fairly large deviation in *chi* from 3 : 1 which is typical for F_2 in crosses with M2004 (Sokolov 1989). Most probably, the changes in PS1 Chl-protein complexes are the primary cause of mutation though there are other causes which show pleiotropic effects.

According to Lichtenthaler (1987) and Knoetzel *et al.* (1988), β-carotene belongs to photosystem cores. Our investigation confirmed this view. The decrease in content of β-carotene in mutant chloroplasts was in accordance with the decrease in number of PS2 RCs per unit fresh mass. However, in M2004 β-carotene may function in the PS1 antenna complex, because the 735 nm band is shifted which provides a connection between the light-harvesting complex and RCs (cf. Öquist *et al.* 1980). Thus the visual phenotypic differences between the mutants and T can be explained by defects both in cell number and in some membrane-bound Chl-protein complexes.

If β-carotene content in M2004 is correlated with the number of RCs, the high content of violaxanthin may be correlated with high rate of noncyclic photophosphorylation. Under these conditions the number of RCs of PS1 was 3 times decreased. This may be an indirect evidence that in the violaxanthin cycle oxygen of

H_2O is used, the formation of which is given by the activity of RCs of PS2. Intense work of these centres is proved by an almost two-fold increase of noncyclic electron transport rate in the mutant chloroplasts. The index ratio P/2e is a very important parameter of NCPP efficiency, allowing to judge the number of phosphorylating centres and the degree of NCPP coupling with the photosynthetic electron transport. This can indicate the best electron transport coupling and phosphorylation (Rubin and Gavrilenko 1977). Apparently the activation of NCPP in mutants is connected with the compensatory effect in response to the decrease in CPP rate, and therefore it is also connected with decrease in photosynthetic pool of ATP. The low-productive M2014 has the maximal rate of NCPP accompanied by the maximal antenna size in PS2 among all the studied forms. At the same time, the number of RCs of PS2 was 4 times decreased in chloroplasts of this mutant than in T, and the ratio P/2e exceeded 1. Hence in the mutant thylakoid membranes additional coupling centres of phosphorylation may exist. A similar compensation is possible for the photosynthetic ATP pool increase. The same results were obtained in other Chl mutants of pea by Roshchina *et al.* (1983) and Schwarz (1983).

In the common pool of photosynthetic ATP in chloroplasts, up to 70 % belong to CPP and 30 % to NCPP (Zalenskiĭ *et al.* 1966, Lawlor 1987). So we can confirm that the formation of ATP in chloroplasts of mutants causes some difficulties. In the mutants the decrease in number of RCs of both photosystems is not proportional to that in T. Thus in M2004 chloroplasts every RC of PS2 corresponds to 0.7 of RC of PS1, but in the T chloroplasts to 1.2 RCs of PS1. Moreover, the rate of noncyclic transport of electrons in mutant chloroplasts is considerably higher, and the rate of cyclic transport of electrons lower than in T. Hence in such conditions the pseudocyclic transport of electrons may be activated: in this case the electron flow is switched from NADP to O_2 with H_2O_2 formation. In contrast to T, in mutant chloroplasts the content of violaxanthin is increased, the rate of NCPP is larger, and there is also an 8-fold increase in endogenous H_2O_2 concentration during 1 min irradiation after keeping chloroplasts in darkness for 10 min (Kalashnikov and Vaishlya 1991). This points to an activation of pseudocyclic transport of electrons in chloroplasts of these mutants. Probably both the activation of violaxanthin cycle and H_2O_2 synthesis serve for moving off the O_2 surplus, which is connected with intensive work of RCs of PS2. It partly compensates the sharp decrease of CPP rate.

CPP actively works when the content of NADP is lower than is necessary for accepting electron from reduced ferredoxin. The large pool of NADP is required for stable functioning of the noncyclic electron transport. The activation of NADPH-MDG is likely to be the source of NADP. NADPH-MDG is an exclusive plant enzyme and it is localized in chloroplasts. Malate, the product of this reaction, is used in different metabolic ways, for example by ME, providing the supply of CO_2 and NADPH in the Calvin cycle (Lance and Rustin 1984). Studies of these and other photosynthetic enzymes and of the contents of oxaloacetic acid, malate, and pyruvate in chloroplasts have shown that switching of carbon flow over organic acids' synthesis occurs in the low-productive M2014 (Vaishlya 1993, 1996). It is provided by the shortage of ATP in CPP, which is used for the synthesis of starch and sucrose. Probably the shortage of ATP and NADP causes the decrease of GAPD activity in

chloroplasts of M2014: the enzyme is very dependent on the rate of primary reactions of photosynthesis. In contrast to this, in the heterotic M2004 the activity of key enzymes of Calvin cycle and enzymes of alternative path of photosynthesis is higher than in T. We suppose a compensatory effect of metabolism is presented here which supplies to chloroplasts the necessary amounts of needed intermediates and energy for CO_2 fixation. According to Scheibe and Beck (1975), Perrot-Rechenmann *et al.* (1982), and Ferte *et al.* (1986), the conversion of organic acids in a cell may be used as the main source of substrate. Activation of these processes in mutant leaves was shown earlier (Vaishlya 1993).

Activation of RuBC in leaves of mutants also implies that besides ATP enough ribulosebisphosphate and CO_2 (Scheibe and Beck 1975, Perrot-Rechenmann *et al.* 1982, Ferte *et al.* 1986) must be present in chloroplasts. The probable supplier of this are dark metabolism reactions functioning in assimilating cells of green plants (Sokolov *et al.* 1983, Singh and Naik 1984, Lawlor 1987). The affinity of carboxylases is higher to CO_2 of endogenic origin in comparison with exogenic CO_2 (Govindjee 1982, Edwards and Walker 1986). This may be one of the causes of activation of RuBPC and PEPC in mutant chloroplasts where the diffusion rate of atmospheric CO_2 to the carboxylating centres is much lower than in the initial line (Sokolov *et al.* 1988).

We can explain the low productivity of M2014 by the absence of compensatory effect which M2004 has. For example, in M2014 the chloroplast number decreased in the spongy and palisade cells, and the sharp decrease in pigment contents, number of photosystem RCs, rate of NCPP and CPP, and CO_2 uptake correlated well with the decrease in Chl-containing cells per unit of assimilating surface. In this case, the volume of cell served by one chloroplast increased, and it indicated the shortage of photoproducts in cytoplasm. In previous papers we have shown that not fully reduced saccharides are quickly involved in oxidative processes. Hence in M2014 they may support the defective structure of photosynthetic apparatus. But what advantage the packing of membranes in grana or agranal thylakoids gives to plants? In both mutants the number of PS1 RCs and the rate of CPP sharply decreased, but under these conditions only in M2004 agranal thylakoids were destroyed, where PS1 is localized. Probably the absence of such serious disturbances inside of M2014 chloroplasts is connected with the absence of compensatory complex of genes. It is the main reason of sharp decrease of yielding in M2014 and also of absence of the heterosis effect.

A clear heredity in the maternal line became apparent in the hybrid M2004×T according to RuBPC, PEPC, NADPH-MDG, and NADP-ME activities. The majority of enzymes of dark stage of photosynthesis is encoded by nuclear and plastid genomes (Mullet 1988). The heterosis advantage of hybrids F_1 is determined by the interaction of non-allelic genes according to the epistasis type as the result of formation in M2004 compensatory complex of genes (Sokolov 1989). Apparently, plastid genome plays a definite role in this process.

The increased activities of carboxylases in mutants and hybrids may be the reason of CO_2 uptake already under a low irradiance. Moreover, it might be the consequence of increase in numbers of spongy cells in hybrids and of chloroplasts in these cells in M2004 and hybrids. The ratio Chl *a/b* is increased in spongy cells in comparison with

palisade cells, and such plastids are characterized as chloroplasts of the shade type. The hybrid M2004×T inherits from M2004 the increased number of chloroplasts in spongy cells, and the reciprocal hybrid T×M2004 contains the same number of chloroplasts as T. The factors that denote the number of chloroplasts are under nuclear control, and chloroplast DNA encodes their ability to division. Such process leads to the existence of additional number of plastids (Butterfass 1973, Tsel'niker 1978, Pyke and Leech 1987). These results demonstrate that the compensatory complex of genes in M2004 probably includes some chloroplast genes that define additional plastid formation in cells.

All these results show that the hybrid vigour is determined by the heredity of normal structure of photosynthetic apparatus from T and of compensatory gene complex from M2004, which is formed as a response to depressive effects of mutation. The specific change of PS1 structure in mutants is likely to be the sign the blocking which primarily depends on mutation. The pleiotropic character of mutation appears at different levels of organization as a compensatory complex of genes' work which is presented in this paper. As a result of this, hybrids contain more photosynthesizing organelles and consequently have an increased rate of CO_2 uptake, more RCs, heightened photophosphorylation rate, and better activation of enzymes of dark stage of photosynthesis than T. All these changes mean the strengthening of exchange of energy and materials in hybrids. It allows them to create more powerful carbon flow for formation of vegetative and reproductive organs in comparison with the best parent line.

Our results help to understand the phenomenon of hybrid vigour. They may be used for creation of a diagnostic test for early forecasting of heterosis progeny under laboratory conditions.

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